Enhancing Tasmanian Clam Resources

Principal Investigator: G. B. Maguire

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1.0 NON-TECHNICAL SUMMARY

This project was undertaken primarily to assess the state of clam fisheries in Tasmania, chiefly for the intertidal stepped venerid *Katelysia scalarina* and to develop technology for hatchery production and growout.

Because of unpredictable settlement of spat, slow growth to commercial size (4-6 years), relatively high rates of natural mortality, very high manual catch rates, and environmental concerns including impact on oystercatchers, there is limited potential for expansion of the *K. scalarina* fishery without stock enhancement.

Mature *K. scalarina* were common in warmer months but did not spawn predictably in hatcheries unless in prime condition. The larval phase was relatively short but in addition to spawning problems, a herpes-like virus often caused mass larval mortality around day 8 and larval settlement was difficult. Researchers in this project were successful in producing small quantities of spat but commercial hatchery trials were unsuccessful. This greatly affected progress towards commercial goals but wild spat collection allowed research on major potential limiting factors (salinity requirements, site effects, natural diet, and effects of density and beach position). The project also facilitated a range of other relevant clam research initiatives including work on environmental impact of clam fishing, genetic issues, predation and parasites and diseases of clams. A range of these potential limiting and other factors may contribute to the periodic occurrence of significant mortalities in the wild which are reflected in the high natural mortality estimate provided in a detailed fishery assessment based on aging, growth and mortality studies. However, mortality in mesh cages in trials lasting 10.5-12 months was very low (<10%) unless located high in the intertidal zone.

Katelysia scalarina is not well suited to aquaculture despite tolerating high stocking densities in cages but the subtidal species *Ruditapes* (=*Venerupis*) *largillierti* shows more promise, based on trails conducted within this project. However, protecting a subtidal clam from predators represents a major technical challenge.

2.0 BACKGROUND

The rapid expansion, and accompanying threat of overfishing, of clam fisheries in Tasmania prompted Tasmanian FRAB and FRDC to facilitate, from trust funds, a broad thrust, into aquaculture technology for two species of Tasmanian clams *Katelysia scalarina* and *Venerupis largillierti.* The aim is to allow future expansion of clam production on a sustainable basis.

This proposal combines the expertise of Dr Greg Maguire (UTAS) who has been monitoring a wild clam population since 1991, four fishermen who have pioneered clam harvesting in Tasmania and Mr Will Zacharin, Division of Sea Fisheries (DSF), Tasmanian DPI who has extensive experience in research and management of bivalve resources in Tasmania.

The proposal is based on a previous application by Dr Maguire to FRDC in 1992 and on a general proposal from one of these fishermen Mr Craig Barnes. That proposal for enhancement of the state's clam fisheries received strong support from the then Minister for Fisheries and key representatives of the Tasmanian aquaculture industry.

3.0 NEED

The production of clams and cockles (*terms often used interchangeably) has become a very significant industry with world production in 1988 (1.75 million tonnes) exceeding that of oysters. International trade in clams and cockles has grown to be worth US $300 million in 1988 (de Franssu, 1990). Considerable progress has been made on aquacultural technology for clams (Manzi and Castagna, 1989) and in Asia "almost all of their cockle and clam landings involve some aspect of culture in the grow-out process" (Manzi, 1991). FAO statistics for 1991 indicate a continuing expansion in the proportion supplied through aquaculture. Economic models indicate that clam farming can be quite profitable overseas e.g. 22 % return on capital in USA, but that returns are sensitive to the farming strategy used (Toba et al., 1992).

Potential exists for Tasmania to foster the development of an industry which would mirror the world situation in which clams and cockles make up around 25% of world mollusc production (Manzi, 1991) and are often a luxury food.

Regrettably, clam fisheries worldwide are declining probably because of a number of factors including pollution and over fishing (Manzi and Castagna, 1989). Fisheries managers in Tasmania consider that the current harvest rate is unsustainable (over 250 tonnes of clams, chiefly *K. scalarina,* during the last two years). It is considered that some form of aquaculture will be necessary to sustain a significant clam industry in Tasmania. These clams can be collected readily by hand or with simple tools in intertidal or shallow subtidal areas.

This project will address the problem of how to ensure that sustainable production of clams can be achieved on a large scale in Tasmania and the results could benefit potential clam industries in other parts of Australia. [Clam fisheries in other parts of Australia are small, except for beach pipis *(Donax deltoides),* but have potential for expansion]. This project will be the first major R&D project on production of Australia's edible temperate clams.

In addition to helping meet the growing demand for clams in Australia, this project could lead to exports; Tasmanian clam fisherman have established that market demand exists in Asia for their product but they have not been able to supply the volume of product required. In general, fisherman have had little trouble selling live clams in Australia for A$ 4/ kg to wholesalers. In comparison, 65 g Tasmanian Pacific oysters *(Crassostrea gigas)* fetch about A$ 4.50/ kg live, ex-farm.

Shellfish assurance programs would be extended to clam farming leases to ensure that the image of farmed bivalves and public safety are not threatened through poisoning of consumers via pollutants, algal toxins, infective organisms e.g. pathogenic bacteria.

From an environmental perspective the orderly harvesting of clams, within leases away from areas of high conservation value, would be preferable to uncontrolled disturbance of large areas of seabed (see Lush, 1992a).

To evaluate and optimise procedures for enhancing clam production it will be necessary to develop hatchery, nursery and growout procedures for these species. Fortunately, technical manuals and other publications are available for species farmed in other countries e.g. Manila clams *Ruditapes philippinarum*(Manzi and Castagna, 1989; de Valence and Peyre, 1990; Spencer et al., 1991; Toba et al., 1992).

Apart from direct aquacultural production, there is a need for more information on the general biology of *K. scalarina* and *V. largillierti.*

(A) Reproductive biology

Bivalves vary in reproductive season, frequency of maturation/spawning cycles, length of cycles, tendency towards hermaphroditism, fecundity, egg size and age at maturity (Eversole, 1989). Dr Maguire has undertaken monthly sampling of a population of *K. scalarina* since January 1991 on the east coast of Tasmania. Condition index data (a measure of the cavity volume occupied by meat) indicate summer peaks in 91/92 and 92/93. However, the detailed histological work has yet to be done. Relatively little equivalent information is available for *V. largillierti* but sampling has commenced.

(B) Habitat requirements

Sediment type can affect burrowing behaviour (Roberts, 1981) and distribution of clams (Malouf and Bricelj, 1989) and hence the requirements of these species should be investigated in relation to tidal position, sediment grain size distribution, organic content and redox potential.

(C) Gut contents

This topic will be investigated to assess potential niche overlap among these species and Pacific oysters. *V. largillierti* held at the University's Aquaculture Centre at Launceston (UTAS at Ltn) have consumed microalgal species normally fed to oysters. Given current concern over carrying capacities of oyster growing areas, it seems prudent to investigate this issue as several Tasmanian farmers have had their aquaculture permits endorsed for clam aquaculture. Gut contents studies may be of additional value if supplementary feeding is required in the nursery phase.

(D) Age studies

A major problem in managing fisheries and in assessing aquaculture potential of species, based on data from wild populations, is the need to understand age structure. This project affords an opportunity to verify ageing techniques in relation to cultured clams of known age as well as helping to establish the age of clams used for determining breeding seasons.

(E) Population structure

If the proposed research is to be of maximum value to Australia it would be useful, using electrophoretic techniques, to establish whether the Tasmanian stock of *K. scalarina* differs from mainland populations.

(F) Salinity tolerance

This factor may limit the range of sites in which these clams can be grown.

[* Explanation of the terms clams and cockles. Manzi (1991) considered clams to be bivalve molluscs of the Order Heterodonta which includes *V. largillierti and K. scalarina* and that cockles belong to Order Taxodonta. Other authorities restrict the term clams to the classificatory rank Veneroidea within the Heterodonta. In general, the taxonomy of clams and cockles is in a confused state (Manzi and Castagna, 1989; Manzi, 1991).]

4.0 OBJECTIVES

1. Identify spawning seasons for *V. largillierti and K. scalarina* in Tasmania.
2. Quantify seasonal changes in meat condition in wild and cultured populations.
3. Using hatchery techniques, produce substantial numbers of clam spat of both species.
4. Conduct any larval and nursery experiments needed to overcome problems experienced during large scale hatchery and nursery production runs.
5. Establish growout trials on a pilot scale (with commercial fishermen in association with DSF) and on a replicated experimental scale (with University staff). Facilitate independent trials by oyster farmers through fostering commercial production of clam spat.
6. Optimise growout variables such as stocking density, frequency of density reduction, and predator protection.
7. Through observations in the wild and experimental studies, assess the importance of various sediment characteristics.
8. Compare the gut contents of these two species and Pacific oysters.
9. Optimise and verify age determination techniques for clams.
10. Establish whether Tasmanian clam populations differ genetically from southern mainland and New Zealand populations.
11. Determine salinity tolerance limits for the two species of clams.
12. Raise public awareness of clams as a desirable food item.
13. Foster the sale of clams harvested in accordance with the Tasmanian Shellfish Quality Assurance Program.
14. Through meeting the above objectives, help provide the information needed to develop an optimum management regime for Tasmania's clam resources.

5.0 METHODS/RESULTS/DISCUSSION

5.1 MANUSCRIPT 1

Gametogenesis and condition index of the stepped venerid, *Katelysia scalarina* (Lamarck 1818), at two sites in Tasmania, Australia.

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5.1.1 ABSTRACT

This study documents changes in gonad maturation and condition index of *Katelysia scalarina* as indicated by monthly morphometric, condition index and histological analyses of wild caught stock from two sites in Tasmania. This is the southern most state within the geographical range for this endemic Australian species. Most of these clams could be ascribed to specific gametogenic stages, based on criteria previously used for oysters. The clams were predominantly (≥50% of sample) mature in spring and summer during the specific periods of November 1991 to February 1992, August 1992 to February 1993 and August 1993 to December 1993 (except September 1993) at Little Swanport (most samples collected prior to the commencement of this grant but processed during the grant period). The equivalent periods for Ansons Bay, where subsequent sampling was undertaken during the grant period in the main commercial fishery, were February 1994 (initial sample) to March 1994, October 1994 to February 1995, and late August to December 1995 (final sample). In summary, this species commences a phase of gonad maturation at Little Swanport in autumn and becomes mature in late winter-spring and regresses in summer - early autumn. These results suggest that peak spawning periods are more likely to be in summer - early autumn whereas other published studies indicate peak spawning periods in September-October in Victoria and April-June in southern Western Australia. Most clams could be sexed, although indeterminate stage clams were evident at Little Swanport but not Ansons Bay where clams were usually larger. No hermaphrodites were observed.

In the 51 monthly samples from Little Swanport, females were more common than males in 25 samples while males were more abundant in only 10 samples. At Ansons Bay, 11 and 6 samples out of 21 samples were skewed towards female and male abundance respectively. Because of the greater monthly sample size used at Ansons Bay it was appropriate to also consider maturation patterns for each sex separately and the seasonal maturation patterns were similar for each sex.

Condition index and moisture content of meats were more stable than for farmed diploid Pacific oysters, *Crassostrea gigas* sampled from Tasmanian waterways and this is a favourable outcome for marketing of this clam. However, declines in condition index were consistent with the likely spawning periods indicated by gonad histology.

Keywords: Clam; venerid; *Katelysia scalarina*; broodstock; spawning; condition; morphometrics

5.1.2 INTRODUCTION

An understanding of reproductive biology of venerid clams is important for aquaculture and fisheries management purposes (Eversole, 1989; Shafee and Daoudi, 1991). Venerid clams

exhibit species-dependent reproductive strategies that include separate sexes through to functional hermaphroditism and, within a single species, reproductive seasonality can vary for different locations (Eversole, 1989). The stepped venerid *Katelysia scalarina* (Lamark 1818) (Fig. 1) is a relatively small clam of 35-50 mm shell length (Lush, 1992) that resides in the upper sediment layer (2-4 cm depth) (Nielsen, 1963; Bellchambers and Richardson, 1995) in both sheltered and moderately exposed intertidal waters of Tasmania and southern mainland Australia (Roberts, 1983; Lamprell and Whitehead 1992). Apart from its ecological significance as an abundant species in coastal inlets (Bellchambers, 1998), this is the major species within the Tasmanian clam fishery (see Manuscript 13) and its aquaculture potential is also being assessed (Kent et al., 1998). Previous investigations have indicated that the major spawning periods for this species in Victoria and Western Australia are September-October (Nielsen, 1963) and April-June (Roberts, 1983) respectively. Tasmanian populations could exhibit a different pattern because of the cooler temperature regime or because these populations are genetically distinct from southern mainland populations (Soh et al., 1998). In Tasmania, Riley et al. (see Manuscript 13) found that *K. scalarina* reaches sexual maturity before reaching the minimum commercial size (32 mm shell length) with advanced gonad development evident in individuals as small as 19 mm, however no research on seasonality of maturation was presented.

Venerid clams can commit "as much as 50% of non-respired assimilated energy annually to reproduction" and this can affect the relative proportions of soft tissue and shell (Eversole, 1989). This latter variable is typically measured as a condition index which may also be a useful guide the marketability of individuals within a bivalve population (Nell et al., 1994). This present study documents changes in gonad maturation and condition index for *K. scalarina* at two sites in Tasmania. It complements an equivalent study (Maguire and Kent, Manuscipt 2) on *Ruditapes largillierti*, the other major venerid clam fished commercially in Tasmania and which is also being evaluated for aquaculture (Kent et al., 1999).

5.1.3 MATERIALS AND METHODS

Adult *K. scalarina* were collected, usually every month, from near the mouth of Little Swanport (1991-1994) during FRDC funded, triploid oyster sampling trips and from the seaward half of Ansons Bay (1994-1995), the site of the major fishery for this species, during a more intensive study focussed specifically on *K. scalarina* (Fig. 2). Both inlets receive input from upstream catchments and are located in a coastal region prone to influence from sporadic periods of high rainfall (Manuscript 5; Bellchambers, 1998). The average shell length for each monthly sample ranged from 36.9 - 42.9 mm (Little Swanport) and from 36.9 - 43.5 mm (Ansons Bay).

All clams collected from natural habitats were transported to the University of Tasmania, Launceston in plastic containers without water or sediment. Standard 4 μm paraffin sections were prepared from a 3 mm slice of the preserved samples using the anterior edge of the foot as a reference (Howard and Smith, 1983). These were stained using Mayer's haematoxylin and eosin Y. Unfortunately, the intensity of staining did not allow image analysis of gonad area. Gonads were staged for gametogenic development using a modified staging system (Fig. 3) based on Dinamani (1974). Whole weight was measured (to 0.01 g) after clams were immersed overnight in a recirculating system approximating oceanic conditions, cleaned if necessary, and dried superficially with paper towel. Condition index [CI=dry meat weight (g) x 1000/cavity volume (g)] was assessed by drying individual meats (soft tissue) at 80^{o}C to constant weight in a forced draft oven (24-48 h); cavity volume was estimated as the difference between whole weight (g) and shell weight (g) (Crosby and Gale, 1990). Dimensions of clams were measured with Vernier callipers to 0.01 mm. Sampling intensity for gonad analyses was 10 and 20 clams

per sampling time for Little Swanport and Ansons Bay respectively. All other sampling intensity data are provided in the relevant graphs below.

5.1.4 RESULTS

Most *K. scalarina* could be ascribed readily to specific gametogenic stages (Figs 4-16). The clams were predominantly (≥50% of sample) mature (G4 stage) in November 1991 to February 1992, August 1992 to February 1993 and August 1993 to December 1993 (except September 1993) at Little Swanport (Figs. 17-18). The equivalent periods for Ansons Bay were February 1994 (initial sample) to March 1994, October 1994 to February 1995, and late August to December 1995 (final sample) (Figs. 19-20). Immediately after these periods most clams were regressed (G5 or GX). Seasonal maturational patterns were similar for males and females at Ansons Bay where the higher monthly sample size allowed meaningful analyses to be conducted for each sex (Figs. 21-22). Most clams could be sexed, although a few indeterminate stage clams were evident at Little Swanport but not at Ansons Bay (Figs. 23-24). No hermaphrodites were observed. In the 39 samples from Little Swanport, females were more common than males in 20 samples while males were more abundant in only 8 samples (Fig. 23). At Ansons Bay, 11 and 6 samples out of 21 samples were skewed towards female and male abundance respectively (Fig. 24).

The size of the clams sampled was relatively consistent within a site although the clams were generally larger at Ansons Bay (Figs. 25-26). The moisture content of the meats was relatively stable although major declines in Condition Index values were evident just prior to the end of each of the above periods when clams were mature. These declines were not evident in wet meat weight data (Figs. 22-23).

5.1.5 DISCUSSION

Histological sections for *K. scalarina* can be classified using a slightly modified staging scheme used for several oyster species (Dinamani, 1974; Gardner et al., 1994; Cox et al., 1996). In contrast, the asynchrony of gamete development within the gonad of *Ruditapes largillierti* necessitated the development of a more species-specific staging protocol (Manuscript 2). The occurrence of some indeterminate clams and the delay of 2-4 months between occurrence of substantial numbers of regressive (G5 or GX) and early developing (G2) stages in *K. scalarina* are consistent with patterns for oysters in which sex change occurs (male to female). The bias towards incidence of females may reflect the relatively large size class sampled and hence greater abundance of females after males change sex. Typically, sex ratios are equal in clams (Eversole, 1989). However, as hermaphrodites were absent, it is not possible to confirm this sex change.

This species commences a phase of gonad maturation at Little Swanport in autumn and becomes mature in late winter-spring and regresses in summer - early autumn. The time series for Ansons Bay is shorter but the maturation pattern is consistent with this summary. Unfortunately, this species is difficult to spawn and thus the attaining of G4 stage may not by itself be indicative of suitability for direct use in a hatchery (Kent et al., 1998). Broodstock for this species have only been conditioned successfully when lower water temperatures than used for conditioning Pacific oysters in Tasmania (Kent et al., 1998) and this may reflect the relatively early seasonal onset of maturation noted above for *K. scalarina.* Temperature is a highly influential variable in conditioning bivalve broodstock (Heasman et al., 1996).

There is a trend towards bimodality in the occurrence of G4 stage clams at Little Swanport (Figs. 17-18) and similar observations have been made by Nielsen (1963) and (Roberts, 1983) for this

species and by other researchers including Shafee and Daoudi (1991) and Toba et al. (1993) for other clam species including *Ruditapes decussatus* and *R. phillipinarum*. Our results suggest that peak spawning periods for *K. scalarina* are more likely to be in summer - early autumn whereas their findings indicated September-October in Victoria (Nielsen, 1963) and April-June in southern Western Australia (Roberts, 1983). Intraspecific variability in reproductive cycles in relation to location and specifically to environmental or genetic differences is evident in other species of clams and in bivalve molluscs in general (Eversole, 1989; Hesselman et al., 1989).

Condition index and moisture content of meats were more stable than for farmed diploid Pacific oysters, *Crassostea gigas* sampled from Little Swanport (Maguire et al., 1994). This is a favourable outcome for marketing of this species. Typically, Condition Index improved as gonad maturation commenced and declined around likely spawning periods as was the case with these Pacific oysters (Maguire et al., 1994). In contrast, Shafee and Daoudi (1991) observed an inverse relationship between Condition and Gonad Indices during seasonal maturation of the clam *Ruditapes decussatus*.

Mature *K. scalarina* are abundant for several months per year over five consecutive summers. Hence broodstock availability should not be a limiting factor for aquaculture provided that broodstock conditioning procedures can be optimised to ensure that these mature individuals spawn readily. It should be noted, however, that over relatively short distances, eg 100m, large differences can occur in maturity at least on the basis of sacrificial visual assessment. The reliability of this maturational phase in wild populations augers well for the sustainability of the fishery. However it is not always possible to distinguish between gonads in postspawning phase or resorption phase after a failure to spawn in the wild. It would also be useful to develop modified histological staining procedures to facilitate image analysis for gonad area in *K. scalarina*.

5.1.6 ACKNOWLEDGMENTS

We are indebted to Oyster Bay Oysters for their assistance with sample collection in Little Swanport.

5.1.7 REFERENCES

Bellchambers, L. M., 1998. Ecology and Ecophysiology of *Katelysia scalarina* (Bivalvia: Veneridae); a commercially exploited clam. Unpublished PhD thesis, University of Tasmania.

Bellchambers, L. M. and Richardson, A. M. M., 1995. The effect of substrate disturbance and burial depth on the venerid clam, *Katelysia scalarina* (Lamarck, 1818). J. Shellfish Res., 14(1): 41-44.

Cox, E., Smith, M. S. R., Nell, J. A. and Maguire, G. B. 1996. Studies on triploid oysters in Australia. VI. Changes in gonad development in diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). J. Exp. Mar. Biol. Ecol., 197: 101-120.

Crosby, M. P. and Gale, L. D., 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. J. Shellfish Res., 9: 233-237.

Dinamani, P., 1974. Reproductive cycle and gonadial changes in the New Zealand rock oyster *Crassostrea glomerata*. N.Z. J. Mar. Freshwater Res., 8 (1): 39-65.

Eversole, A. G., 1989. Gametogenesis and spawning in North American clam populations: Implications for culture. In: J. J. Manzi and M. Castagna (Editors), Clam mariculture in North America. Developments in Aquaculture and Fisheries Science, vol 19. Elsevier, Amsterdam, pp. 75-109.

Gardner, N. C., Smith, M. S. R., Maguire, G. B., Kent, G. and Nell, J. A., 1993. Studies on triploid oysters in Australia. III. Gametogenesis of diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania. In: J.A. Nell and G.B. Maguire (Editors), Evaluation of Triploid Sydney Rock Oysters (*Saccostrea commercialis*) and Pacific Oysters (*Crassostrea gigas*) on Commercial Leases in New South Wales and Tasmania. FRDC Grant Report.

Heasman, M. P. O'Connor, W. A. and Frazer, A. W., 1996. Temperature and nutrition as factors in conditioning broodstock of the commercial scallop *Pecten fumatus* Reeve. Aquaculture, 143(1): 75-90.

Hesselman, D. M., Barber, B. J. and Blake, N. J., 1989. The reproductive cycle of adult hard clams, *Mercenaria* spp. in the Indian River lagoon, Florida. J. Shellfish Res., 8(1): 43-49

Howard, D. W. and Smith, C. S., 1983. Histological techniques for marine bivalve mollusks. NOAA Technical Memorandum NMFS-F/NEC-25.

Kent, G. N., Maguire, G. B., John, M., Cropp, M. and Frankish, K. 1998. Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina* (Lamark 1818). J. Shellfish Res., 17(4): 1065-1070.

Kent, G. N., Maguire, G. B., and Duthie, I., 1999. Spawning, larval rearing, settlement and growth of the New Zealand Venerid *Ruditapes largillierti* (Philippi 1849). NZ J. Mar. and Freshwater Res., 33: 55-62.

Lamprell, K. and Whitehead, T. 1992: Bivalves of Australia. Volume 1. Crawford House Press, Bathurst, NSW, Australia.

Lush, G., 1992. The life history of the common cockle with particular reference to the Tasmanian genus *Katelysia*. Internal Report, Tasmanian Dept. of Sea Fisheries, Taroona, Australia.

Maguire, G. B., Gardner, N. C., Nell, J. A., Kent. G. and Kent, A., 1994. Studies on triploid oysters in Australia. II. Growth, condition index, glycogen content and gonad area of triploid and diploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania. In: J.A. Nell and G.B. Maguire (Editors), Evaluation of Triploid Sydney Rock Oysters (*Saccostrea commercialis*) and Pacific Oysters (*Crassostrea gigas*) on Commercial Leases in New South Wales and Tasmania. FRDC Grant Report., pp. 37-71.

Nell, J. A., Cox, E., Smith, I. R. and Maguire, G. B. 1994. Studies on triploid oysters in Australia. 1. The farming potential of triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). Aquaculture, 126: 243-255.

Nielsen, B.J., 1963. Studies of the genus *Katelysia* Romer 1857 (Mollusca, Lamellibranchiata), Mem. Nat. Mus. Vic., 26: 219-257.

Riley, S., Green, R., Zacharin W. and Maguire, G. B. Growth models and age determination for the intertidal venerid clam *Katelysia scalarina* (Lamarck 1818) from three sites in Tasmania, Australia. In: G. B. Maguire, ed. Enhancing Tasmanian Clam Resources. Final Report to Fisheries Research & Development Corporation, Project 93/232. FRDC, Canberra. Submitted.

Roberts, D., 1984. The genus *Katelysia* (Bivalvia: Veneridae) in southern Australia. J. Aust. Malacol. Soc., 6: 191-204.

Shafee, M. S. and Daoudi, M., 1991. Gametogenesis and spawning in the carpet-shell clam, *Ruditapes decussatus* (L.) (Mollusca: Bivalvia), from the Atlantic coast of Morocco.

Soh, S. W. L., Maguire, G. B. and Ward, R. D. 1998.Genetic studies of the venerid clam genus *Katelysia.* J. Shellfish Res., 17(4): 1057-1064.

5.1.8 FIGURES

Figure 1. *Katelysia scalarina.*

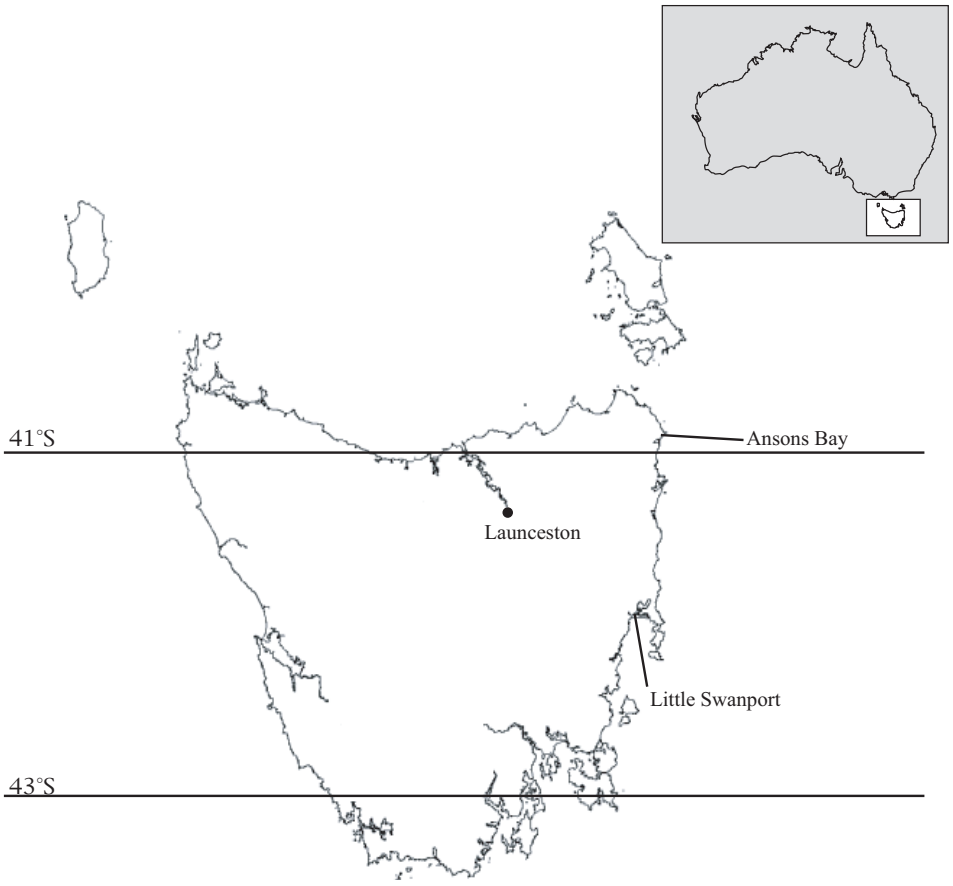

Figure 2. Collection and sample processing sites within Tasmania, Australia for the clam *Katelysia scalarina*.

G1. Gonad occupies a relatively small proportion of total cross sectional area. Male follicles primarily contain spermatogonia and some primary spermatocytes, females contain oogonia and some primary oocytes.

G2. Gonad still occupies a relatively small proportion of total cross sectional area. Male follicles contain all stages of spermatogenesis including a very small number of spermatozoa. Female follicles contain primary oocytes and secondary oocytes attached to follicle by stalk like connection.

G3. Gonad has increased substantially in size from G2 stage. Male follicles contain all stages of spermatogenesis however follicles are not densely packed and are predominated occupied by spermatids. In females this stage is characterised predominantly by well developed secondary oocytes attached to follicles and some free ova.

G4. Gonad occupies large proportion of total cross sectional area. In males follicles are densely packed primarily with spermatozoa which are surrounded by small bands of spermatids and secondary spermatocytes. In females, follicles are less well defined and gonad consists mainly of round to oval ova which are free in the lumen and a few secondary oocytes.

G5. In both genders, many follicles have discharged. Some follicle walls show evidence of rupture and in most instances small numbers of phagocytes will be present. Note in this stage immature germ cells will not be released hence some sections will be characterised by large follicle size containing a small number of immature germ cells.

GX. This stage characterised primarily by large numbers of phagocytes. Discharged follicles have collapsed. Female follicles which have not discharged are characterised by gametes with a granular/porous texture indicative of deterioration. In males follicles will only contain residual spermatozoa.

I. Indeterminate sexual phase. Follicles generally contracted. Any germ cells present will not be sexually differentiated. Characteristically, few phagocytes are present.

Staging data were also condensed to 3 stages; Developing (G1 + G2 + G3), Advanced (G4), and Regressive (G5 + GX + I).

Figure 3. Staging description for *Katelysia scalarina.*

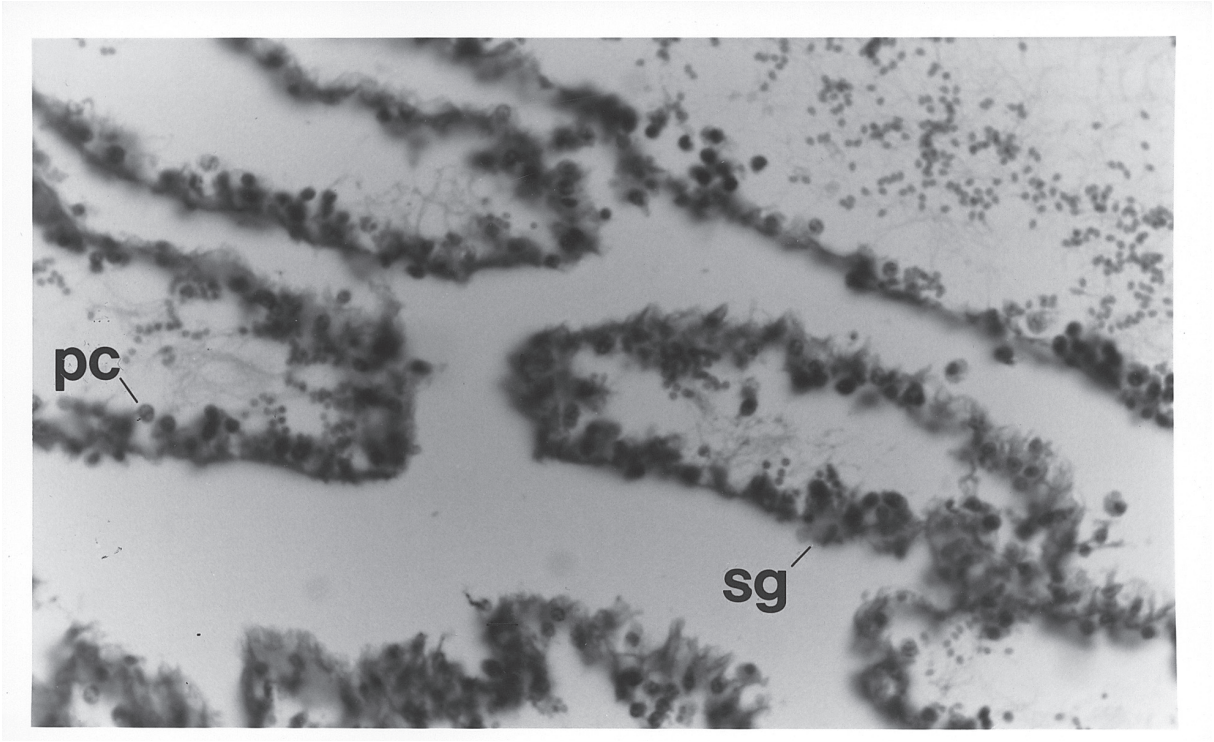

Figure 4. Immature male (M1) Gonad. Follicles primarily contain spermatogonia (sg) and some primary spermatocytes (pc). (x400).

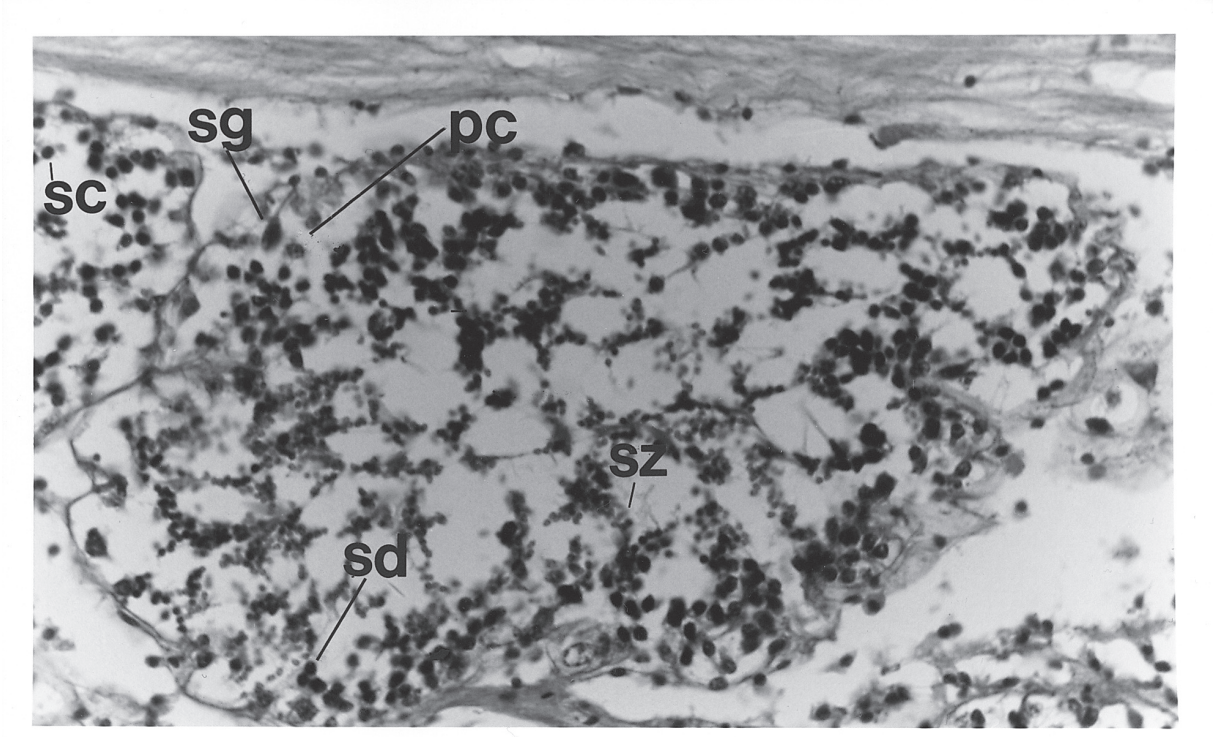

Figure 5. Developing male (M2) gonad stage. Follicles contain all stages of spermatogenesis including spermatogonia (sg), primary spermatocytes (pc), secondary spermatocytes (sc), spermatids (sd), and a very small number of spermatozoa (sz). (x400). (x400).

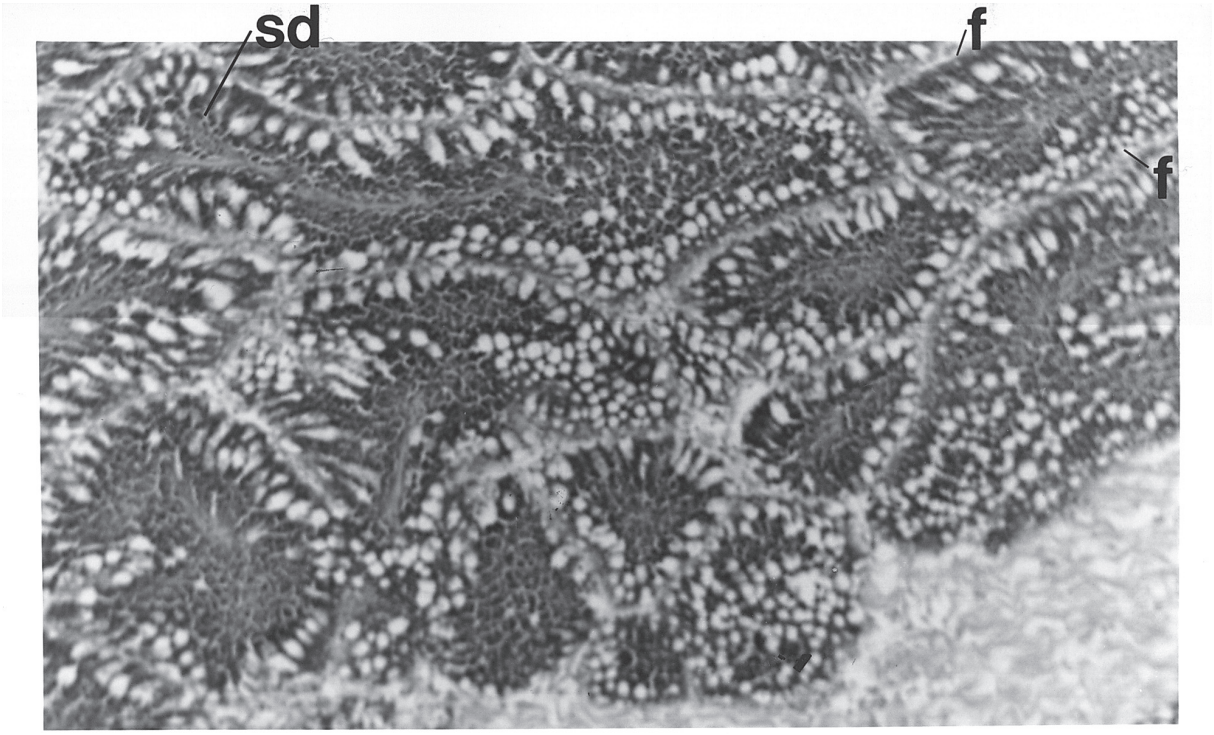

Figure 6. Developing male (M3) gonad stage. Follicles (f) are not densely packed and are predominated occupied by spermatids (sd). (x100).

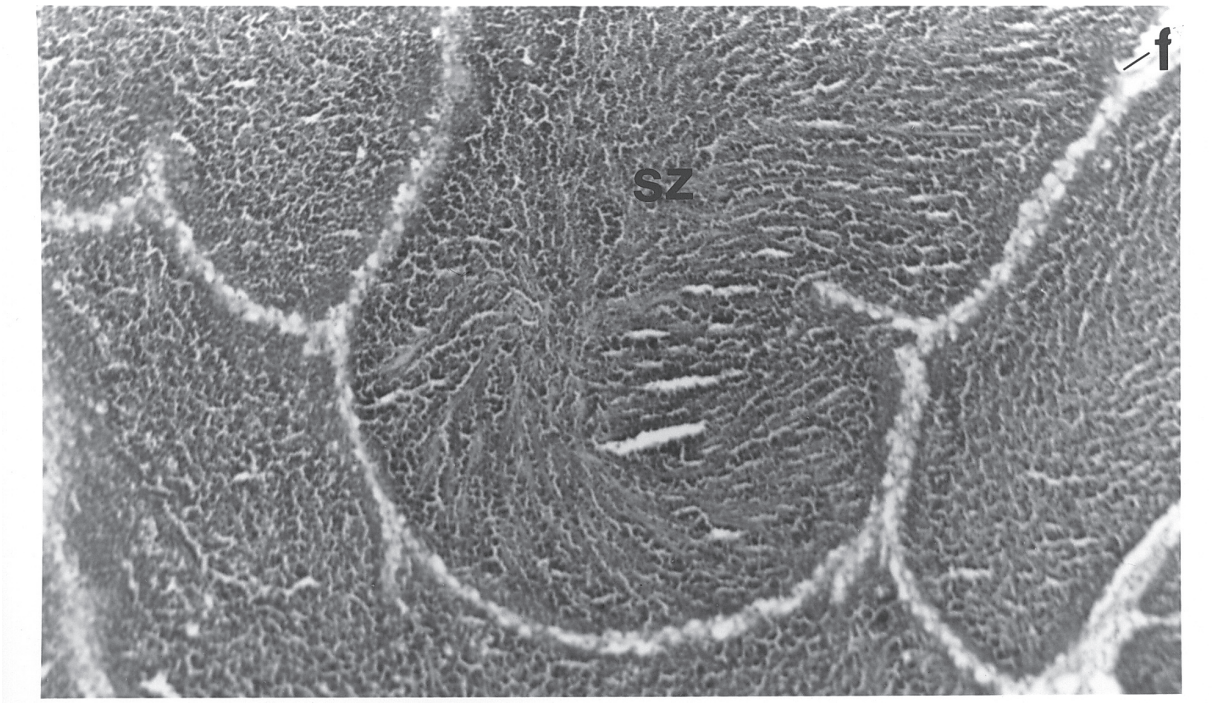

Figure 7. Mature male (M4) gonad stage. Follicles are densely packed primarily with spermatozoa (SZ). (x400).

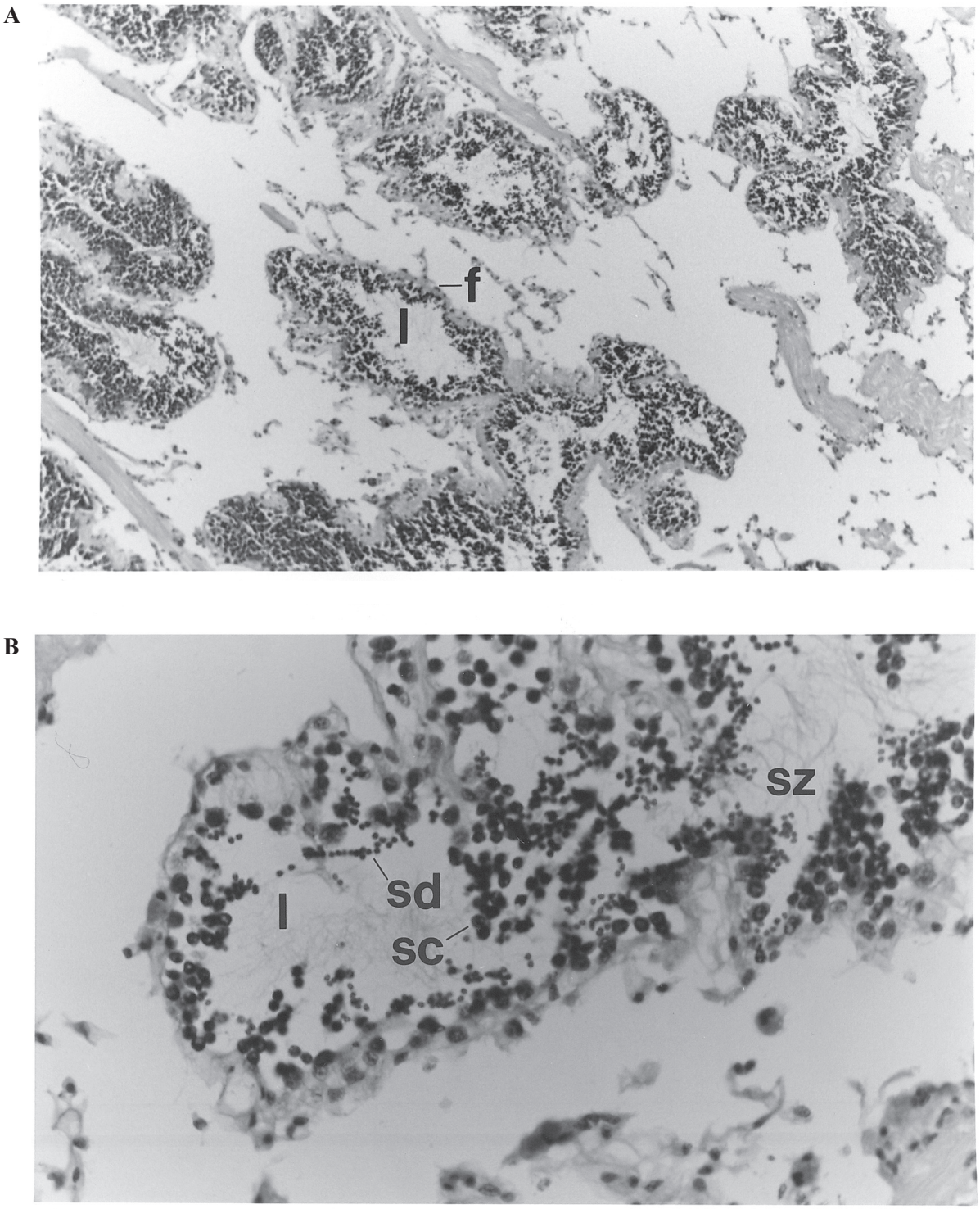

Figure 8. Postspawned male (M5) gonad stage. Follicles (f) have discharged with the lumen (l) largely devoid of cells. **a** (x100). **b** (x400). Secondary spermatocytes (sc), spermatids (sd), and spermatozoa (sz) present but lumen (l) is quite apparent.

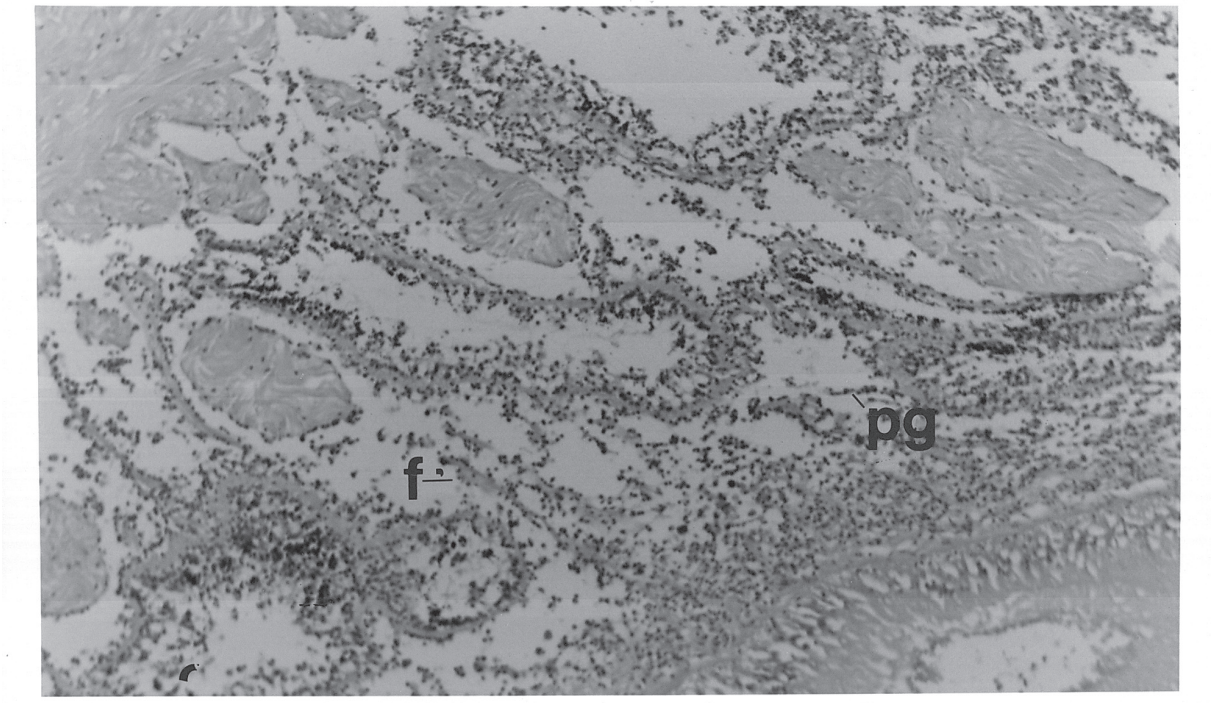

Figure 9. Fully regressed male (MX) gonad stage. There are numerous phagocytes (pg). Discharged follicles (f) have collapsed. (x100).

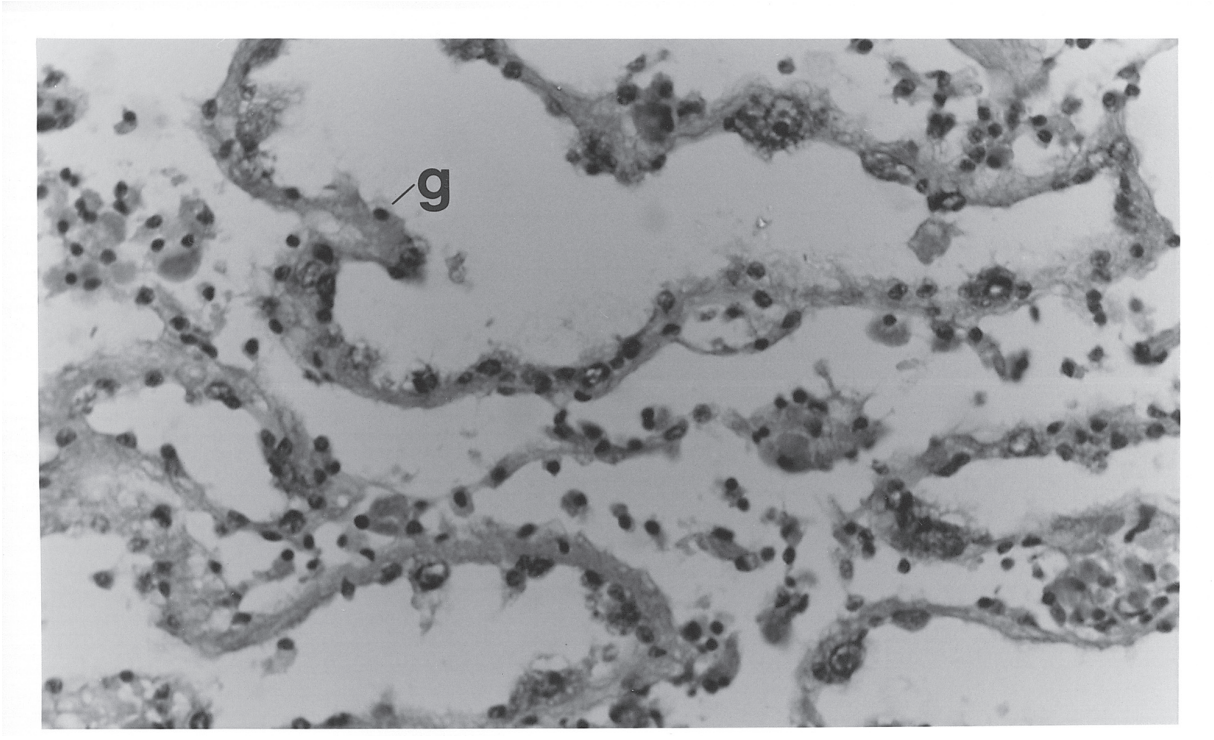

Figure 10. Indeterminate (I) sexual phase. Follicles generally contracted. Germ cells present are not sexually differentiated (gonia = g). (x400).

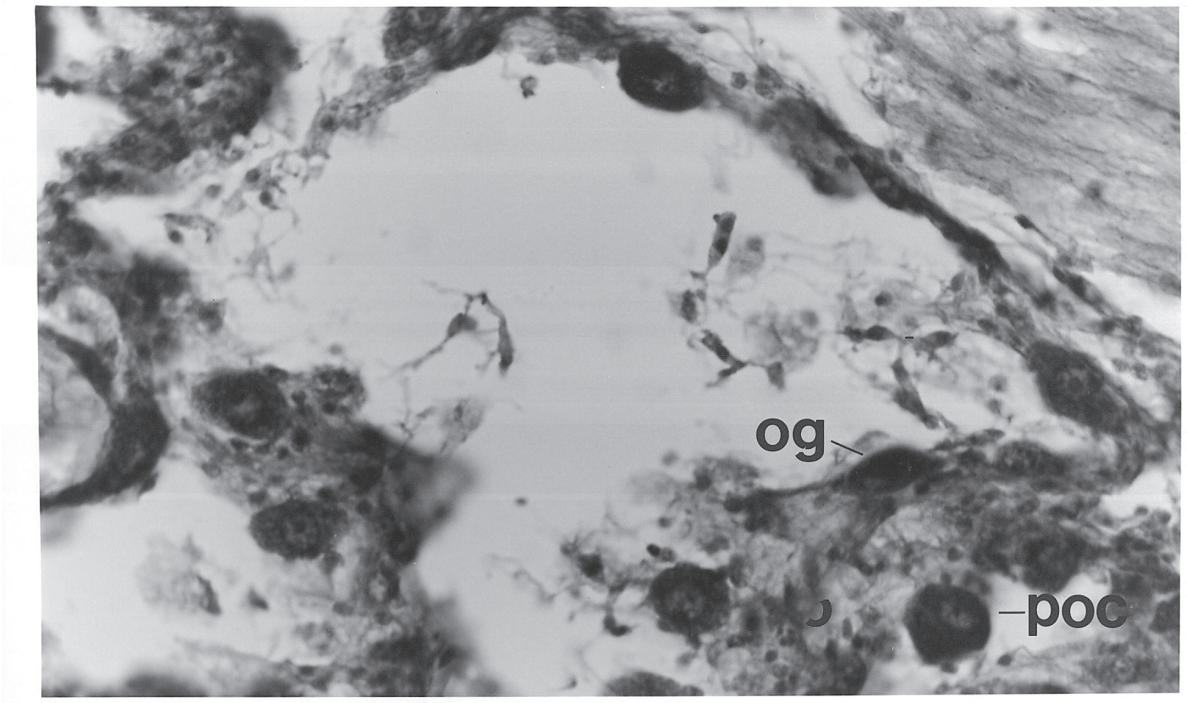

Figure 11. Immature female (F1) gonad containing oogonia (og) and some primary oocytes (poc). (x400).

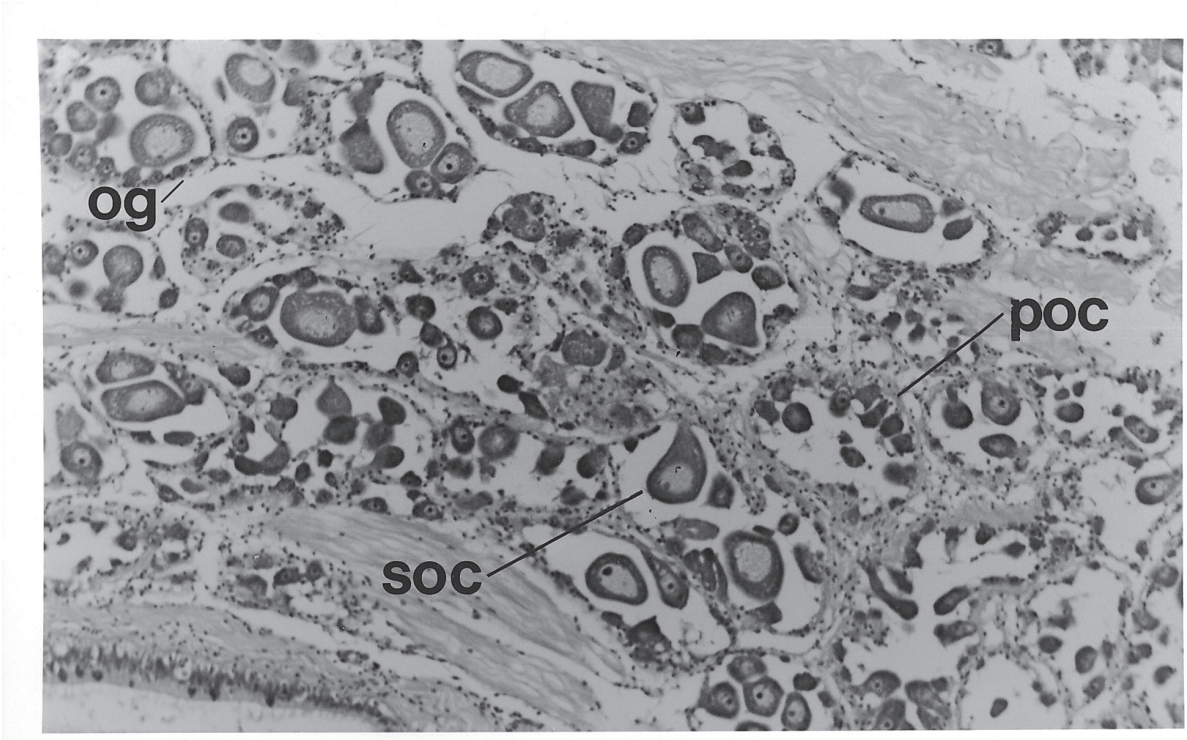

Figure 12. Developing female (F2) gonad. In addition to oogonia (og), follicles contain primary oocytes (poc) and secondary oocytes (soc) attached to follicle by stalk like connection. Follicles contain few cells. (x100).

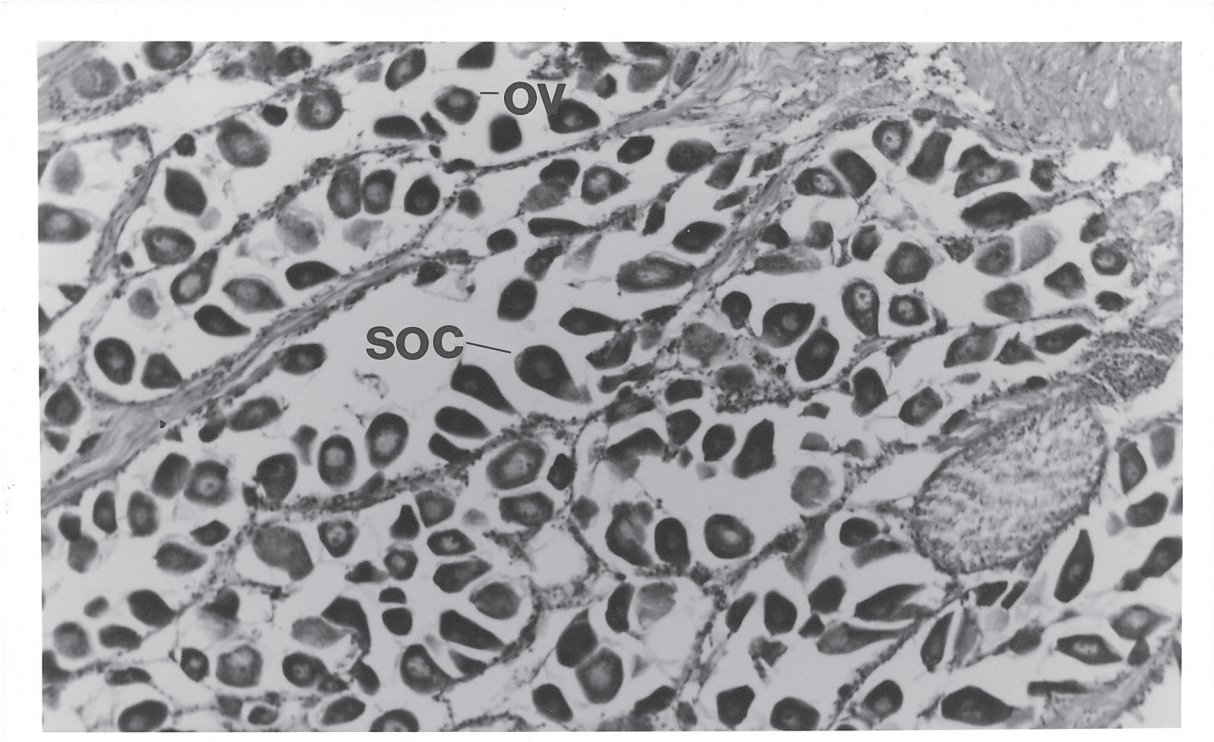

Figure 13. Developing female (F3) gonad. Characterised predominantly by well developed secondary oocytes (soc) attached to follicle and some free ova (ov) in the lumen of the follicle. (x100).

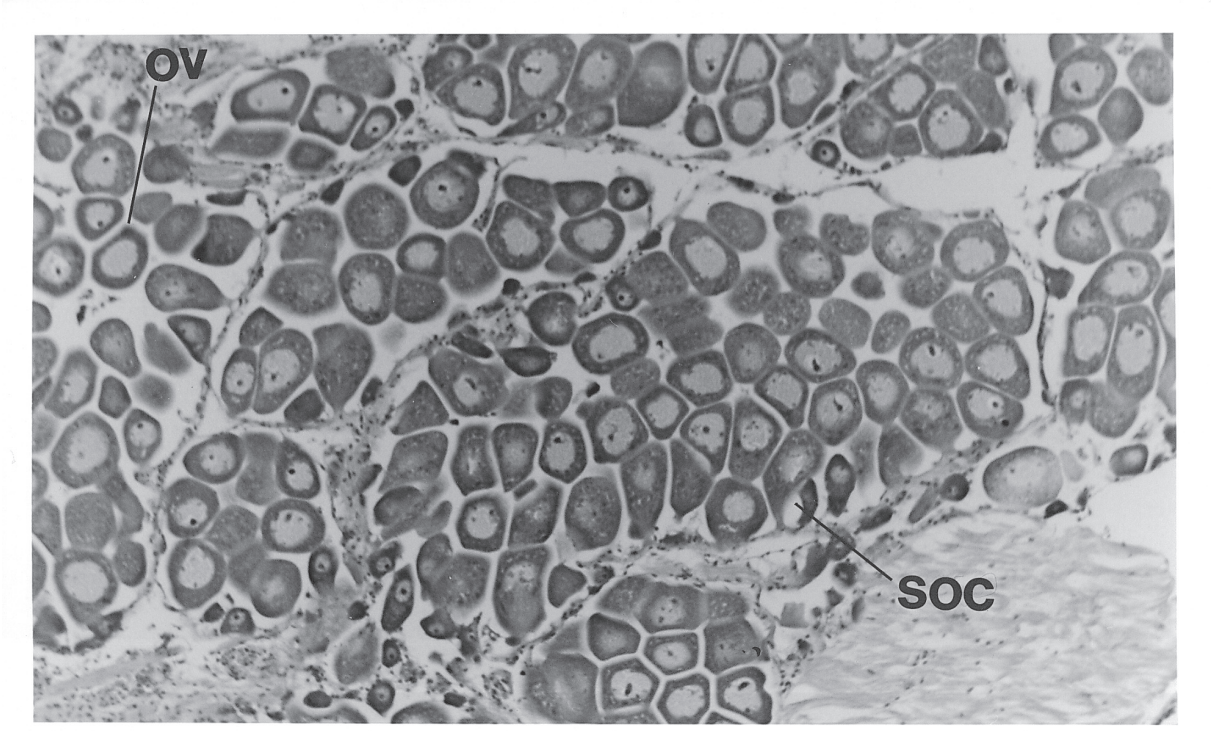

Figure 14. Mature female (F4) gonad with densely packed follicles containing mostly round to oval ova (ov) which are free in the lumen and a few secondary oocytes (soc). (x100).

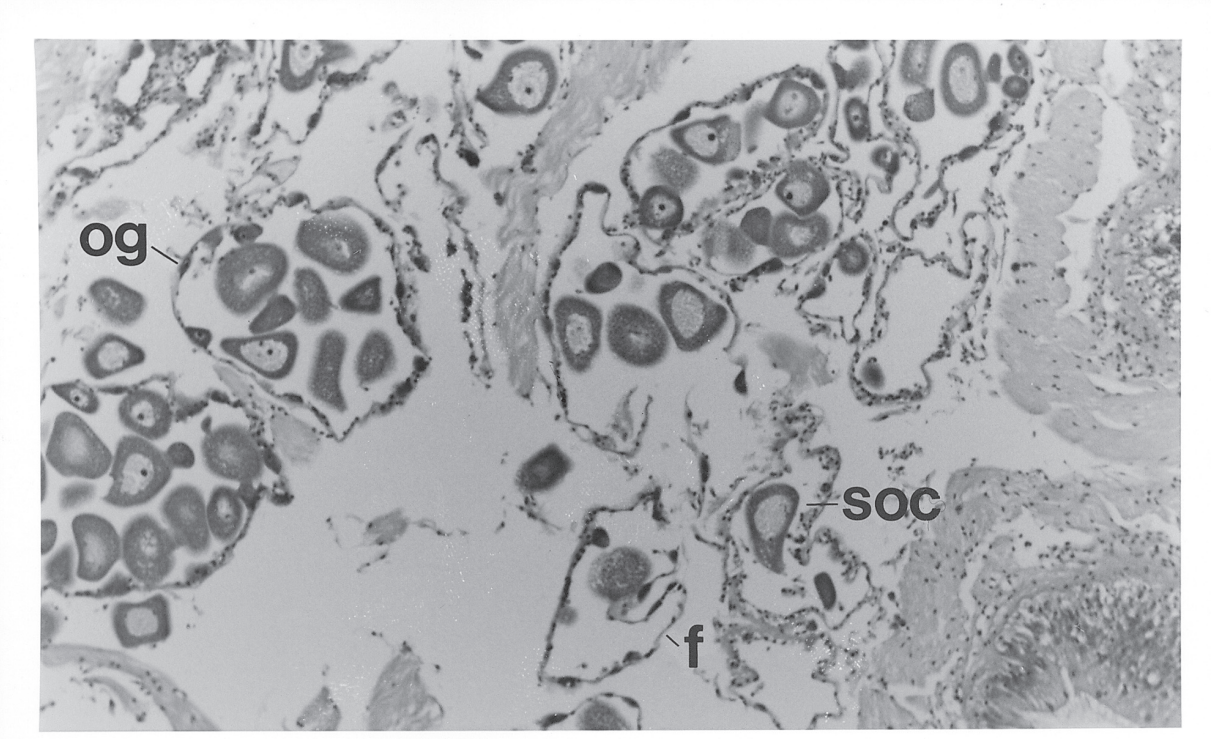

Figure 15. Postspawned female (F5) gonad containing immature germ cells (oogonia = og) within large follicles (f) which may have been disrupted. Few phagocytes present but secondary oocytes (soc) still evident. (x100).

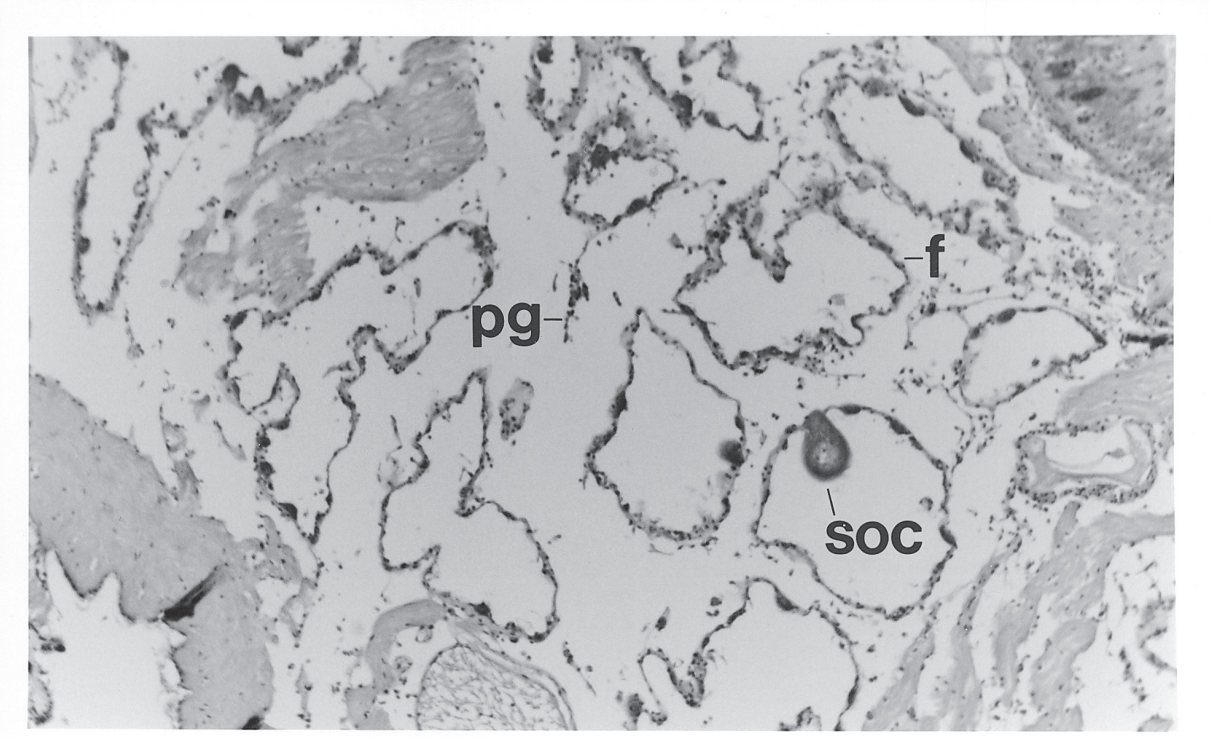

Figure 16. Fully regressed female (FX) gonad containing large numbers of phagocytes (pg). Discharged follicles (f) have collapsed. The follicle which has not completely discharged contains gametes (soc) with a granular/porous texture indicative of deterioration.

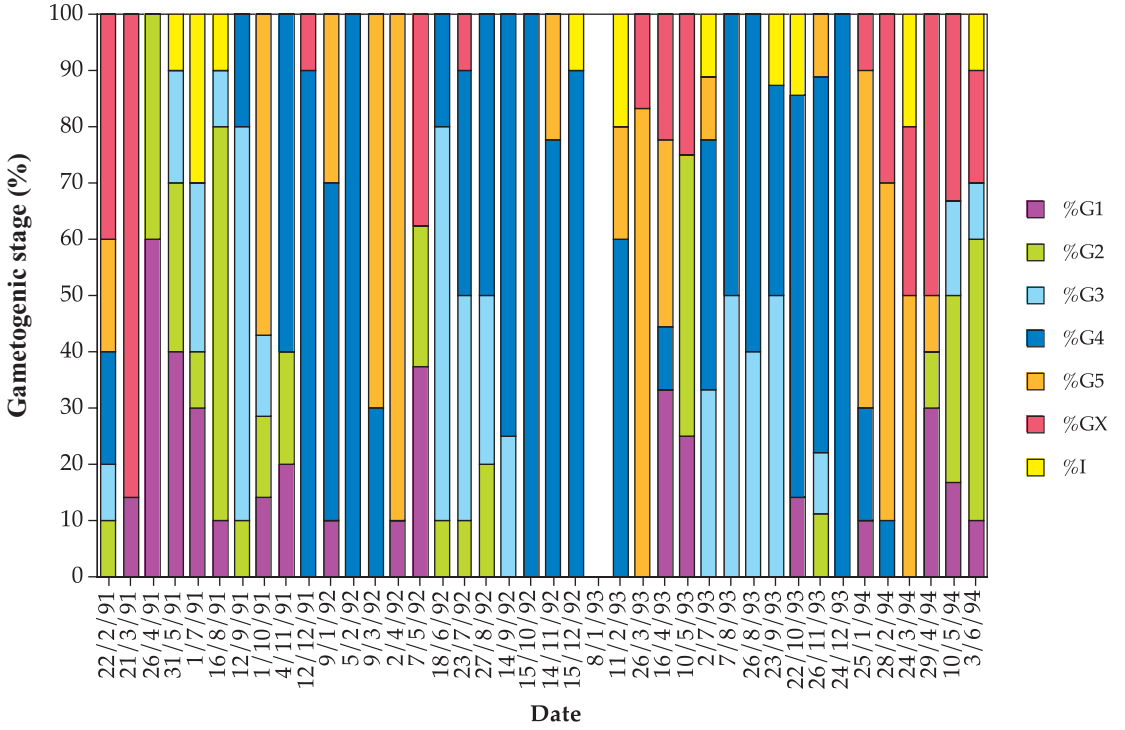

Figure 17. Incidence of gametogenic stages for *Katelysia scalarina* at Little Swanport 1991-1994 (sexes pooled).

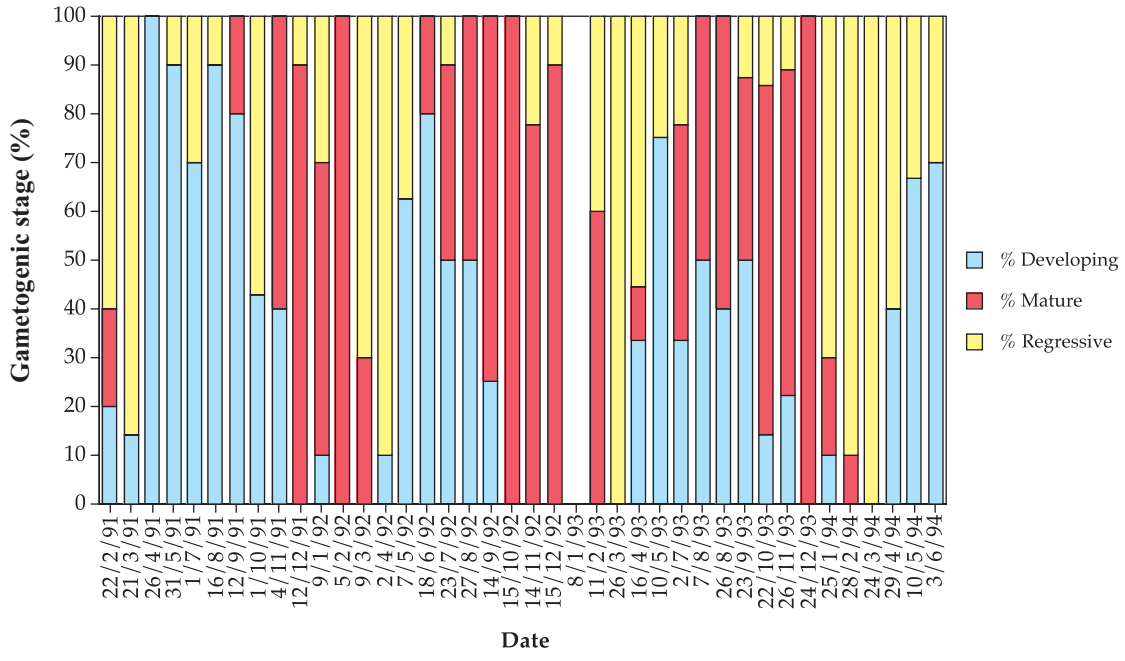

Figure 18. Incidence of condensed gametogenic stages for *Katelysia scalarina* at Little Swanport 1991-1994 (sexes pooled).

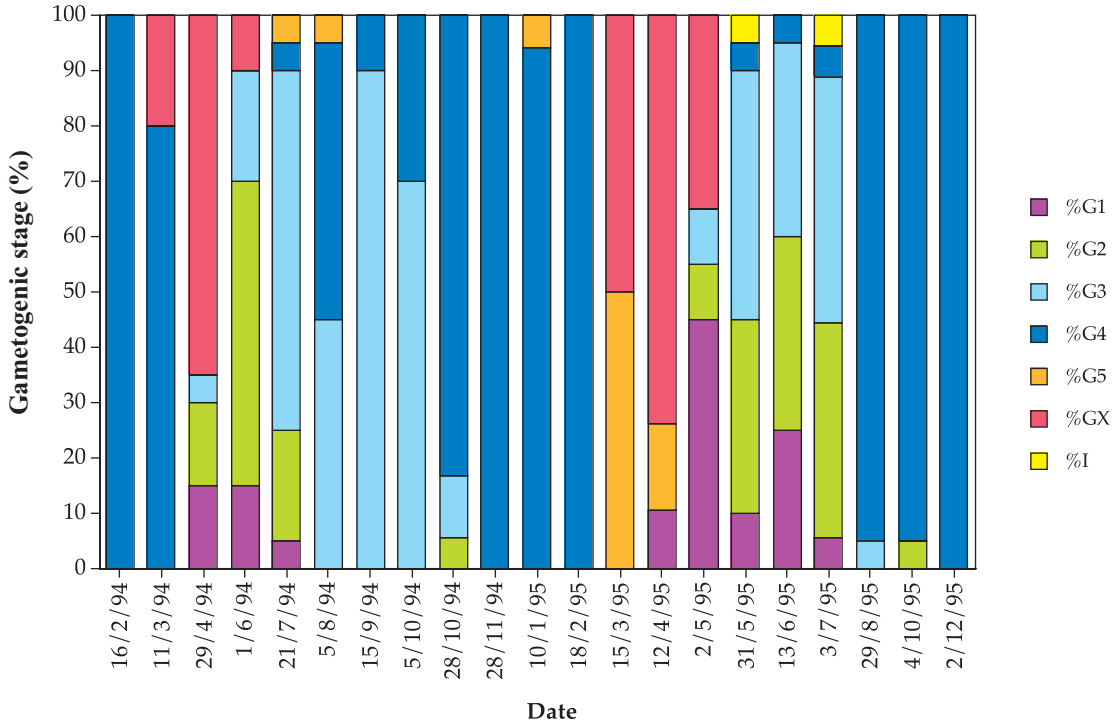

Figure 19. Incidence of gametogenic stages for *Katelysia scalarina* at Ansons Bay 1994-1995 (sexes pooled).

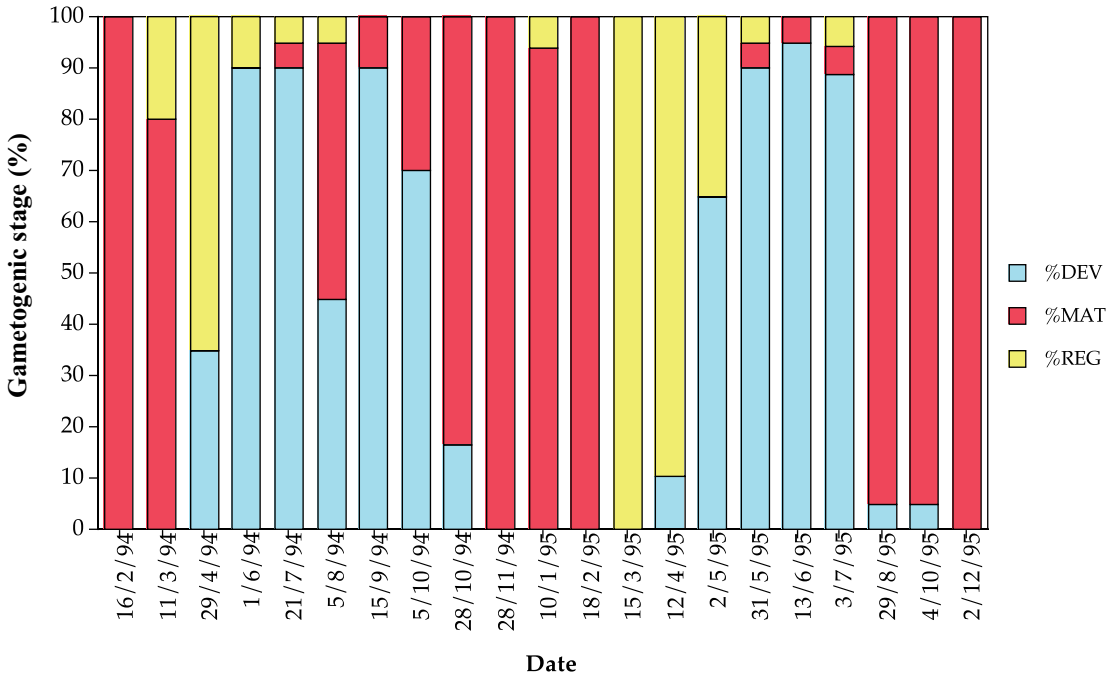

Figure 20. Incidence of condensed gametogenic stages for *Katelysia scalarina* at Ansons Bay 1994-1995 (sexes pooled).

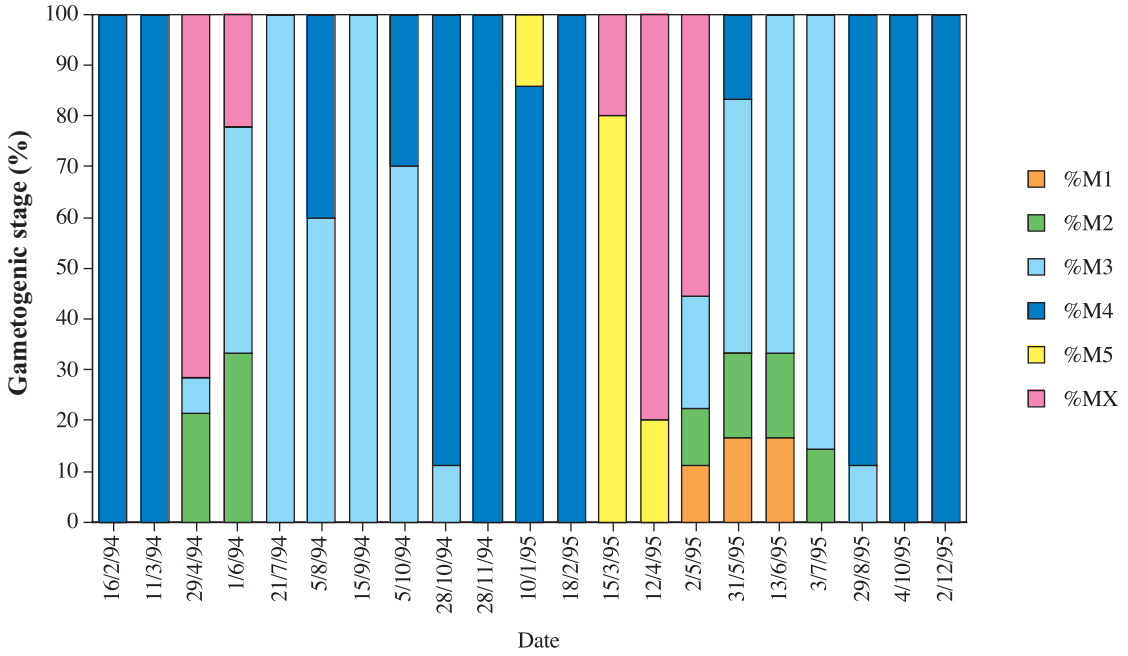

Figure 21. Incidence of male gametogenic stages for *Katelysia scalarina* at Ansons Bay 1994-1995.

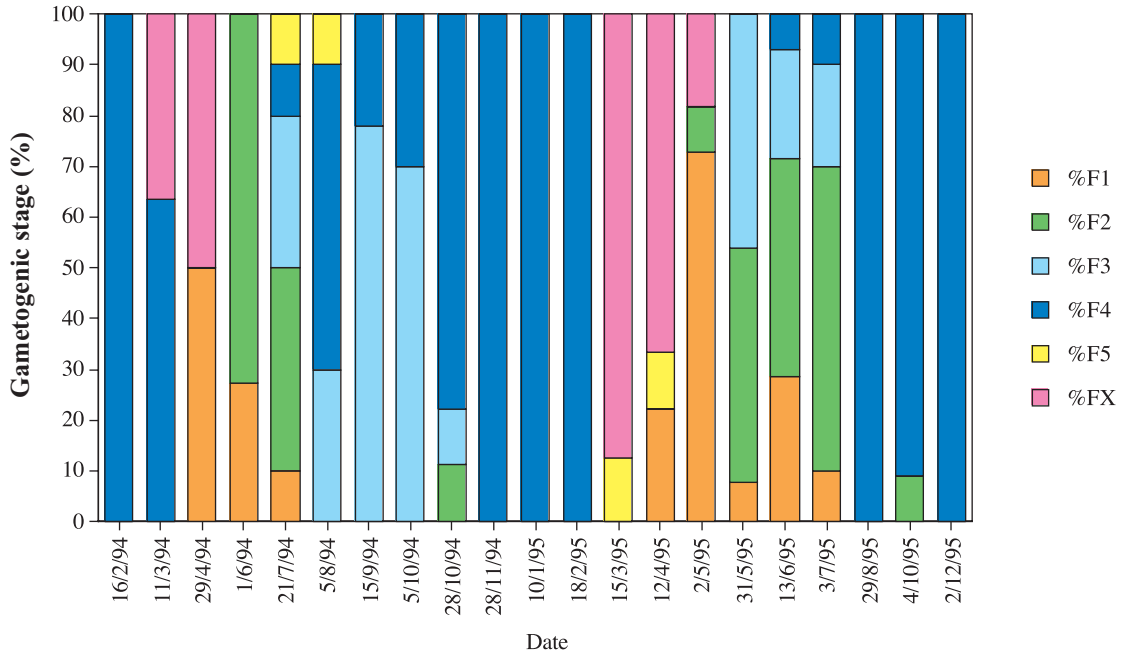

Figure 22. Incidence of female gametogenic stages for *Katelysia scalarina* at Ansons Bay 1994-1995.

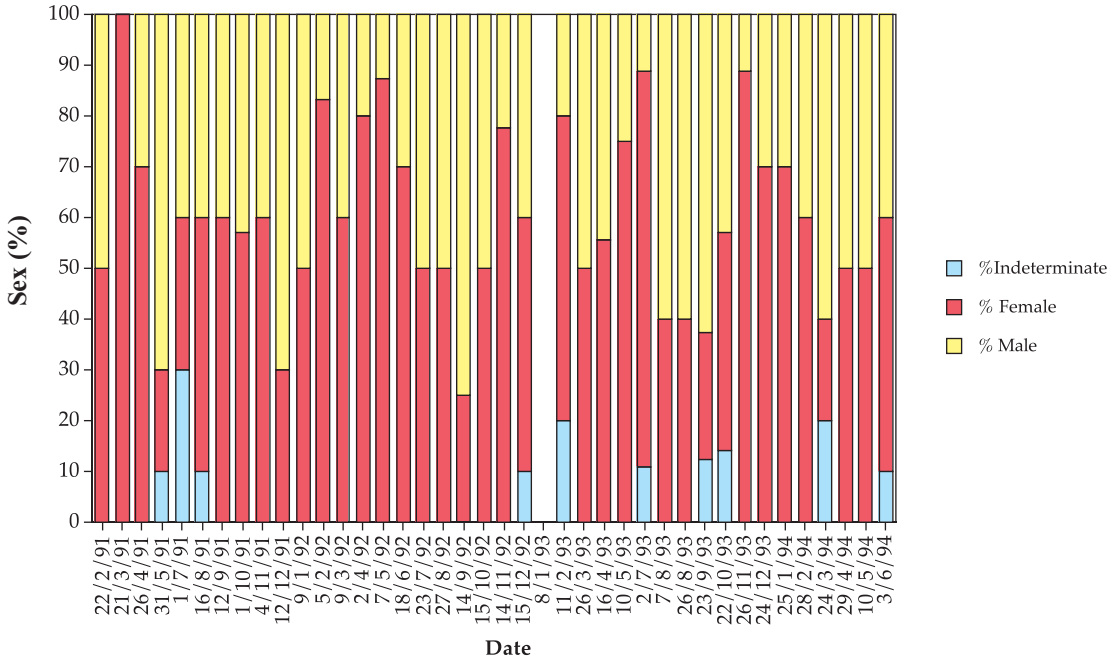

Figure 23. Sexual differentiation for *Katelysia scalarina* at Little Swanport 1991-1994.

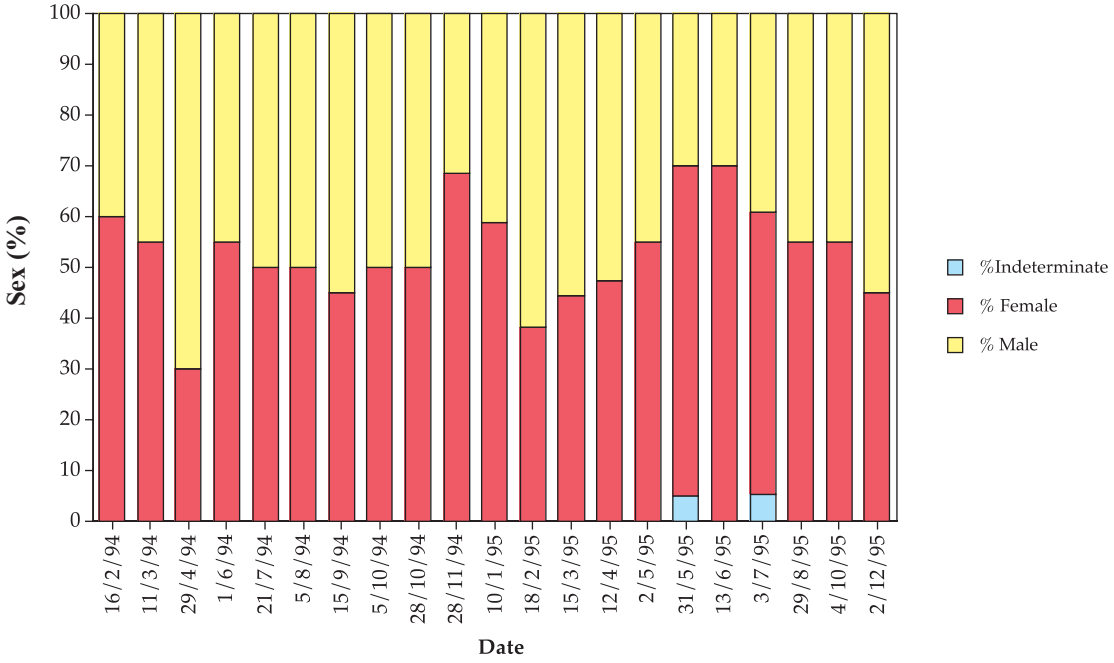

Figure 24. Sexual differentiation for *Katelysia scalarina* at Ansons Bay 1994-1995.

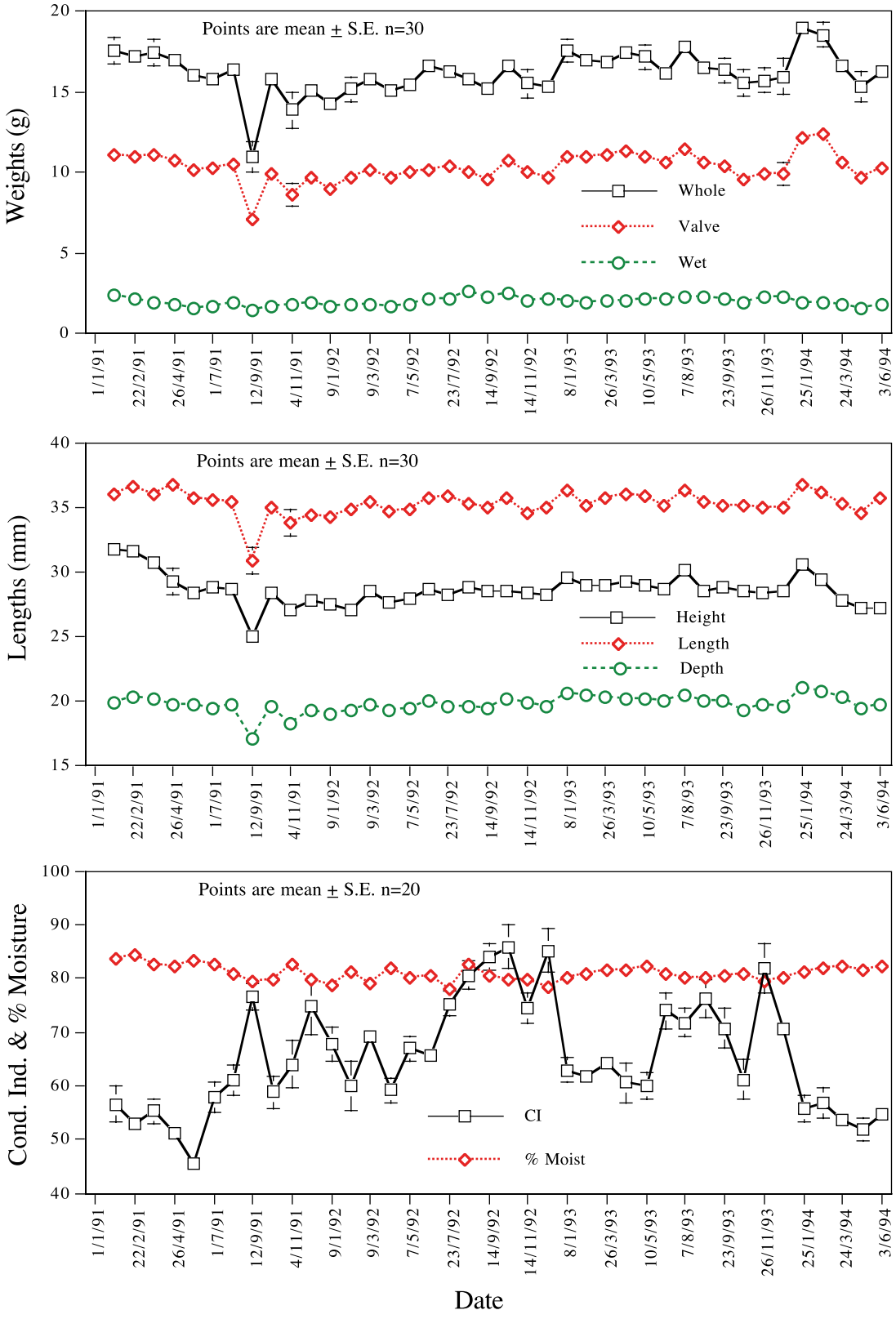

Figure 25. Morphometric characteristics and Condition Index for *Katelysia scalarina* at Little Swanport 1991-1994 (sexes pooled).

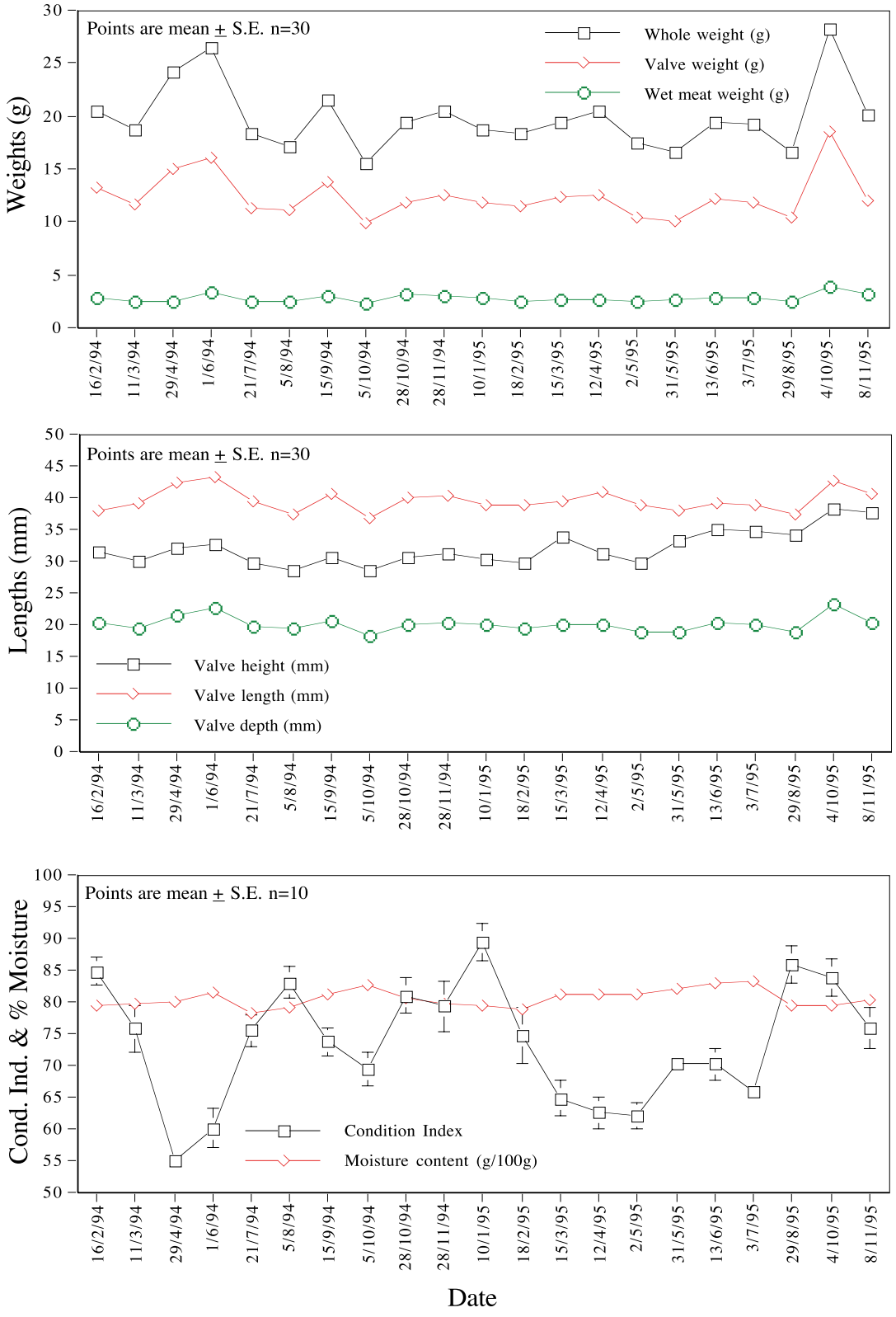

Figure 26. Morphometric characteristics and Condition Index for *Katelysia scalarina* at Ansons Bay 1994-1996 (sexes pooled).

5.2 MANUSCRIPT 2

Gametogenesis and condition index of the New Zealand venerid *Ruditapes largillierti* (Philippi 1849) from St Helens, Tasmania, Australia.

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5.2.1 ABSTRACT

This study documents changes over two years in gonad maturation and condition index of *Ruditapes (=Venerupis) largillierti* from St Helens, the site of the only commercial fishery in Tasmania for this species which is considered to have been an inadvertent introduction from New Zealand. Most of these clams could be ascribed to specific gametogenic stages and no hermaphrodites were observed. However, *R. largillierti* gonads are relatively difficult to stage because of the tendency towards co-occurrence of several gamete development stages within the one gonad section. Mature individuals were found in most months but only in September (spring) samples were most individuals mature. However, the incidence of mature individuals differed greatly between the summers of 1993/94 (rare) and 1994/95 (still common in December). All of the samples comprised relatively large individuals (mean shell length for each monthly sample ranged 45.6 - 56.7 mm) and in most months the sex ratio was skewed towards females.

Condition index of *R. largillierti* was more stable than for farmed diploid Pacific oysters *Crassostea gigas* from other Tasmanian waterways or *Katelysia scalarina*, a commercially exploited venerid clam that is sympatric with *R. largillierti* at St Helens, and this is a favourable outcome for marketing of this species.

Keywords: Clam; venerid; *Ruditapes largillierti*; *Venerupis*; broodstock; spawning; condition; morphometrics

5.2.2 INTRODUCTION

An understanding of the reproductive biology of venerid clams is important for aquaculture and fisheries management purposes (Eversole, 1989; Shafee and Daoudi, 1991). Species-dependent reproductive strategies that include separate sexes through to functional hermaphroditism occur within this group and, within a single species, reproductive seasonality can vary for different locations (Eversole, 1989). The genus *Ruditapes* is one of the most commercially significant venerid taxa largely because of the importance of the carpet shell clam *Ruditapes decussatus* in Atlantic and Mediterranean fisheries (Shafee and Daoudi, 1991) and the Manila clam *R. phillipinarum* which is farmed widely (Toba et al., 1992). *R. largillierti* is endemic to New Zealand but its range has extended to Tasmania, probably during the last 100 years, where it remains indistinguishable from New Zealand populations, on the basis of allozyme analyses (Manuscript 16). *R. largillierti* (Fig. 1) grows to a length of 70 mm and a height of 50 mm and is found subtidally in both muddy and sandy substrates in shallow estuarine waters (Gabriel and Macpherson 1962). The only commercial fishery for this species in Tasmania is at St Helens and it is also being evaluated for aquaculture (Paturusi, 1994; Kent et al., 1999). This species occurs sympatrically in Tasmania with native *Katelysia scalarina*, the latter occurring more commonly in the intertidal or shallow subtidal zone. Paturusi (1994) sampled *R. largillierti*, every 2 months, from experimental cages containing a wide size range over 9 months (April - December 1993). He found most mature individuals in the August samples and overall there was little difference between sexes in relative incidence of maturational stages during the study.

This present study assessed large individuals (mean size >50 mm shell length) directly from the fishery on a monthly basis for two years.

Venerid clams can commit "as much as 50% of non-respired assimilated energy annually to reproduction" and this can affect the relative proportions of soft tissue and shell (Eversole, 1989). This latter variable is typically measured as a condition index which may also be a useful guide to the marketability of individuals within a bivalve population (Nell et al., 1994). This present study documents changes in gonad maturation and condition index for *R. largillierti* at St Helens and complements an equivalent study on *K. scalarina* (manuscript 1), the other major venerid clam which is fished commercially in Tasmania and is also being evaluated for aquaculture (Kent et al., 1998).

5.2.3 MATERIALS AND METHODS

Each month 30 clams were collected from Georges Bay and transported in plastic containers without water or sediment to the University of Tasmanian at Launceston (Fig. 2) where they were held at least overnight in an ambient recirculating seawater system (35-37‰). Morphometric analyses, condition index and soft tissue moisture content analyses, and histological assessment of gametogenesis were performed on 30, 10 and 20 clams, in that order, each month for two years (October 1993 to November 1995).

Standard 4 µm paraffin sections were prepared from a 3 mm slice of the preserved samples using the anterior edge of the foot as a reference (Howard and Smith, 1983). These were stained using Mayer's haematoxylin and eosin Y. Unfortunately, the intensity of staining did not produce sufficient tissue differentiation to allow image analysis of gonad area. Gonads were staged for gametogenic development using a heavily modified staging system (Table 1) based on Dinamani (1974). Whole weight was measured (to 0.1 g) after clams were immersed overnight in a recirculating system approximating oceanic conditions, cleaned if necessary, and dried superficially with paper towel. Condition index [CI=dry meat weight (g) x 1000/cavity volume (g)] was assessed by drying individual meats (soft tissue) at 80°C to constant weight in a forced draft oven (24-48 h); cavity volume was estimated as the difference between whole weight (g) and shell weight (g) (Crosby and Gale, 1990). Dimensions of clams were measured with Vernier callipers to 0.1 mm.

5.2.4 RESULTS

R. largillierti were relatively difficult to stage because of the occurrence of germ cells in several different stages of maturation within the one histological section and the staging system developed (Table 1) was based on the most common maturational stage. On this basis, most gonad samples could be ascribed to specific gametogenic stages (Figs 3-17). Mature individuals were found in most months but only in September (spring) samples (1994 and 1995) were most individuals mature and few mature individuals were found in mid or late summer samples (January - February) (Figs. 18-19). However, the incidence of mature individuals differed greatly between the summers of 1993/94 (rare) and 1994/95 (still common in December). No hermaphrodites were observed. All of the samples comprised relatively large individuals (mean shell length for each monthly sample ranged 45.6 - 56.7 mm) and in 18 of the 22 monthly samples the sex ratio was skewed towards females (Fig. 20). Because of the low numbers of males in many samples, data for seasonal maturational patterns for each sex are not presented separately.

The moisture content of the soft tissue and Condition Index values were relatively stable (Fig. 21) with the latter index ranging from 86.5 - 110.6 except for one sample (125.1).

5.2.5 DISCUSSION

The coexistence of different developmental stages does occur in gonads in many bivalves (Manuscript 1) but this tendency is very pronounced in *R. largillierti*, making it much more difficult to stage definitively. Hence a simplified staging system based on the dominant developmental stage was developed. This tendency towards more extreme divergence in developmental stages within the one gonad of a clam, which does not exhibit synchronous hermaphroditism, has also been reported for *Donax trunculus* (Tirado and Salas, 1998). When *R. largillierti* from the fishery are spawned under hatchery conditions, the initial survival of the larvae is much lower than for *K. scalarina* and this may reflect the more synchronous development of gametes within the gonad of individual *K. scalarina* (Fig. 22).

The sex ratio of the relatively large individuals sampled in this study was strongly skewed towards females (usually >60%) (Fig. 20). This could be due to inherently unequal sex ratios but this is atypical in clams (Eversole, 1989). Alternatively, sexes could be independent with size at maturatity differing with sex or this species may exhibit protandry. Paturusi (1994) found that the incidence of females at St Helens was much higher (usually >60%) in a larger size class of *R. largillierti* (43.5 - 47.7 mm shell length) compared to <45% for the smaller size class (27.4 - 39.0 mm). Protandric development seems the more likely explanation, as occurs in Pacific oysters *Crassostrea gigas*, with some of the males changing sex as they grow. Within an initial sample more than 50% of the smaller size class (mean length 27.4 mm) in the study by Paturusi (1994) could not be sexed, indicating that size at maturity is well below the average size of clams in the present study.

Seasonal maturation patterns indicated considerable interannual variation (mature individuals common from August to December 1994 but only in September 1995) or perhaps localised spatial variability in maturational stage. The September 1995 peak in abundance of mature individuals is both preceded and followed by an abundance of spawned individuals; this is indicative of a small scale uneveness in the spatial distribution of mature individuals. Such spatial variation was commonly encountered by the authors when collecting *K. scalarina* broodstock directly from a fishery. The occurrence of mature individuals in cooler months is consistent with the finding by Paturusi (1994) that for *R. largillierti* sampled every two months in the period April to December 1993, mature individuals were most common in August. This general inconsistency in maturational patterns in this wild population and the tendency towards partial spawning under hatchery conditions indicates that further research on conditioning protocols for hatchery stock, with indoor, controlled temperature systems with sediment, may be important (Kent et al., 1999). Initial conditioning trials with indoor, controlled temperature systems without sediment and outdoor, ambient systems with sediment were unsuccessful (C. O'Meley and G. Maguire, unpublished data).

The relatively large size and higher and more stable condition index of *R. largillierti* compared to *K. scalarina* (manuscript 1) may afford a marketing advantage for the former species. Condition index and moisture content of meats were also more stable than for farmed diploid Pacific oysters, *Crassostea gigas* sampled from another east coast Tasmanian estuary Little Swanport, (Maguire et al., 1994).

5.2.6 ACKNOWLEDGMENTS

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5.2.7 REFERENCES

Crosby, M.P. and Gale, L.D., 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. J. Shellfish Res., 9: 233-237.

Dinamani, P., 1974. Reproductive cycle and gonadial changes in the New Zealand rock oyster *Crassostrea glomerata*. N.Z. J. Mar. Freshwater Res., 8 (1): 39-65.

Eversole, A. G., 1989. Gametogenesis and spawning in North American clam populations: Implications for culture. In: J. J. Manzi and M. Castagna (Editors), Clam Mariculture in North America. Developments in Aquaculture and Fisheries Science, vol 19. Elsevier, Amsterdam, pp. 75-109.

Gabriel, C.J. and Macpherson, J.H., 1962. Marine Molluscs of Victoria, Melbourne University Press, 475 pp.

Howard, D. W. and Smith, C. S., 1983. Histological techniques for marine bivalve mollusks. NOAA Technical Memorandum NMFS-F/NEC-25.

Kent, G. N., Maguire, G. B., John, M., Cropp, M. and Frankish, K. 1998. Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina* (Lamark 1818). J. of Shellfish Res., 17(4): 1065-1070.

Kent, G. N., Maguire, G. B., and Duthie, I., 1999. Spawning, larval rearing, settlement and growth of the New Zealand Venerid *Ruditapes largillierti* (Philippi 1849). N.Z. J. of Mar. and Freshwater Res., 33: 55-62.

Nell, J.A., Cox, E., Smith, I.R. and Maguire, G.B. Studies on triploid oysters in Australia. 1. The farming potential of triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). Aquaculture, 126: 243-255, 1994.

Paturusi, A., 1994. Effects of stocking density and initial size on the performance of the clam *Venerupis largillierti* (Phillipi, 1849) in experimental cages in Georges Bay, Tasmania. Unpublished thesis, University of Tasmania.

Shafee, M. S. and Daoudi, M., 1991. Gametogenesis and spawning in the carpet-shell clam, *Ruditapes decussatus* (L.) (Mollusca: Bivalvia), from the Atlantic coast of Morocco. Aquaculture and Fisheries Management, 22(2): 203-216.

Tirado, C. and Salas, C., 1998. Reproduction and fecundity of *Donax trunculus* L., 1`758 (Bivalvia:Donacidae) in the littoral of Málaga (southern Spain). J. of Shellfish Res., 17(1): 169-176.

Toba, D.R.; Thompson, D.S.; Chew, K.K.; Anderson, G.J.; Miller, M.B., 1992. Guide to Manila Clam Culture in Washington. Washington Sea Grant Program, University of Washington, 80 pp.

5.2.8 FIGURES

Table 1. Gonad staging description for *Ruditapes largillieti.*

D1. Developing 1. In the male all stages of spermatogenesis are present. However the vast majority of germ cells consist of spermatogonia, with decreasing numbers of primary and secondary spermatocytes. Females will be characterised by the presence of oogonia, and primary oocytes. A small number of secondary oocytes attached to follicle wall by an elongated peduncle will also be present.

D2. Developing 2. Gonad area as a % of total cross sectional area has increased substantially from D1. In a male all stages of spermatogenesis are present. Primary and secondary spermatocytes form a loose band at the periphery of the follicle with the central follicle area consisting primarily of spermatids and some spermatozoa. In females, gametes within the follicle primarily consist of advanced secondary oocytes attached to the follicle wall and some ova lying free in the lumen.

A. Advanced. Gonad occupies relatively large proportion of overall cross sectional area. Male follicles are densely packed substantially with spermatozoa, with some spermatids and secondary spermatocytes at the periphery. The females consist primarily of large numbers of mature ova free in the lumen however some secondary oocytes attached to the follicles may still be present.

PS. Post Spawned. Partial or complete spawning has occurred. Follicles tend to have a disorganised appearance. Some follicles will show evidence of rupture. Small numbers of phagocytes often present. Developing germ cells will still be present lining the follicles.

R. Regressive. This stage characterised primarily by large numbers of phagocytes. Discharged follicles have collapsed. Female follicles which have not discharged are characterised by gametes with a granular/porous texture indicative of deterioration. In males follicles will generally only contain residual spermatozoa.

I. Indeterminate. Indeterminate sexual phase. Follicles generally contracted. Any germ cells present will not be sexually differentiated. Phagocytes characteristically present.

Staging data was condensed to 3 stages; Developing (D1 + D2), Advanced (A), and Regressive (PS + R + I).

Figure 1. *Ruditapes largillieti.*

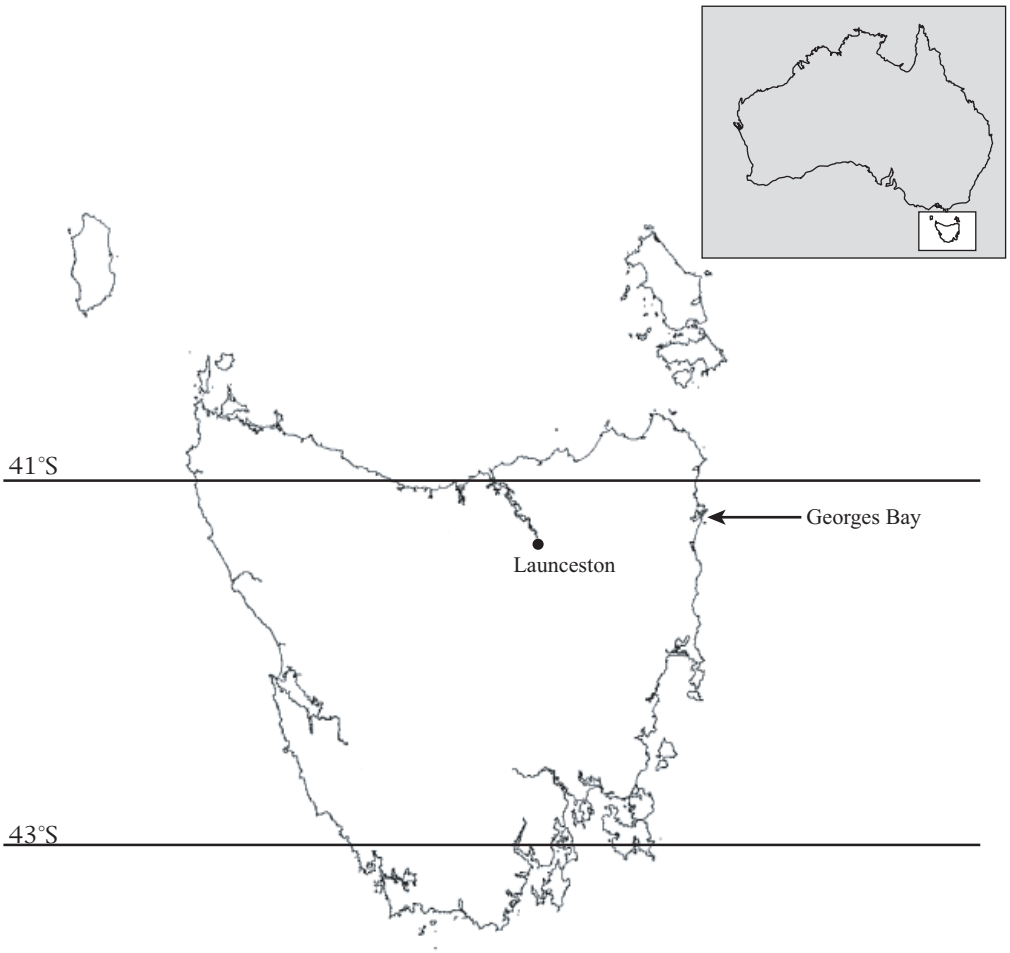

Figure 2. Collection and sample processing sites for *Ruditapes largillierti* in Tasmania.

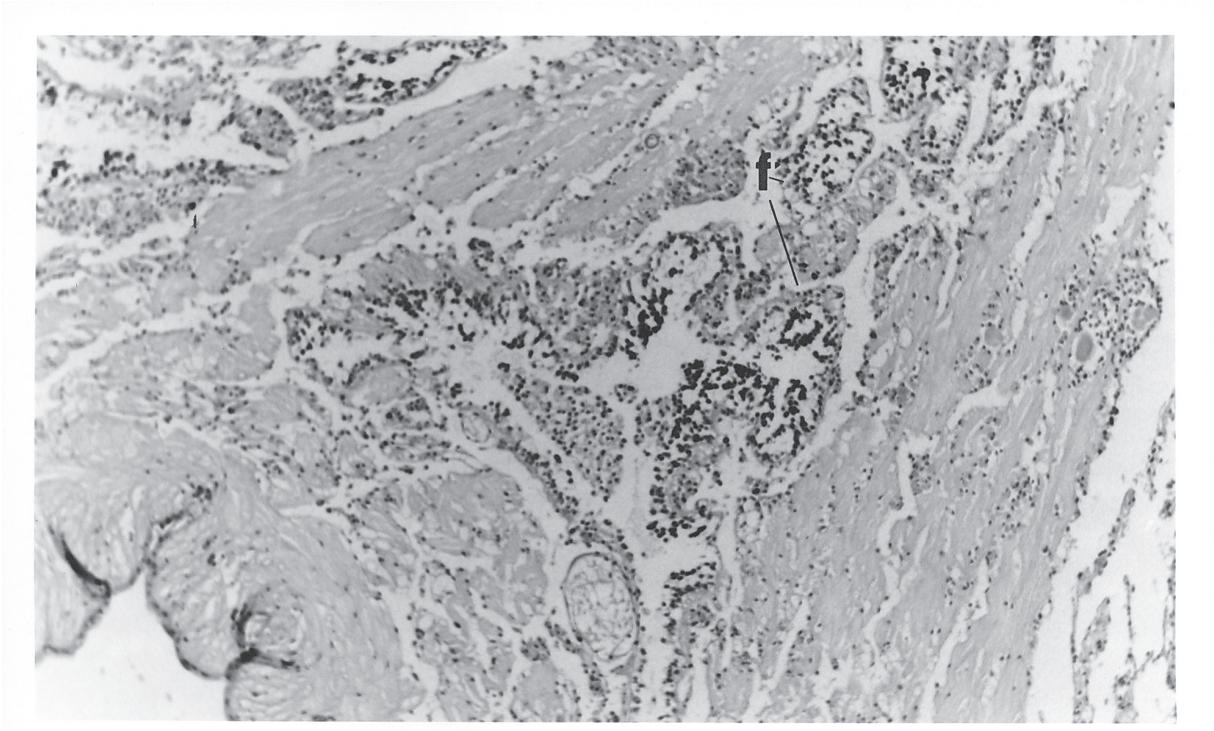

Figure 3. Developing male 1 stage (MD1) showing early development of follicle (f). (x100) (See Figure 4 for higher magnification.)

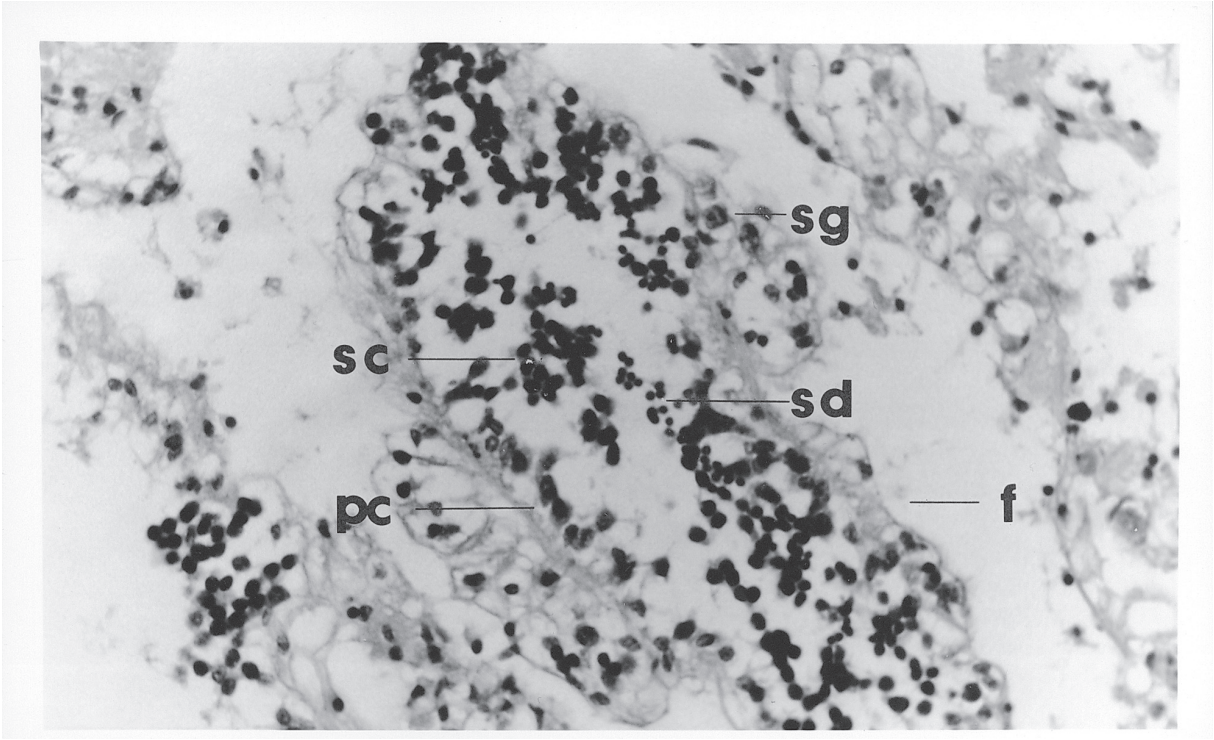

Figure 4. Developing male 1 (MD1). Most stages of spermatogenesis are present but most germ cells consist of spermatogonia (SG), with decreasing numbers of primary (pc) and secondary spermatocytes (sc). A few spermatids (sd) are apparent. (x400)

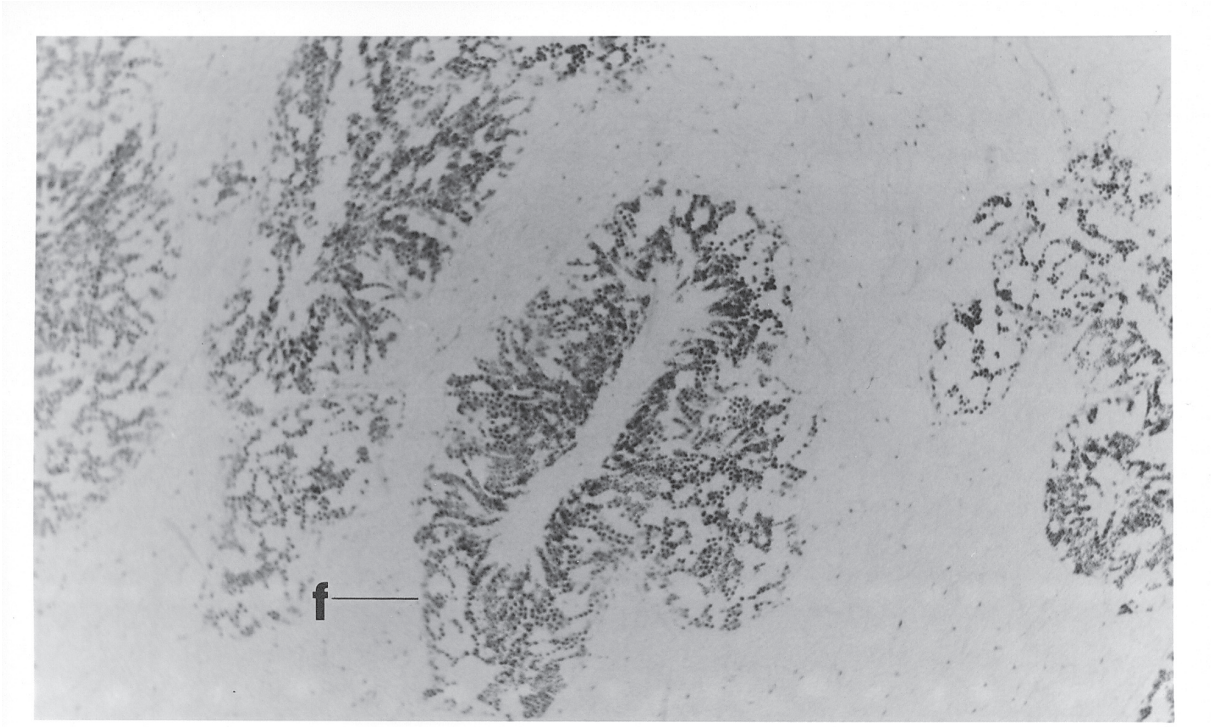

Figure 5. Developing male 2 (MD2). Follicles (f) are larger. (x100) (See Figure 6 for higher magnification.)

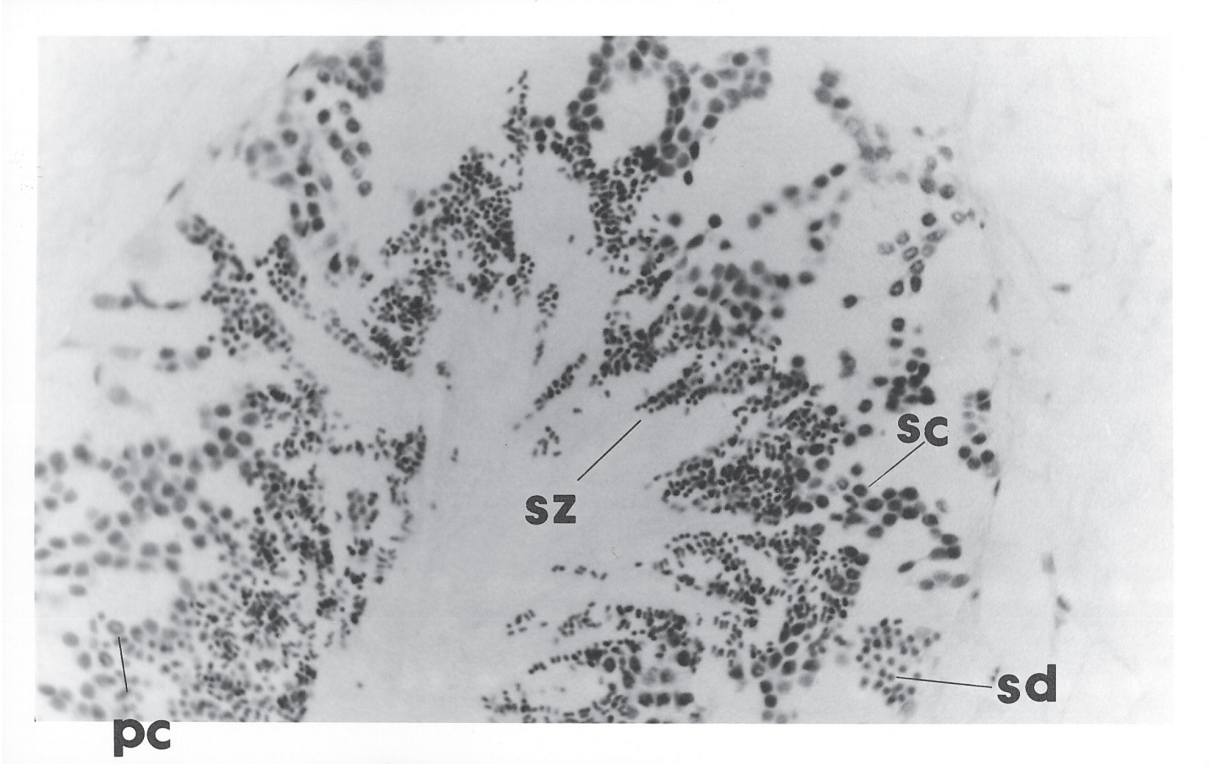

Figure 6. Developing male 2 (MD2). All stages of spermatogenesis are present. Primary (pc) and secondary spermatocytes (sc) form a loose band at the periphery of the follicle with the central follicle area consisting primarily of spermatids (sd) and some spermatozoa (sz). (x400)

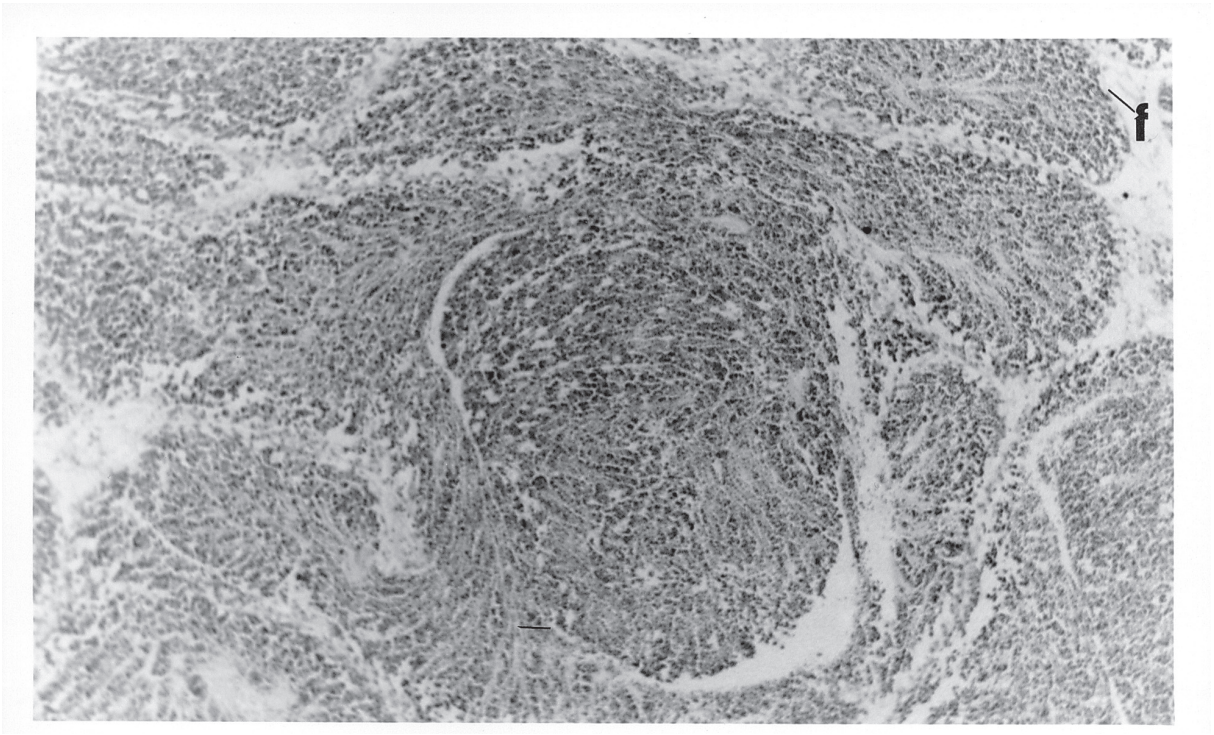

Figure 7. Advanced male (MA) with densely packed follicles. (x100) (See Figure 8 for higher magnification.)

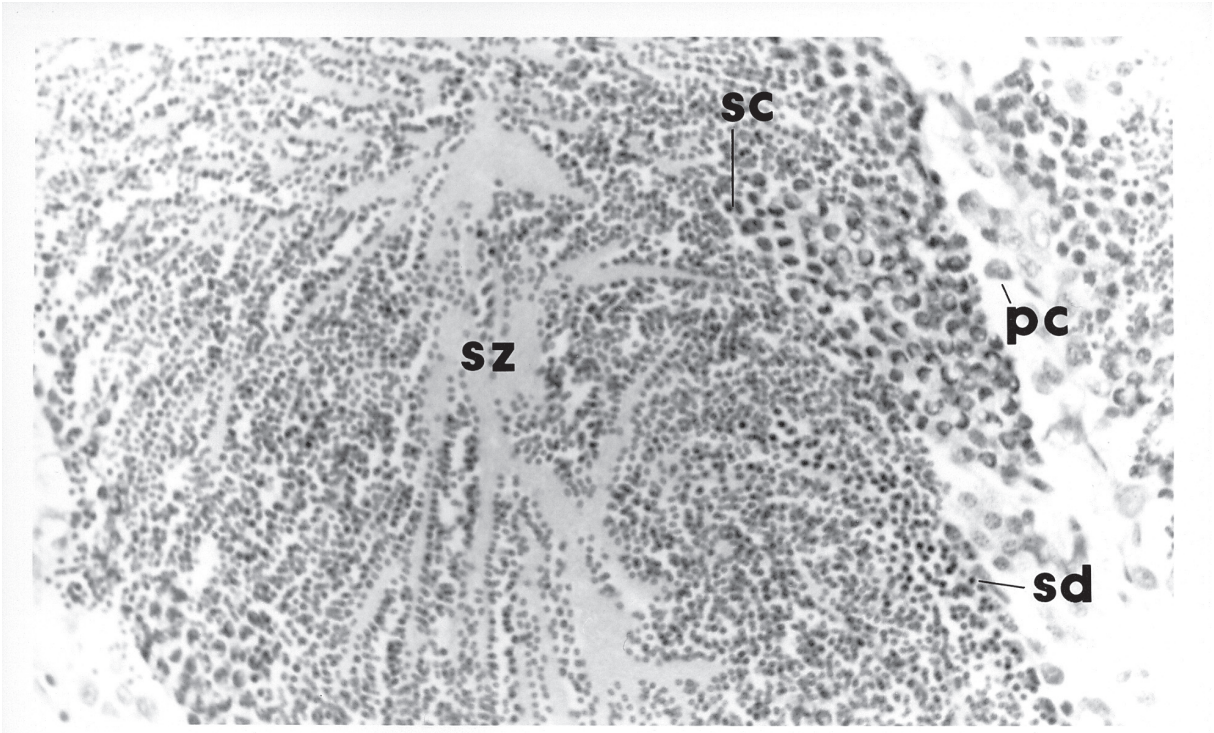

Figure 8. Advanced male (MA) with follicles packed largely with spermatozoa (sz) but with some spermatids (sd) and primary (pc) and secondary spermatocytes (sc) at the periphery. (x400)

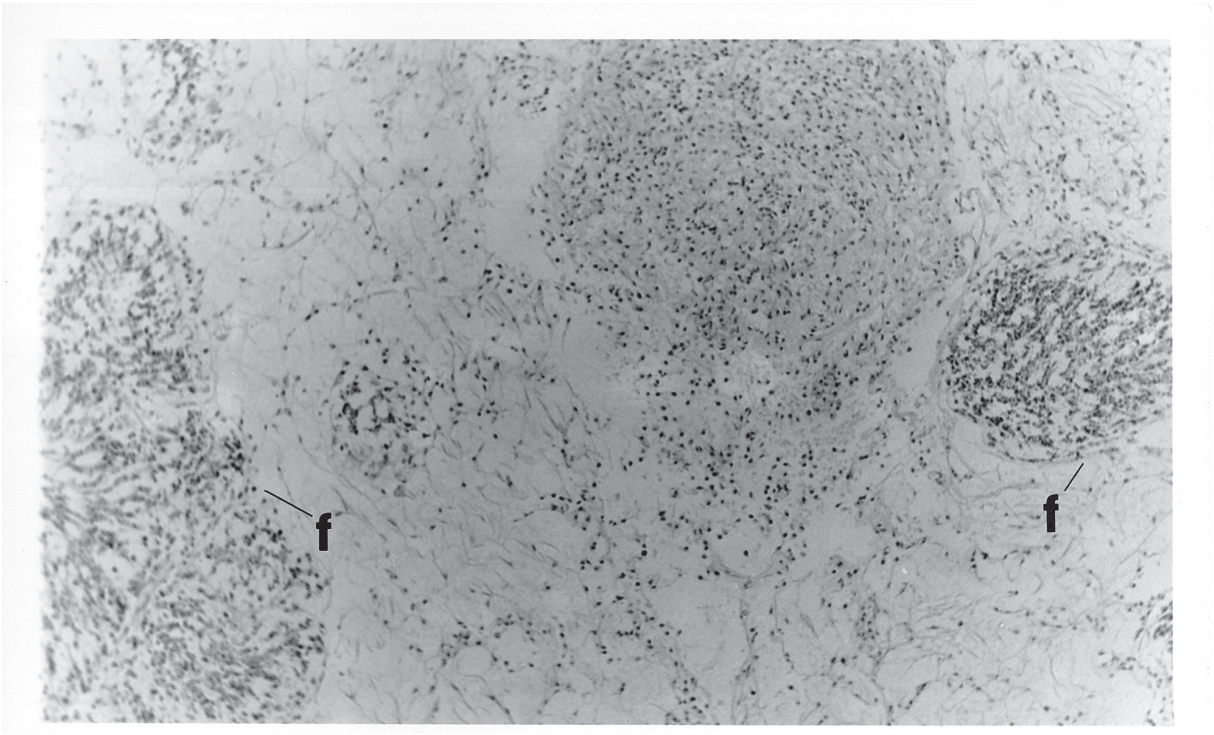

Figure 9. Post spawned male (MPS). Some follicles (f) have ruptured or degenerated while in this individual some follicles still retain significant numbers of gametes. (x100)

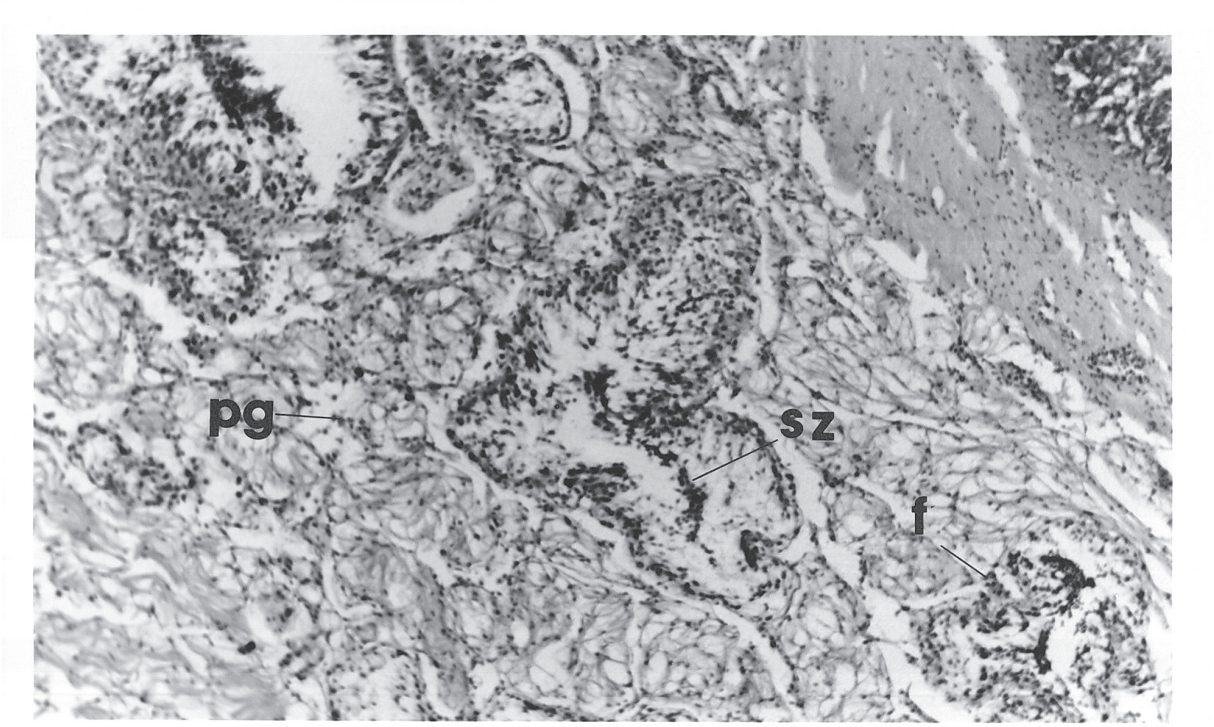

Figure 10. Regressive male (MR). Large numbers of phagocytes (pg) are evident as well as discharged, collapsed follicles containing residual spermatozoa (sz). (x100)

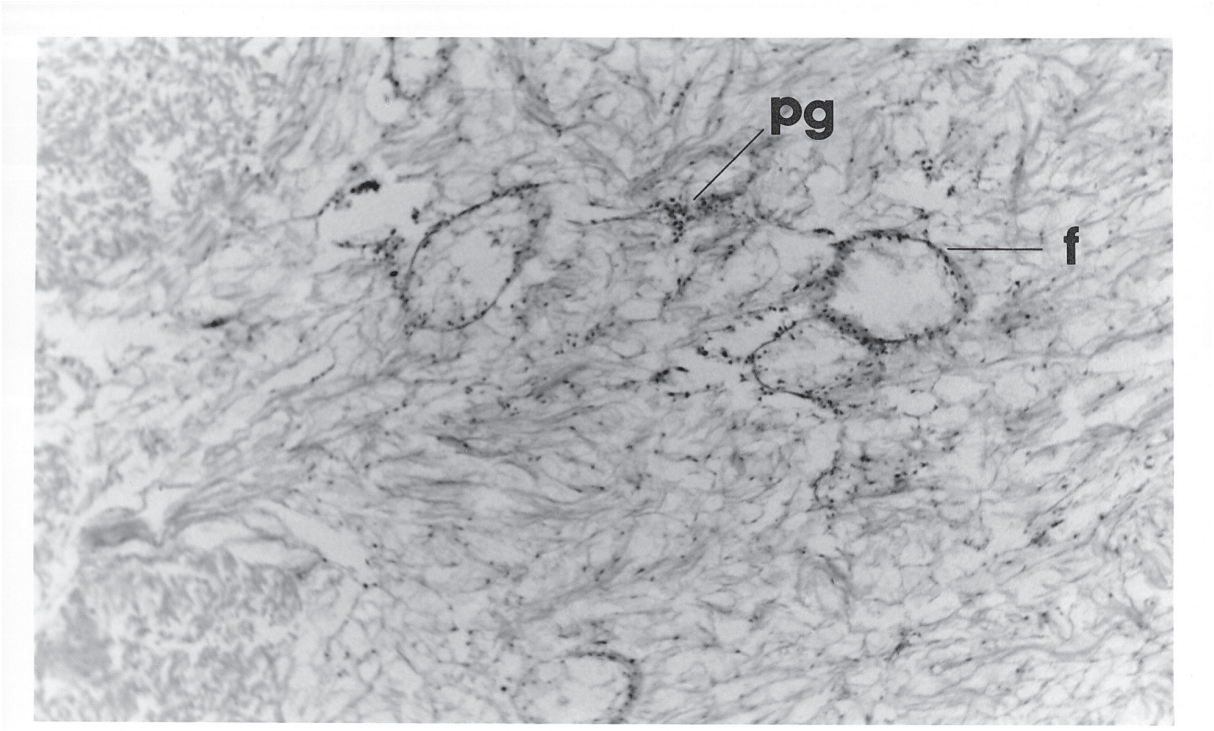

Figure 11. Indeterminate sexual phase (I). Follicles (f) are contracted and germ cells not sexually differentiated. Phagocytes (pg) are present. (x100)

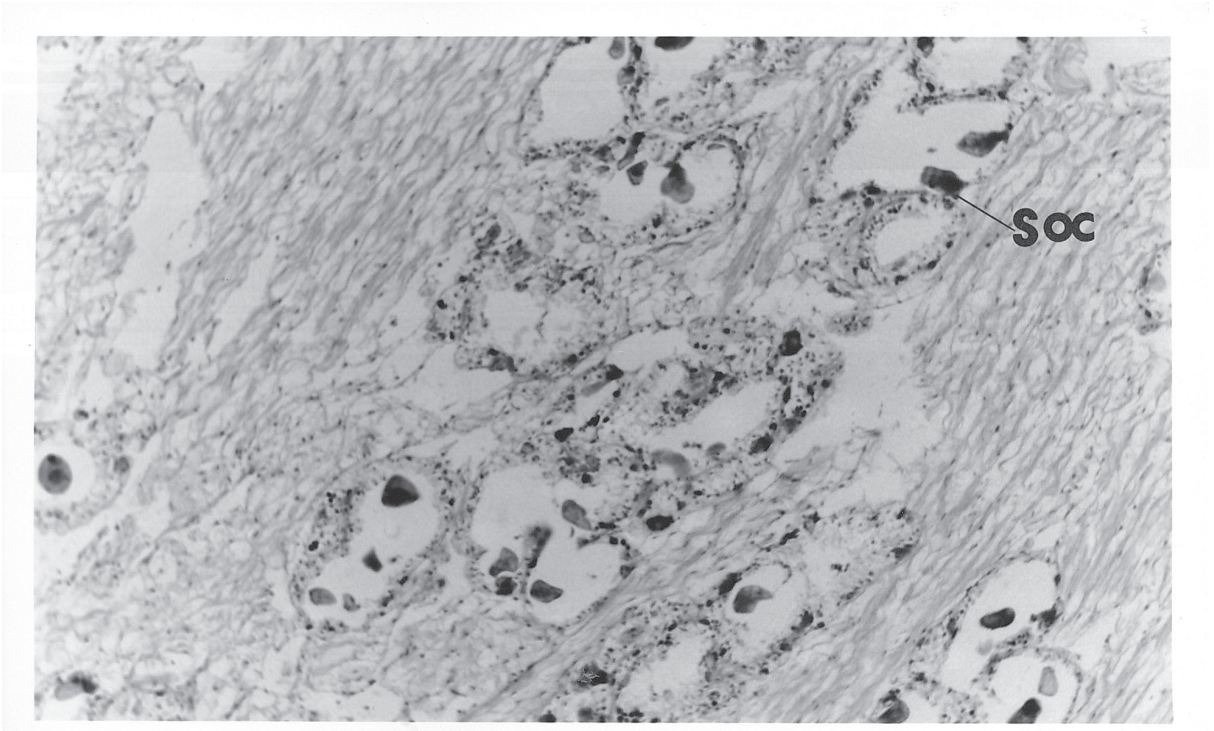

Figure 12. Developing female 1 (FD1). Characterised by the presence of relatively immature female germ cells. A few distinctive secondary oocytes (soc) are attached to follicle walls by an elongated peduncle. (x100)

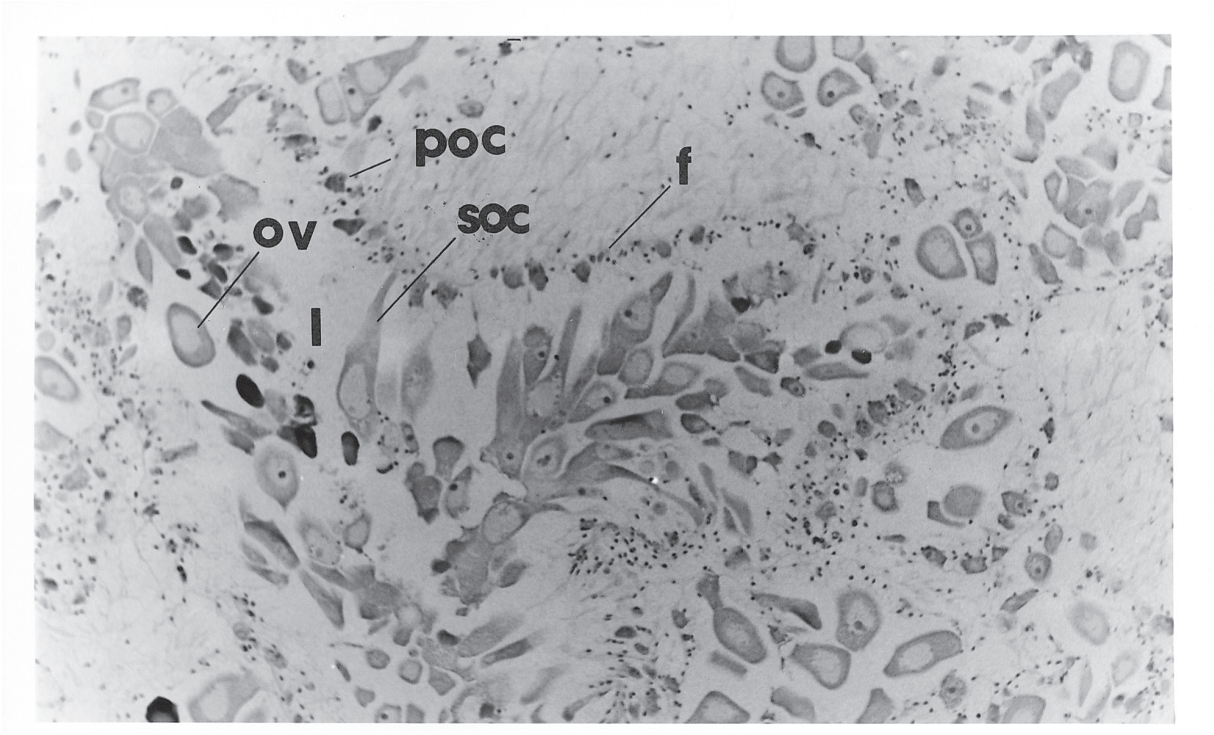

Figure 13. Developing female 2 (FD2). As well as primary oocytes (poc), secondary oocytes (soc) are attached to the follicle wall but there are some ova (ov) lying free in the lumen (l) of the follicle (f). (x400)

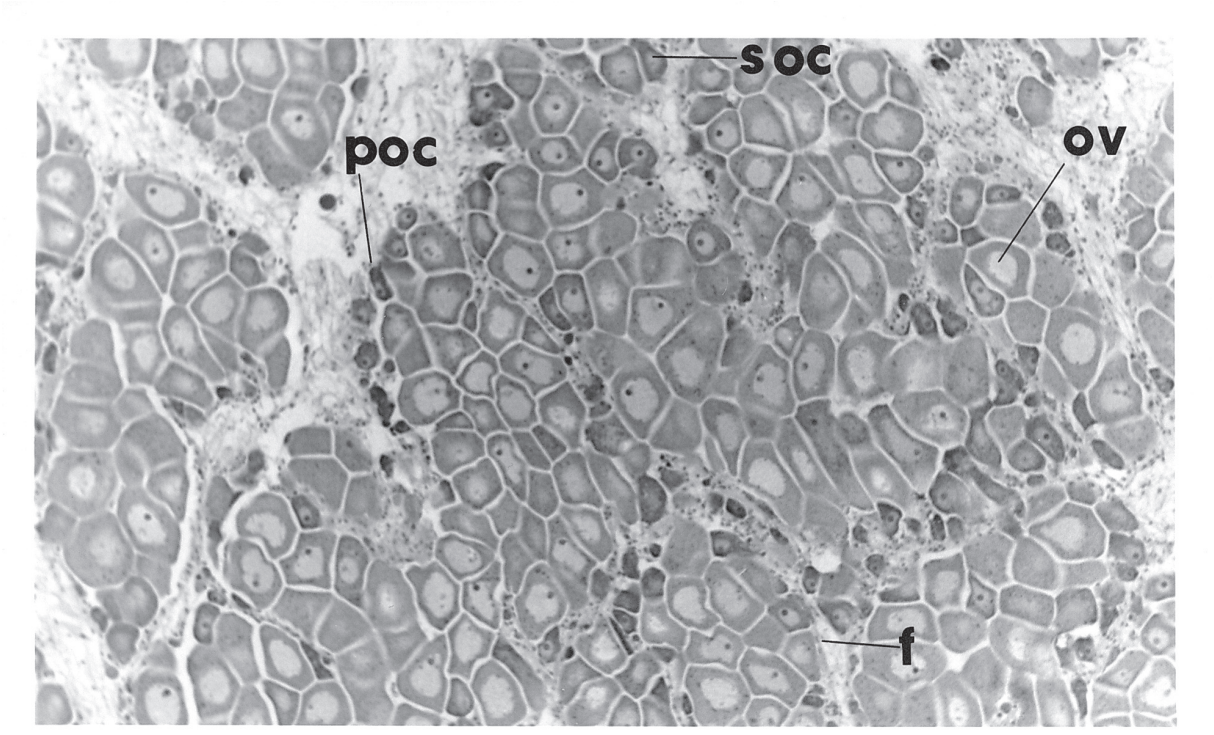

Figure 14. Advanced female (FA). While some primary (poc) and secondary oocytes (soc) are evident, most of the lumen of the follicles (f) is occupied by mature ova (ov). (x100)

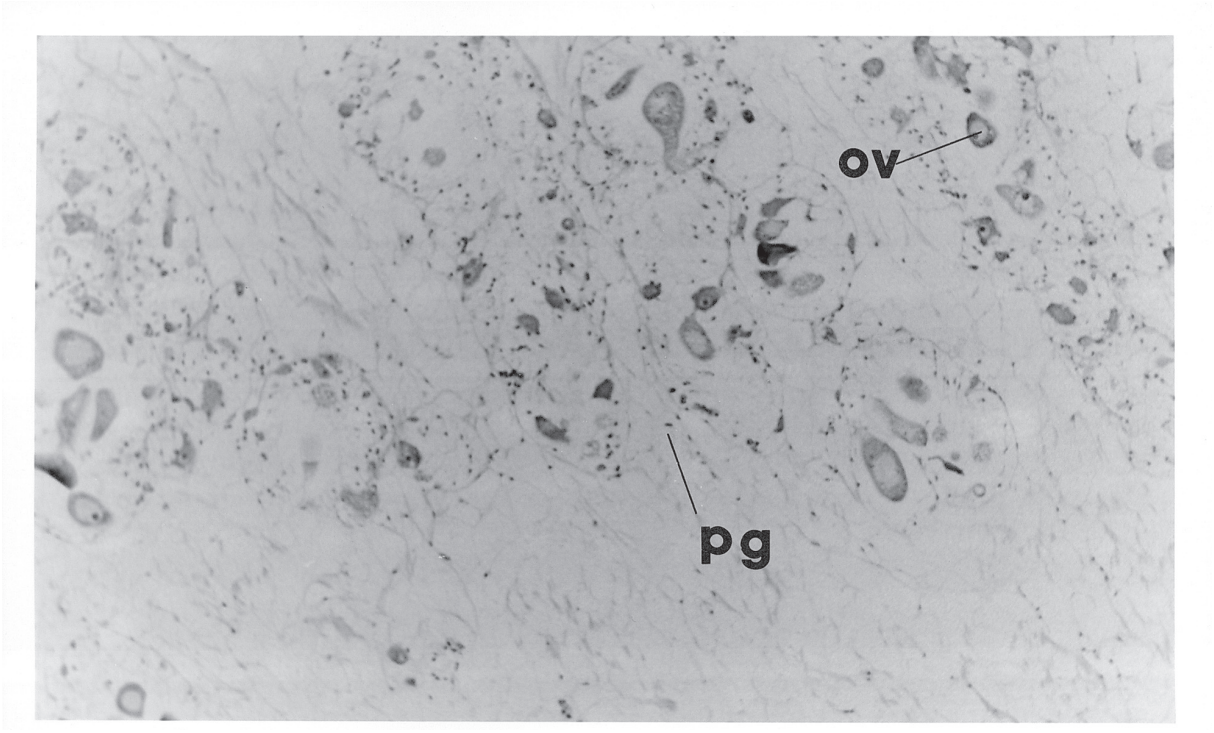

Figure 15. Post spawned female (FPS). Partial or complete spawning has occurred. Some follicles (f) show evidence of rupture or have a disorganised appearance. Small numbers of phagocytes often present and some residual but intact ova (ov) are still evident. (x100)

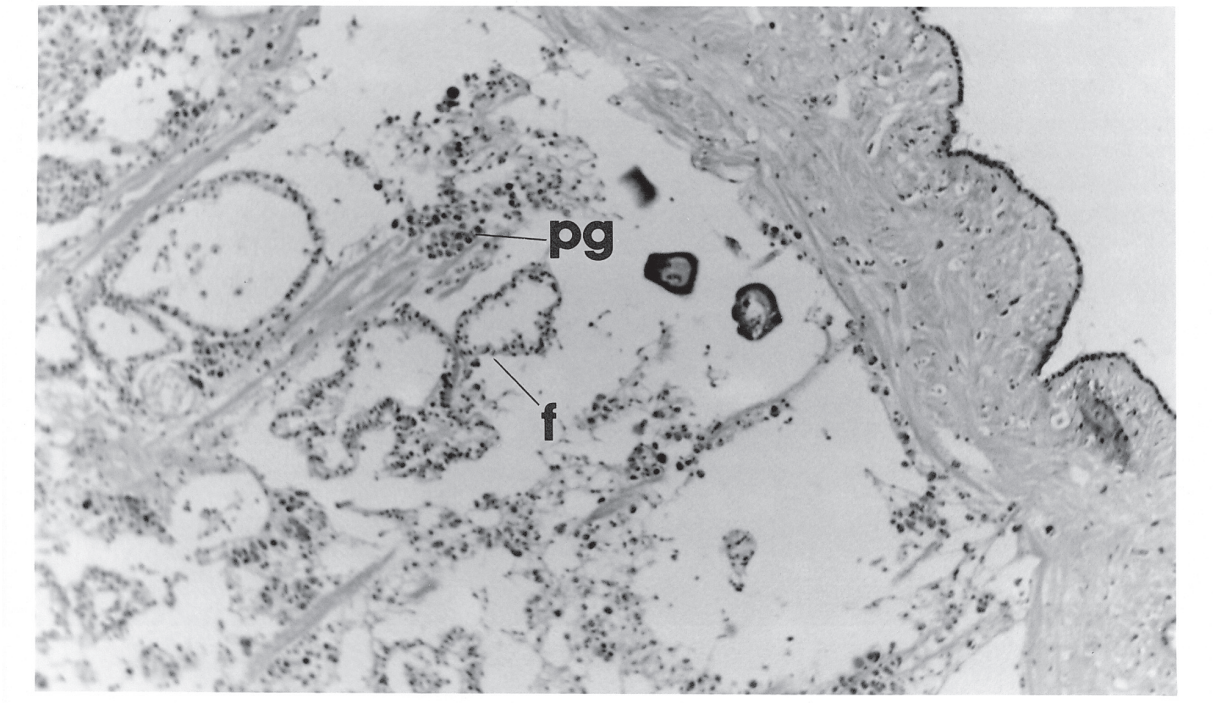

Figure 16. Regressive female (FR). Follicles (f) have largely degenerated. There are a few undischarged ova and large numbers of phagocytes (pg). (x100) (See Figure 17 for higher magnification.)

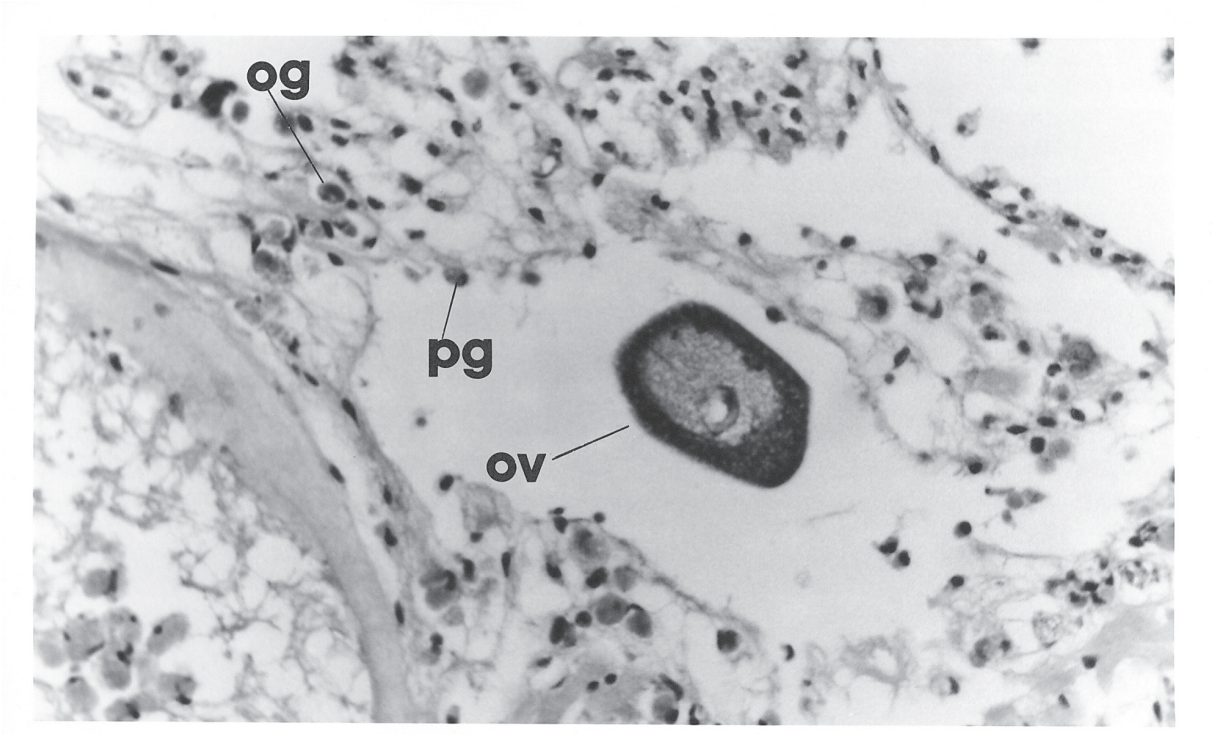

Figure 17. Regressive female (FR). The few undischarged ova (ov) have a granular/porous texture that is indicative of deterioration but a few immature cells (oogonia, og) are evident along with numerous phagocytes (pg). (x400)

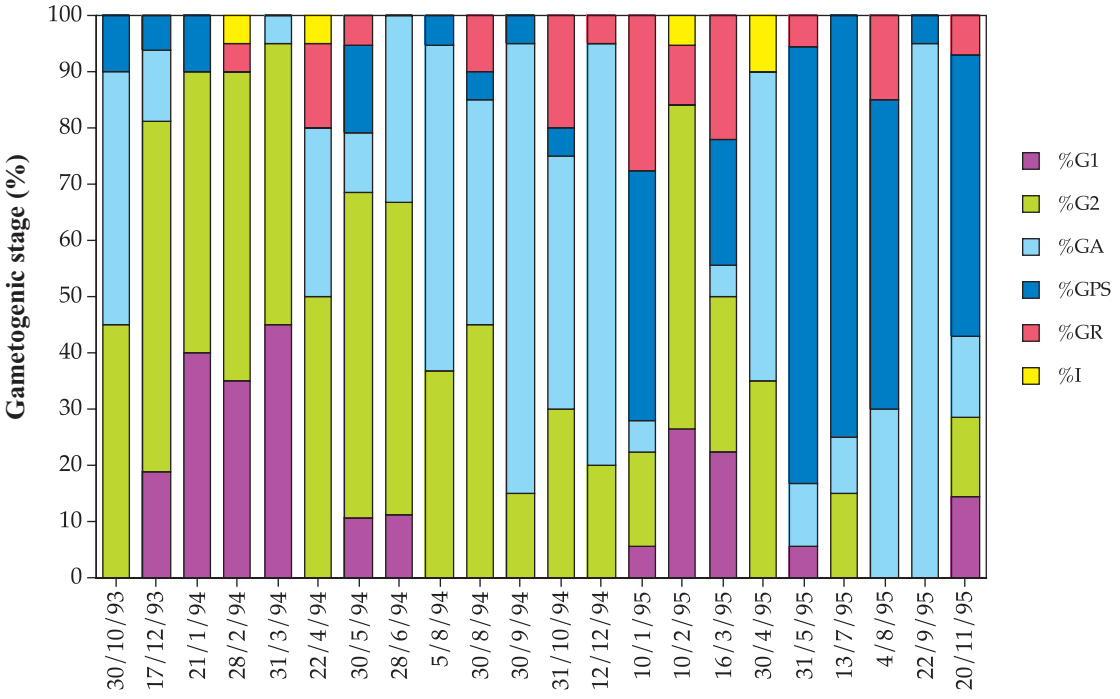

Figure 18. Seasonal variation in gonad development of *Ruditapes largillierti* from St Helens, Tasmania. Sexes are pooled but stages not condensed.

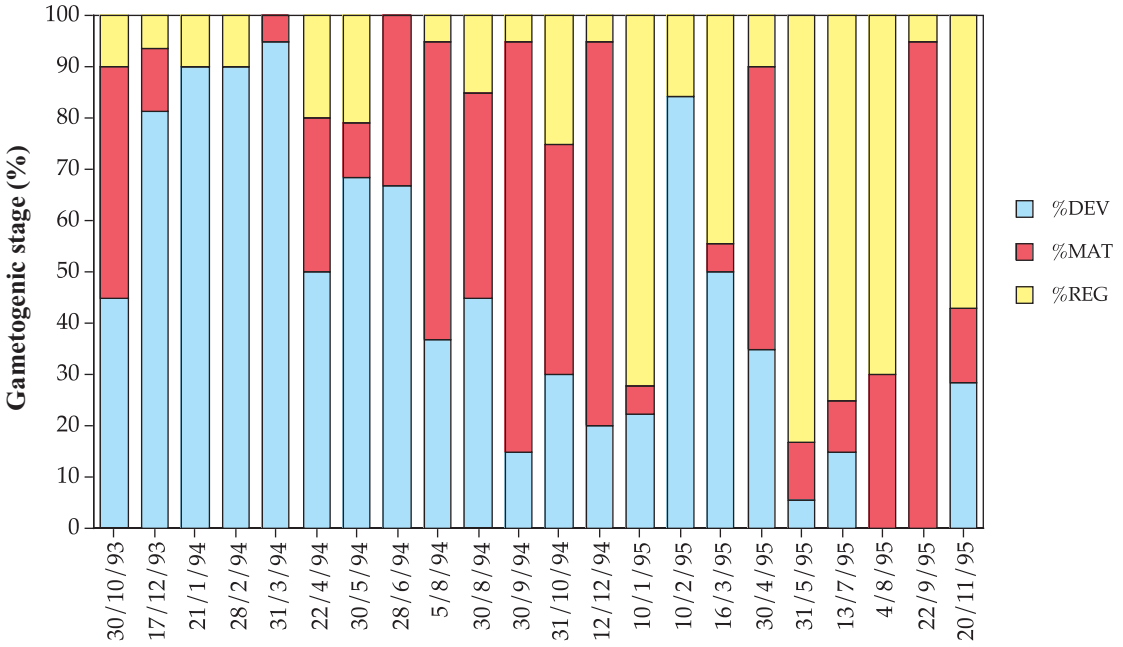

Figure 19. Seasonal variation in gonad development of *Ruditapes largillierti* from St Helens, Tasmania. Sexes are pooled and stages are condensed. Developing (DEV) = G1 + G2. Mature (MAT) = GA. Regressed (REG) = GPS + GR + I.

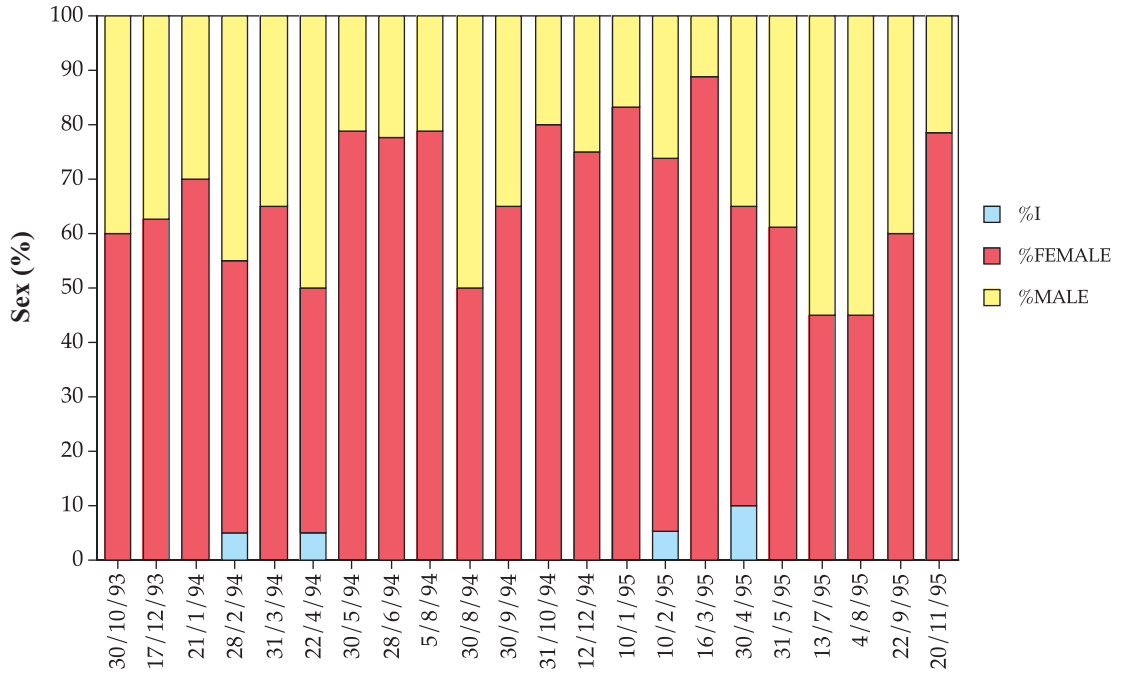

Figure 20. Sex ratio of *Ruditapes largillierti* from St Helens, Tasmania. I = Indeterminate

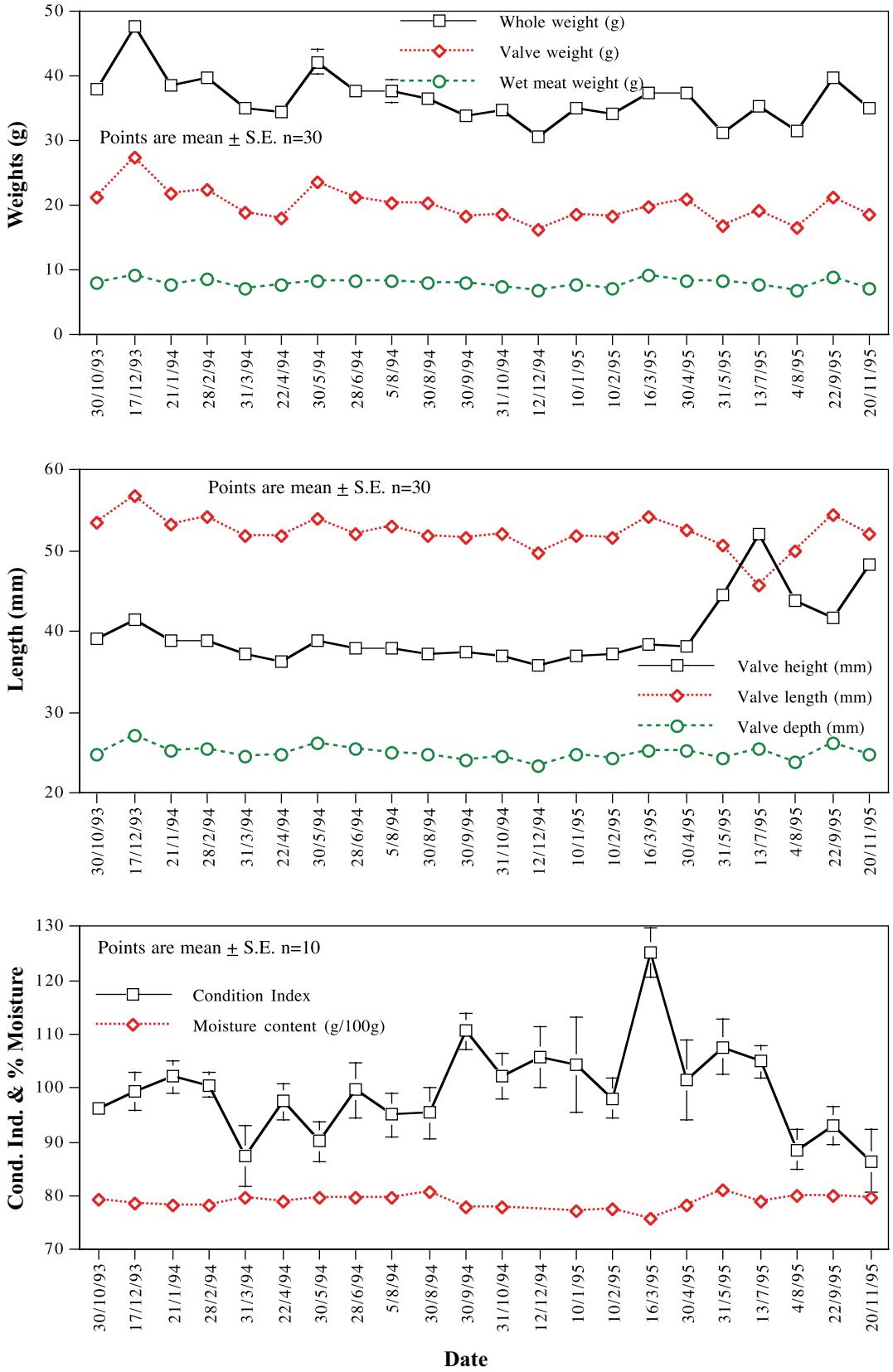

Figure 21. In descending order, Whole weight, dimensions, Condition index CI (dry meat weight.100/cavity volume) and moisture content of meat for *Ruditapes largillierti*. from St Helens, Tasmania.

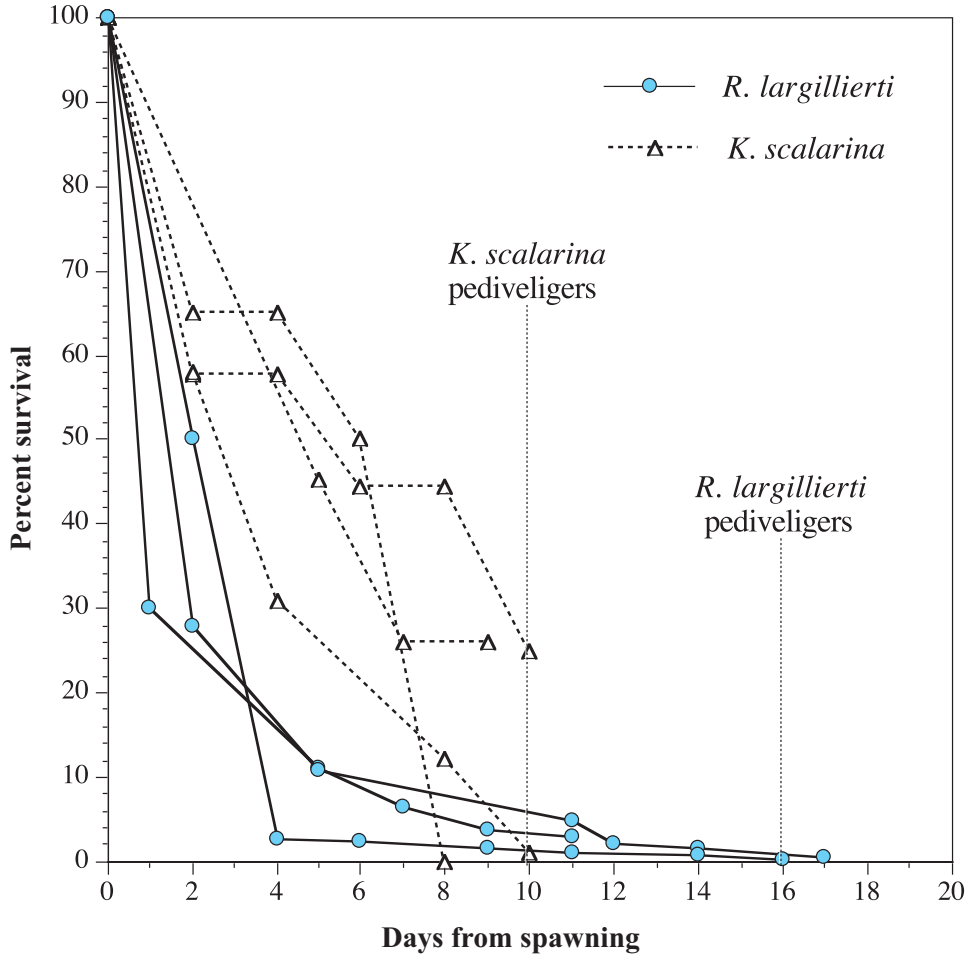

Figure 22. Survival of 3 batches of *Ruditapes largillierti* larvae to metamorphosis, expressed as a percentage of total number of eggs obtained at spawning. Data for 4 batches of *Katelysia scalarina* provided for comparison (data from Kent et al., 1998, 1999.)

5.3 MANUSCRIPT 3

Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina* (Lamark 1818).

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5.3.1 ABSTRACT

In a series of trials, broodstock venerid clams *Katelysia scalarina* were conditioned, or obtained directly from fishing grounds, and stimulated to spawn using thermal cycling. Broodstock, initially with only moderately developed gonad, were sufficiently conditioned for mass spawnings after 8 weeks at 13°C in an indoor recirculating seawater system. Ripe wild broodstock maintained at 20°C could still be spawned after 6 weeks in a similar system. Mean fecundity from mass spawning trials ranged between 0.7 and $2.4x10^6$ eggs.female^{-1} whilst fecundity estimates for strip spawned individuals varied from 0.3 to $2.2x10^6$ eggs.female^{-1}. Neither intramuscular serotonin injections ($6x10^{-8}$ to $1x10^{-6}$ mol, total dose), nor strip spawning were reliable methods for producing significant numbers of healthy larvae. Eggs (69 ± 2 µm) developed to D veliger larvae (110 ± 1.3 µm) within 48 h at 20°C. Metamorphosis to spat (210.9 ± 2.1 µm) was observed from day 20 following treatment with 10^{-4} M norepinephrine for 60 min on day 19. Larval rearing was not always successful because of bacterial and viral problems and the extended settlement period for this species.

Keywords: Clam; venerid; *Katelysia scalarina*; broodstock conditioning; spawning; larval rearing.

5.3.2 INTRODUCTION

The stepped venerid, *Katelysia scalarina* (Lamark 1818) is a relatively small clam of 35-50 mm shell length (Lush, 1992). It is a shallow (2-4 cm) burrower (Nielsen, 1963; Bellchambers and Richardson, 1995), and is found throughout both sheltered and moderately exposed intertidal waters of Tasmania and southern mainland Australia (Gabriel and Macpherson, 1962). *K. scalarina* may be distinguished from another closely related species *K. rhytiphora* (Lamy 1937) by the absence of radiating striae and more oblique shape (Gabriel and Macpherson, 1962) as well as with allozyme analyses (Soh et al. 1998). In addition, *K. scalarina* is often distributed higher up the intertidal zone than *K. rhytiphora* (Roberts, 1984).

In Tasmania, *K. scalarina* is the major species in a small clam fishery and is exported to mainland niche markets and occasionally overseas (Treadwell et al., 1992; Zacharin, 1993). In recent years, concerns over a decrease in abundance of this species in some areas have necessitated a reduction in catch quotas and given impetus to research into the feasibility of producing hatchery-reared stock for culture or fisheries enhancement.

Currently there is no commercial aquaculture production of *Katelysia* spp. within Australia, although research into hatchery production and growout of *K. rhytiphora* has been undertaken (Nell et al., 1994, Paterson and Nell, 1997). Whilst oyster hatchery techniques have much in common with clam culture (Maguire, 1992), and in the USA have required little modification for clam production (Castagna and Manzi, 1989), further investigation into hatchery techniques for temperate clams, including these endemic species, is still required (Maguire, 1992; Nell et

al., 1995). Potential bottlenecks exist with supply of broodstock, spawning, larval rearing and metamorphosis, and this study addresses aspects of these topics for *K. scalarina*.

5.3.3 MATERIALS AND METHODS

All clams collected from natural habitats were transported to Tasmanian culture facilities in plastic containers without water or sediment. All seawater used was drawn from exposed sections of coastline largely unaffected by freshwater runoff. However the quality of seawater delivered to the University of Tasmania's (UTAS) bivalve hatchery at Launceston is periodically affected by seaweed debris.

Broodstock Conditioning (Broodstock Trial 1)

Two separate groups of 200 broodstock clams were collected from Ansons Bay in August 1994 (Broodstock Trial 1) and transported to Marine Shellfish Hatcheries, Bicheno (Figure 1). Gonad condition was visually assessed as intermediate (++) (Garland et al., 1993). Clams were scrubbed and rinsed with fresh water and placed in an indoor conditioning system consisting of a 1,100 L holding trough, recirculating pump and header tank. Clams were held in plastic mesh baskets lined with 400 µm screen and filled with 2-4 mm quartz gravel. Water temperature was maintained at 13°C and clams were fed a mixture of *Pavlova lutheri*, (Droop) and Tahitian *Isochrysis* sp., supplemented with *Chaetoceros muelleri* (Lemmermann) at approximately 8-12 x 10^{10} cells.day^{-1}. After approximately 8 weeks conditioning, clams were assessed as ripe (+++) (Garland et al., 1993) and 50 animals from each group were successfully spawned by cycling water temperatures between 13°C and 20°C.

Broodstock Maintenance (Broodstock Trial 2)

In December 1996 approximately 200 clams were collected from Ansons Bay. Twenty clams were fixed in 10% phosphate buffered formalin for later histology and the remainder were reproductively conditioned in an indoor recirculating seawater system. The system at UTAS Launceston consisted of a 300 L reservoir and 4 x 50 L plastic bins (45 animals per bin) each with a floor area of 0.19 m^2. Beach sand (6 cm deep) was placed on the floor of two bins, so that clams could burrow while clams in the other two bins were maintained without substrate. Water temperature was maintained at 20°C ± 0.5°C, and salinity remained constant at 34.5 ± 0.2 ppt. Clams were fed a daily ration of *P. lutheri*, Tahitian *Isochrysis* sp., and *Tetraselmis suecica* (Butcher) such that all feed was cleared within 24 h (Toba et al., 1992).

In January 1997 (six weeks later), another sample of 20 clams was collected from the same site at Ansons Bay and preserved. A sample of 10 clams from each bin was also taken and preserved as above for later histology. A further 72 clams from the substrate bins were held out of water overnight and placed in 1 µm (nominal) filtered seawater (FSW) at 19°C the following morning, 45 minutes after which spawning commenced. Animals were allowed to spawn out in the tray yielding a total of 24 x 10^6 eggs with a fertilisation rate of 99 percent. These subsequently developed into active, healthy D larvae within 48 h.

Standard 4 µm paraffin sections were prepared from a 3 mm slice of the preserved samples using the anterior edge of the foot as a reference (Howard and Smith, 1983). These were stained using Mayer's haematoxylin and eosin Y. Gonads were staged for gametogenic development using a modified staging system based on Dinamani (1974).

Spawning Induction

In Summer 1995, clams from three sites (Georges Bay, Ansons Bay and Swanwick) were collected for Spawning Trials 1-3 at UTAS, Launceston. Gonad condition by visual observation

was assessed as moderate (++) (Garland et al., 1993). Clams failing to spawn after temperature cycling (20°C- 26°C, at 30 min intervals), were injected via the anterior adductor muscle with various concentrations and volumes of serotonin (5-HT) (Table 1) (Gibbons and Castagna (1984 and 1985), Heasman et al. (1994), and O'Connor and Heasman (1995)) and placed in 1 µm FSW at 25°C.

In Spawning Trial 4, strip spawnings were also attempted with moderate to ripe stock (++/+++) (Garland et al., 1993) that failed to spawn with temperature fluctuations. Ten females and six males were strip spawned by lacerating the gonad with sterile scalpel blades and flushing eggs and sperm into separate 1 L beakers with 1 µm FSW. Eggs were collected, retained on a 63 µm screen and resuspended in 1 L fresh FSW. Eggs were then treated with 0.25 mL of 2 M NH_4OH ($0.5x10^{-3}$ M final concentration) for 45 minutes to aid breakdown of vesicle after Wada (1953) and Minaur (1969). Following this, eggs were resuspended in fresh FSW and a dilute sperm solution was added. Fertilised eggs were placed in a 300 L larval rearing tank (LRT) at 20°C with low aeration.

Larval Rearing

Larval Trials 1-4 (Table 2), were conducted in summer 1993-1994 at Shellfish Culture Pty. Ltd., a commercial bivalve hatchery at Bicheno. Broodstock were collected from various estuaries (Figure 1), including Musselroe Bay (north coast), Swanwick on the east coast and Cockle Creek in the south of the state. Water used in spawning and larval rearing was 1 µm FSW (spawning and day 0) and 10 µm FSW thereafter. Disodium ethylenediaminetetra-acetic acid (EDTA) was added to day 0 water at $1mg.L^{-1}$ as per Utting and Helm (1985). With the exception of trial 1, ripe broodstock were held out of water for 12 h (Castagna and Manzi, 1989) prior to being placed in a dark flat fibreglass trough (100 x 600 x 900 mm) filled with recirculating filtered seawater. Clams were encouraged to spawn by elevating water temperature by 3 - 6°C as indicated in Table 2 (Loosanoff and Davis, 1963). A UV light was included in the recirculating system to help maintain low bacterial levels and stimulate spawning (Bourne et al., 1989). Numbers of broodstock and spawning details are outlined in Table 2.

Larvae for trial 1 were obtained after broodstock spawned overnight in their holding tank. Eggs were collected the following morning and were rinsed through a 53 µm screen and collected on 25 µm mesh. Larval rearing then proceeded as for other trials. Larvae were cultured in 3000 L and 15000 L flat-bottom fibreglass tanks. For each trial, eggs were stocked at 7-10 $eggs.mL^{-1}$ and larval densities were between 3-4 $larvae.mL^{-1}$. Water temperatures were maintained at 24°C ± 0.5°C. Tanks were cleaned and water changed every 48 h after larvae had been retained on appropriate sized nylon mesh screens. Larvae were fed a 1:1 (by cell number) mixture of *P. lutheri* and Tahitian *Isochrysis* sp. at approximately 10,000 $cells.larvae^{-1}.day^{-1}$ (Toba et al., 1992).

For Larval Trial 5 in February 1996, ripe broodstock from Ansons Bay were encouraged to spawn at UTAS, Launceston via thermal fluctuations. Males and females were left to spawn together in the spawning tray. Fertilised eggs were screened through a 75 µm screen to remove faeces and other debris, and retained on 25 µm screen. Eggs were resuspended in fresh FSW and stocked in 300 L tanks at 25 $eggs.mL^{-1}$. Equal quantities (by cell number) of *P. lutheri*, Tahitian *Isochrysis* and *Chaetoceros calcitrans* (Paulsen) were fed at approximately 5,000 $cells.larvae^{-1}.day^{-1}$. Water temperatures were maintained at 20.5°C ± 0.2°C. Tanks were cleaned and water changed every 48 h with 1 µm UV sterilised FSW. On day 8 larvae were transferred to a 1,000 L fibreglass flat bottom tank and stocking densities reduced to 1.2 $larvae.mL^{-1}$. At Day 18, 20,000 larvae were placed in a 200 mm diameter plastic downweller pot (with 132 µm mesh)

and treated with 10^{-4} M solution of the catecholamine norepinephrine for 60 min (Coon et al., 1986) to promote metamorphosis. Water was circulated within a 40 L bin by means of an air lift pump. Spat and juvenile clams were maintained for a further 48 days in the above system, with cleaning, feeding and water changed every 48 hours.

5.3.4 RESULTS

K. scalarina can be reproductively conditioned in a relatively simple recirculating seawater system and ripe broodstock can be maintained for more than a month for subsequent spawnings. Histological examination of clams collected from the wild for Broodstock Trial 2 in December 1996, revealed gonads to be ripe with large numbers of mature ova or sperm in the gonad follicles. Subsequent retention of these animals for six weeks in a conditioning system did little to improve the extent of gonad development. However, examination of gonad tissue from wild stock in January 1997, revealed that at least a partial spawning had occurred which was not mirrored by animals held in the broodstock system (Figure 2).

In spawning trials 1-3, intramuscular injection of 5-HT encouraged some moderately conditioned males to spawn, releasing sperm with normal activity. However, in all cases females failed to spawn (Table 1), despite variations in concentration and volume of 5-HT administered. Controls that were not injected did not spawn.

Stripped females (35-45 mm shell length) in spawning Trial 4 yielded a total of 8×10^6 eggs with a mean diameter of 70 µm ± 2.5 µm. Fecundity of individuals ranged from 0.3×10^6 to 2.2×10^6. Observed eggs were pear shaped with a prominent germinal vesicle. Survival of larvae from eggs treated with NH_4OH, was very low falling to less than 1% by day 2 post-fertilisation. Moreover, many of the larvae that remained alive were abnormal and exhibited extensive fouling of the velum.

In Larval Rearing Trials 2 - 5, ripe *K. scalarina* spawned readily in response to thermal stimulations of 3 - 6°C above ambient. Mean egg diameter was 69 µm ± 2 µm and average fecundity ranged between 0.7×10^6 and 2.4×10^6 eggs.female^{-1} (Table 2). Controls, not subjected to thermal stimulation, did not spawn.

Larval rearing was not always successful (Figure 3). However, in all trials, development of fertilised eggs to trochophore stage occurred within 24 h and to D veligers, with a mean shell length of 110 µm ± 1.3 µm, by 48 h. Survival of larvae to day 5 was between 30-50% but in all cases was less than 1% by settlement. Growth of *K. scalarina* was rapid at 24°C, with larvae reaching a mean shell length of 200 µm by day 8 (Figure 3a). Pediveligers of approximately 200 µm shell length, were first observed between day 9 and day 15 depending on rearing temperature which were 24°C and 20.5°C respectively (Figure 3b, 4), and metamorphosis to spat was first observed by day 20 at 20.5°C (Figure 4). Following metamorphosis, growth was exponential until day 53 after which it slowed abruptly, possibly in response to reduced water flows caused by fouling of the downweller screen.

5.3.5 DISCUSSION

Broodstock

Broodstock conditioning systems are advantageous for maturing bivalves both within (Castagna and Kraeuter, 1981; Castagna and Manzi, 1989; Toba et al., 1992), and outside of the normal spawning season (Numaguchi, 1997). *K. scalarina* broodstock held in clean sediment, could be conditioned at a relatively low temperature (13°C) (Broodstock Trial 1). Also mature clams

collected from the wild could be maintained in ripe condition within a recirculating system despite indication of spawning of stock in the fishery during the experimental period (Broodstock Trial 2), (Figure 2).

Spawning

Fecundity of *K. scalarina* was comparable to that for *K. rhytiphora* (Nell et al., 1994) and the Manila clam *Ruditapes philippinarum* Adams and Reeve (Utting et al., 1996, Laing and Lopez-Alvarado, 1994), but less than that of the hard clam *Mercenaria mercenaria* (Castagna and Kraeuter, 1981).

During spawning trials, both isolation of spawning male and female stock, and mass spawnings in the tray were undertaken. We detected no deleterious effects from the latter, even though more than 50 sperm per egg were observed on some occasions. Indeed, larvae displaying the greatest degree of activity were produced using this method. Nevertheless, *K. scalarina* has not always responded well to spawning induction protocols. On occasions, spawning has occurred up to two days after thermal stimulation, and neither serotonin injections nor strip spawnings have provided appropriate results. These difficulties may have arisen largely from variability in the quality of broodstock obtained directly from the wild. Spawning in two additional trials in the summer of 96-97 was relatively predictable (within 60 minutes of initiation of thermal stimulation) when broodstock in excellent condition were available.

Gibbons and Castagna (1985) found that injections of between 80-800 μmol of 5-HT into the adductor muscle of gravid *M. mercenaria* encouraged spawning, however, males were more responsive than females and both concentration and absolute dose significantly affected spawning success. Numaguchi (1997) induced spawning in the common oriental clam *Meretrix lusoria* with a 125 μmol intragonadal injection of 5-HT and other workers have induced spawning in bivalves with 10^{-3} M external applications (Ram et al., 1992). During our trials, the application of various concentrations of 5-HT failed to induce spawning in female broodstock, although some males did spawn. This may have been as a result of inadequate broodstock condition, although Ram and Nichols (1993) reported that injection of serotonin into zebra mussels induced ripe males to spawn but not females.

Strip spawnings using moderate to ripe broodstock were not successful and larval mortality was very high (99%). In addition, many of the remaining larvae were deformed. In the rearing of *Pinctada maxima*, using stripped eggs treated with ammonium hydroxide, Minaur (1969) also reported up to 95% mortality of larvae to pediveliger stage, with some larvae showing deformed valves or other gross abnormalities. At this stage it is unclear whether the deficiencies of this technique are primarily due to the method itself, such as possible toxic side effects of the NH_4OH used to promote breakdown of the germinal vesicle, or inadequately matured gametes. Stripped gametes from both this study and Minaur (1969) were primarily oocyte 3 as defined by Dinamani (1974). These oocytes are smaller and more irregular in shape compared to free oocytes at final maturity (Dinamani, 1974) and as such may have contributed to poor larval survival.

Larval Rearing

Our results suggest that *K. scalarina* cannot be reared reliably in hatcheries by simply adopting existing oyster hatchery techniques. However, at 20°C, in Trial 5, larval growth was comparable to *K. rhytiphora* (Nell et al., 1994) and similar to that for the Manila clam *R. philippinarum* (Utting and Spencer, 1991).

In a number of the early trials run at 24°C, mass mortality occurred between days 8 and 9. Larval crashes (between days 5 and 8) of commercial cultures of *K. scalarina* run at 16-18°C have also been reported (R. Pugh, pers. comm.). In both instances a herpes-like, viral pathogen has been found in larvae from some of the cultures investigated (see Manusript 17-19). Other failed cultures have been associated with bacterial infestations a common problem with rearing of bivalve larvae (Gibbons and Blogoslawski, 1989). Optimal temperatures for each successive stage in the early life history of *K. scalarina* need to be identified. This should facilitate improved reliability of hatchery rearing as has been the case for other bivalves such as the commercial scallop *Pectin fumatus* (Reeve) (Heasman et al., 1996).

Results from Larval Trial 2 (Figure 3b), suggest that further investigation of setting behaviour should also be undertaken. Unlike *Crassostrea gigas* larvae which may settle over a 24 - 48 h period (Beiras and Widdows, 1995), our observations of *K. scalarina* appear similar to data for Manila clams *R. philippinarum* which may indicate an extended settlement period (Utting and Spencer, 1991). Transferring *K. scalarina* pediveligers to downweller oyster set systems, containing a layer of ground scallop shell cultch, for extended periods (as adopted in Trial 2) proved to be unsatisfactory for this species as larvae were eventually challenged by ciliates. Similarly, larvae from Broodstock Trial 1 developed well but did not metamorphose.

Although nor-epinephrine hastened permanent settlement (Trial 5, Figure 4), larval survival was low. Many larvae were invaded by a marine ciliate (resembling *Uronema nigricans*, see Munday et al., 1997) probably in response to increased bacterial numbers (Plunket and Hidu, 1978). Since the effect of epinephrine or nor-epinephrine can vary with different species of oyster (Coon et al. 1986), specific work to determine appropriate concentration and duration for *K. scalarina* should be undertaken. In addition, larvae must be competent to metamorphose prior to inducement with epinephrine (Coon et al., 1986). With low larval numbers this proved difficult to achieve for all larvae, as animals need to be graded into tight size groups prior to induction.

5.3.6 CONCLUSION

Based on some of the criteria outlined by Kraeuter and Castagna (1989), *Katelysia scalarina* have the potential of being an economically viable culture species. However, whilst broodstock can be adequately conditioned and subsequently spawned, larval rearing, and survival through metamorphosis, are still unreliable. The latter has primarily been due to the extended settlement period observed for this species, and the former due to the presence of a herpes-like viral pathogen. Further investigation of alternate types and dosing regimes for catecholamines as a means of enhancing settlement response are needed. Additional research is also required into the prevention and epidemiology of the herpes-like virus encountered during the course of this study. It is also recommended that the optimal rearing temperatures for the early life stages of this clam be identified.

5.3.7 ACKNOWLEDGMENTS

The authors would also like to thank Ms Elizabeth Cox and Mr Ian Duthie for their assistance with spawning and larval rearing trials, Mr Derek Cropp for help with broodstock conditioning, and Mr Dale Ridges and Ms Lynda Bellchambers who assisted in collection of broodstock. We are also indebted to Dr Mike Heasman for his valuable editorial comments during the preparation of this manuscript.

5.3.8 REFERENCES

Bellchambers, L.M. and Richardson, A.M.M., 1995. The effect of substrate disturbance and burial depth on the venerid clam, *Katelysia scalarina* (Lamarck, 1818). *J. Shellfish Res*., 14(1): 41-44.

Beiras, R. and Widdows, J., 1995. Induction of metamorphosis in larvae of the oyster *Crassostrea gigas* using neuroactive compounds. *Mar. Biol*., 123(2): 327-334.

Bourne, N., Hodgson, C.A. and Whyte, J.N.C., 1989. A manual for scallop culture in British Columbia. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1694. Canadian Dept. of Fisheries and Aquatic Sciences Branch.

Castagna, M. and Kraeuter, J.N., 1981. Manual for growing the hard clam *Mercenaria*. Special report in Applied Marine Science and Ocean Engineering N. 249, Virginia Institute of Marine Science, Gloucester Point, VA, 110 pp.

Castagna, M., and Manzi, J.J., 1989. Clam culture in North America: Hatchery production of nursery stock clams. In: J.J. Manzi and M. Castagna (Editors), Clam mariculture in North America. Developments in Aquaculture and Fisheries Science, vol 19. Elsevier, Amsterdam, pp. 111-125.

Coon, S.L., Bonar, D.B. and Weiner, R.M., 1986. Chemical production of cultchless oyster spat using epinephrine and norepinephrine. *Aquaculture*, 58:255-262.

Dinamani, P., 1974. Reproductive cycle and gonadial changes in the New Zealand rock oyster *Crassostrea glomerata*. *N.Z. J. Mar. Freshwater Res*., 8 (1): 39-65.

Gabriel, C.J. and Macpherson, J.H., 1962. Marine Molluscs of Victoria, Melbourne University Press, 475 pp.

Garland, C.D., O'Brien, D.T., Fraser, M.I., Tong, P.E. and Ward, C., 1993. Rapid induction of spawning by Pacific oysters *Crassostrea gigas* in still seawater containing H_2O_2. *Tasmanian Fish. Res*., No. 28: 20-27.

Gibbons, M.C. and Castagna, M., 1984. Serotonin as an inducer of spawning in six bivalve species. *Aquaculture*, 40: 189-191.

Gibbons, M.C. and Castagna, M., 1985. Responses of the hard clam *Mercenaria mercenaria* (Linne) to induction of spawning by serotonin. *J. Shellfish Res.,* 5(2): 65-67.

Gibbons, M.C. and Blogoslawski, W.J., 1989. Predators, pests, parasites and diseases. In: J.J. Manzi and M. Castagna (Editors), Clam mariculture in North America. Developments in aquaculture and fisheries science, vol 19. Elsevier, Amsterdam, pp. 167-200.

Heasman, M.P., O'Conner, W.A. and Frazer, A.W., 1994. Improved hatchery and nursery rearing techniques for *Pectin fumatus* Reeve. *Mem. QLD. Mus.,* 36(2): 351-356.

Heasman, M.P., O'Conner, W.A. and Frazer, A.W., 1996. Ontogenetic changes in the optimal rearing temperatures for the commercial scallop *Pectin fumatus* (Reeve). *J. Shellfish Res.,* 15(3): 627-634.

Howard, D.W. and Smith, C.S., 1983. Histological techniques for marine bivalve mollusks. NOAA Technical Memorandum NMFS-F/NEC-25.

Kraeuter, J.N. and Castagna, M., 1989. Factors affecting the growth and survival of clam seed planted in the natural environment. In: J.J. Manzi and M. Castagna (Editors), Clam mariculture in North America. Developments in Aquaculture and Fisheries Science, vol 19. Elsevier, Amsterdam, pp. 149-165.

Laing, I. and Lopez-Alvarado, J., 1994. Effect of dried algae diets on conditioning and fecundity of Manila clam, *Tapes philippinarum* (Adams and Reeve). *Aquaculture Fish. Manag*. 25: 157-166.

Loosanoff, V.L. and Davis, H.C., 1963. Rearing of bivalve molluscs. *Adv. Mar. Biol*., 1: 1-136.

Lush, G., 1992. The life history of the common cockle with particular reference to the Tasmanian genus *Katelysia*. Internal Report, Tasmanian Dept. of Sea Fisheries, Taroona, Australia.

Maguire, G.B., 1992. Temperate clam farming. In: D. O'Sullivan (Editor), Multi-skilling in Aquaculture: A Hands-on Training Workshop. University of Tasmania, Launceston, Australia. June 28 - July 2, Session 4.B, pp. 24-33.

Minaur, J., 1969. Experiments on the artificial rearing of the larvae of *Pinctada maxima* (Jameson) (lamellibranchia). *Aust. J. Mar. Freshwater Res.,* 20(2): 175-178.

Munday, B. L., O'Donoghue, P. J., Watts, M., Rough, K. and Hawkesford, T., 1997. Fatal encephalitis due to the sctoicociliate *Uronema nigricans* in sea-caged, southern bluefin tuna *Thunnus maccoyii*. *Dis. Aquat. Org*. 30: 17-25.

Nielsen, B.J., 1963. Studies of the genus *Katelysia* Romer 1857 (Mollusca, Lamellibranchiata), *Mem. Nat. Mus. Vic*., 26: 219-257.

Nell, J. A., O' Connor, W. A., Heasman, M. P. and Goard, L. J., 1994. Hatchery production for the venerid clam *Katelysia rhytiphora* (Lamy) and the Sydney cockle Anadara trapezia (Deshayes). *Aquaculture*, 119: 2-3.

Nell, J.A., O'Conner, W.A., Hand, R.E. and McAdam, S.P., 1995. Hatchery production of diploid and triploid clams, *Tapes dorsatus* (Lamarck 1818): A potential new species for aquaculture. *Aquaculture*, 130: 389-394.

Numaguchi, K., 1997. A preliminary trial to induce maturity and spawning of the common oriental clam *Meretrix lusoria* out of spawning season. *J. World Aquaculture Soc.,* 28(1): 118-120.

O'Connor, W.A. and Heasman, M.P., 1995. Spawning induction and fertilisation in the doughboy scallop *Chlamys* (*Mimachlamys*) *asperrima*. *Aquaculture*, 136: 117-129.

Paterson, K.J. and Nell, J.A., 1997. Effects of different growing techniques and substrate types on the growth and survival of the clams *Tapes dorsatus* (Lamark) and *Katelysia rhytiphora* (Lamy). *Aquaculture Res*., 28: 707-715.

Plunket, L. and Hidu, H., 1978. The role of *Uronema marinum* (Protozoa) in oyster hatchery production. *Aquaculture*, 15: 219-225.

Ram, J.L. and Nichols, S.J., 1993. Chemical regulation of spawning in the zebra mussel (*Dreissena polymorpha*). In: T.F. Nalepa and D.W. Schloesser (Editors), Zebra mussels. Biology, impacts, and control. Lewis Publishers, Boca Raton, Florida, pp. 307-314.

Ram, J.L., Crawford, G.W. and Walker, J.U., 1992. Zebra mussel spawning: Release of eggs and sperm in response to external applications of serotonin. *J. Shellfish Res*., 11(1): 236.

Roberts, D., 1984. The genus *Katelysia* (Bivalvia: Veneridae) in southern Australia. *J. Aust. Malacol. Soc.,* 6: 191-204.

Toba, D.R., Thompson, D.S., Chew, K.K., Anderson, G.J. and Miller, M.B., 1992. Guide to Manila clam culture in Washington. Washington Sea Grant Program, University of Washington.

Treadwell, R., McKelvie, L. and Maguire, G.B., 1992. Potential for Australian aquaculture. Australian Bureau of Agricultural and Resource Economics., Research report 92.2. Canberra, 81 pp.

Utting, S.D. and Helm, M.M., 1985. Improvement of seawater quality by physical and chemical pre-treatment in a bivalve hatchery. *Aquaculture*, 44: 133-144.

Utting, S.D. and Spencer, B.E., 1991. The hatchery culture of bivalve mollusc larvae and juveniles. Lab. Leafl. No. 68., MAFF Fish. Res., Lowestoft, UK.

Utting, S.D., Millican, P.F. and Laing, I., 1996. The breeding potential and biochemical composition of triploid Manila clams, *Tapes philippinarum* Adams and Reeve. *Aquaculture Res*. 27: 573-580.

Wada, S., 1953. Biology of the silver lip pearl *Pinctada maxima* (Jameson). I. Artificial fertilisation and development. *Margarita*, 1: 1-15.

Zacharin, W., 1993. Interim management plan for the clam fishery released. *Fishing Today*, 6(4): 32-33.

5.3.9 TABLES AND FIGURES

Table 1. Numbers of male and female *Katelysia scalarina* induced to spawn by injection of serotonin at different volumes and concentrations.

Spawning Trial	Number of broodstock[1]	Collection site	Injection volume (μL)	Concentration[2] (mM)	Number spawned	
					Male	Female
1	10	Georges Bay	100	10	1	0
1	10	Georges Bay	30	10	2	0
1	10	Ansons Bay	30	10	2	0
1	10	Swanwick	30	10	2	0
2	40	Ansons Bay	30	2	9	0
3	39	Ansons Bay	40	15	6	0

[1] Sexes could not be visually differentiated prior to spawning. Hence Number of broodstock = ∑(male + female).
[2] Range of total amount of serotonin injected was ($6x10^{-8}$ to $1x10^{-6}$ moles).

Table 2. Mean fecundity, and numbers of male and female *Katelysia scalarina* induced to spawn by thermal fluctuations.

Larval Trial	Number of broodstock	Thermal stimulus (°C)	U.V. light	Number spawned		Number of eggs ($x10^6$)	Mean Fecundity (eggs $x10^6$.clam^{-1})
				Male	Female		
1	no data[1]	20	No	no data[1]	no data[1]	4.5	no data[1]
2	186	24-27	Yes	50	62	150	2.4
3	98	20-26	Yes	40	29	20	0.7
4	230	20-26	Yes	22	26	26	1.0

[1] Fertilised eggs collected following inadvertent overnight spawning of captive broodstock.

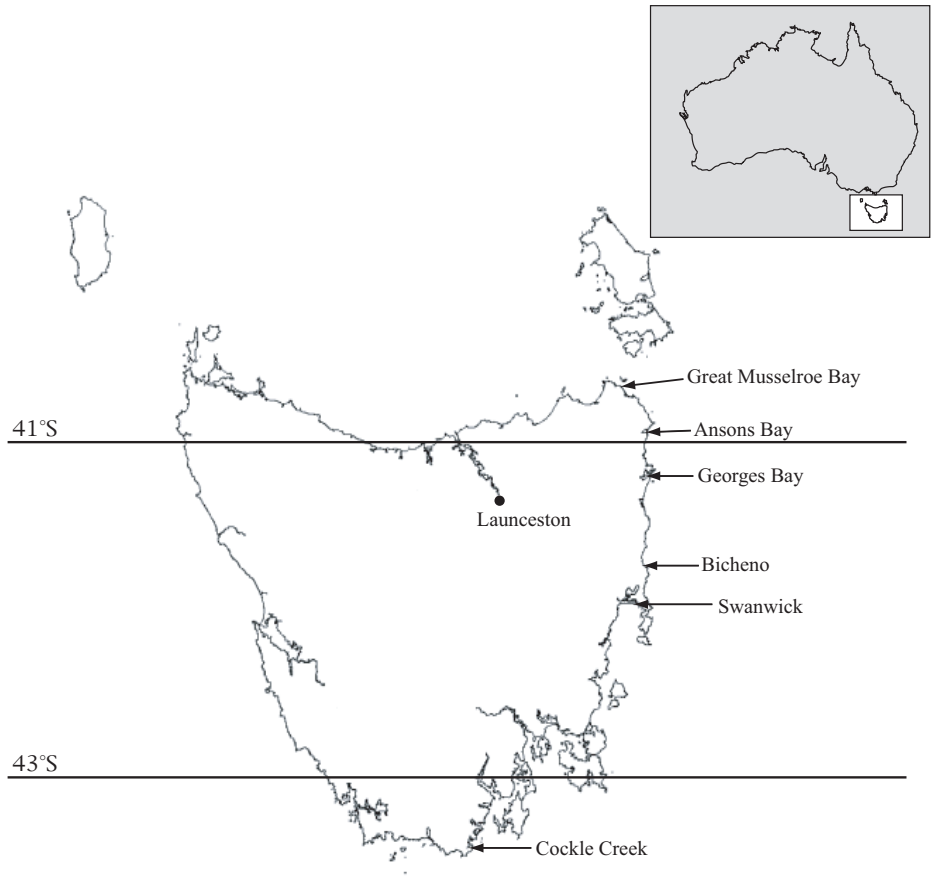

Figure 1. Collection and larval rearing sites within Tasmania, Australia for the clam *Katelysia scalarina.*

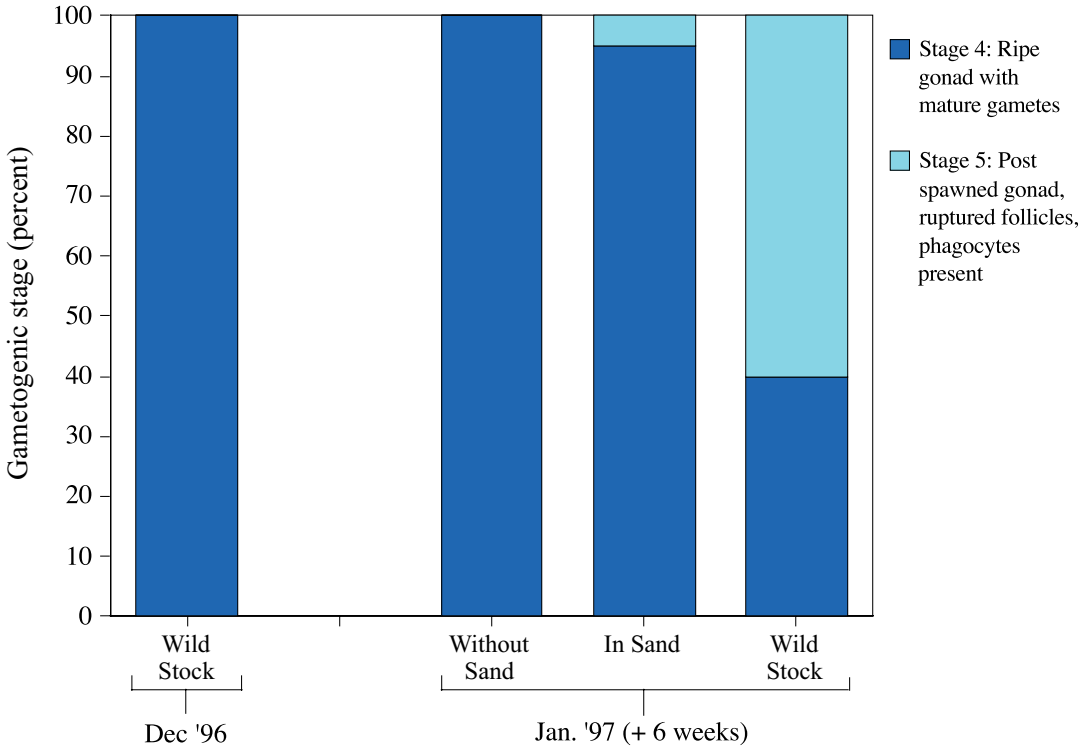

Figure 2. Gonad development in wild and captive *Katelysia scalarina* held for six weeks in a recirculating conditioning system, with and without substrate. n = 10.

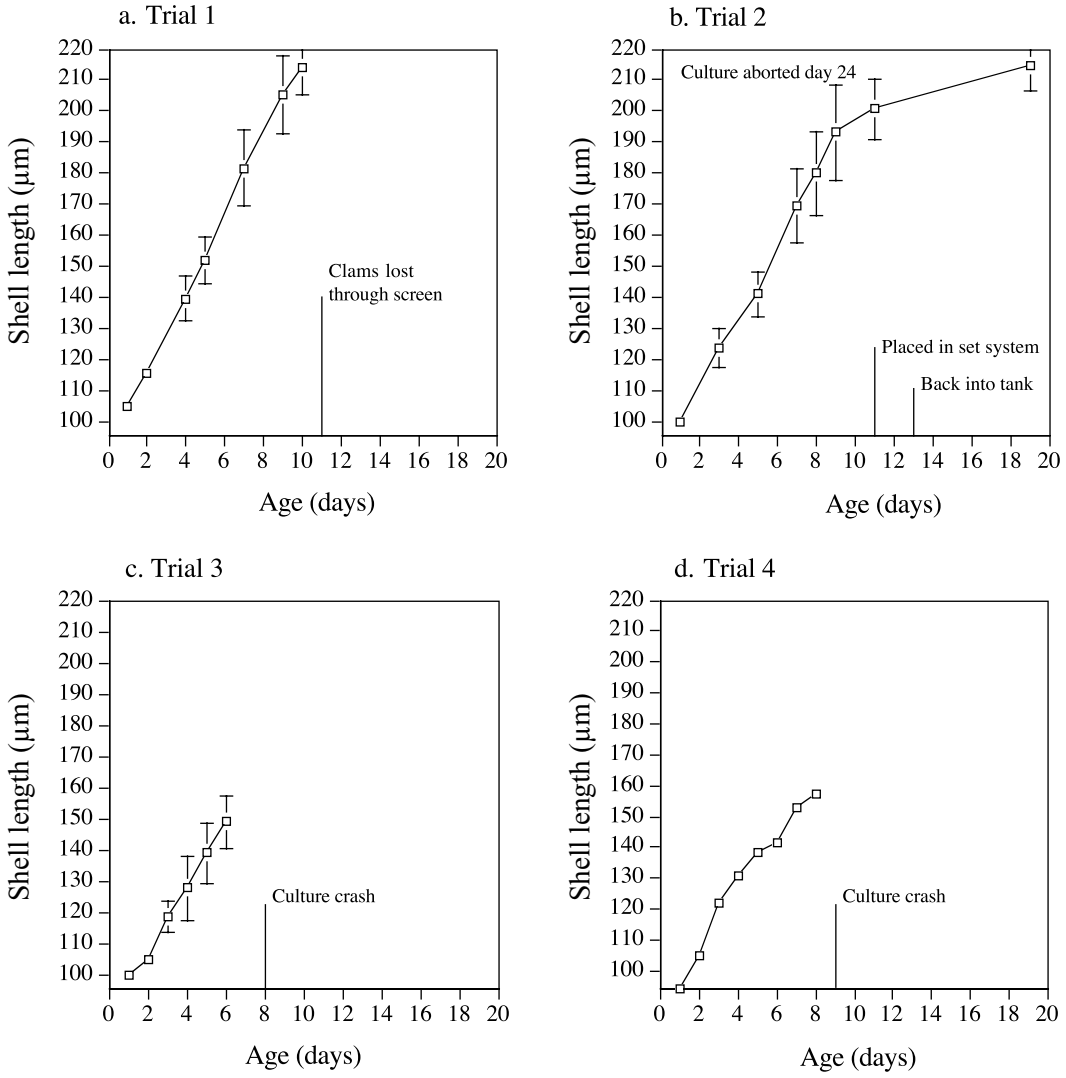

Figure 3. Results of four larval rearing trials of *Katelysia scalarina*, conducted at Bicheno during summer 1993. Points are mean ± s.d. n = 20-38. a. and b. indicate rapid development (Trials 1 and 2), whilst c. and d. indicate characteristic larval crashes around days 6 - 8 (Trials 3 - 4).

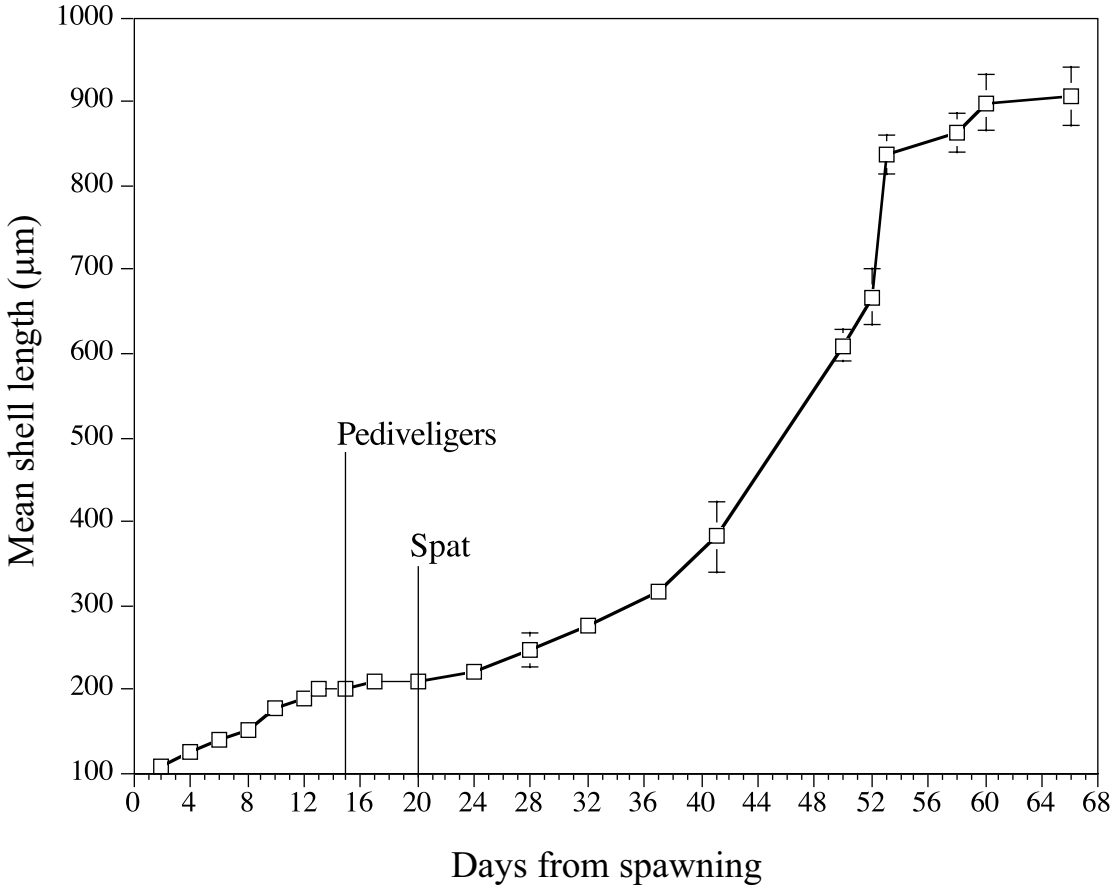

Figure 4. Growth (expressed as shell length) of *Katelysia scalarina* reared at UTAS, Launceston (Trial 5) during February - April 1996. Points are mean ± s.e. (n = 5-20).

5.4 MANUSCRIPT 4

Spawning, settlement and growth of the New Zealand venerid *Ruditapes largillierti* (Philippi 1849) in culture

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5.4.1 ABSTRACT

Spawning, larval rearing, and growout of *Ruditapes largillierti* were investigated in a series of trials conducted at the University of Tasmania (UTAS), Launceston and Georges Bay, St Helens, Tasmania, Australia. Intramuscular injection of serotonin (3 x 10^{-7} to 1.5 x 10^{-6} moles) failed to induce spawning in female *R. largillierti*, although some males did spawn. Fecundity of *R. largillierti* induced to spawn by thermal stimulus ranged from 0.5 - 0.9 x 10^6 eggs.female^{-1}. Fertilised eggs developed into trochophore larvae by 24 h at 20°C and D veligers with a mean shell length of 85.3 ± 4.7 µm within 48 h. Early larvae were frequently deformed and their mortality rates were very high. Development to pediveliger stage (mean shell length 200.3 ± 7.3 µm) took between 11 and 16 days at 20°C, and metamorphosis to spat (mean shell length 240 µm) occurred between Days 16 and 19. There was no significant difference in efficacy of epinephrine, nor-epinephrine or untreated groups for inducing larval settlement. Average growth of juveniles held subtidally within trays or baskets (mesh size 1.7 -12.0 mm) was 1.3 mm.month^{-1}. The aquaculture potential of this subtidal venerid clam warrants further investigation.

Keywords clam; venerid; *Venerupis*; *Ruditapes*; *largillierti*; spawning; larval rearing; survival; growth

5.4.2 INTRODUCTION

Clam aquaculture is a significant industry in many countries and accounted for 29% by value and 26% by weight of world molluscan aquaculture production in 1995 (FAO/FIDI 1997). In Australia, however, commercial interest in clams has been largely restricted to a relatively small fishery, although, the aquaculture potential of several venerid species is being evaluated (Nell et al. 1994, 1995; Patterson & Nell 1997; Kent et al. 1998). The major venerid species of interest in Tasmania for fisheries and aquaculture research are the stepped venerid *Katelysia scalarina* (Lamark (1818) and the New Zealand venerid, *Ruditapes largillierti* (Philippi 1849) (Treadwell et al. 1992; Kent et al. 1998). A lack of information on appropriate hatchery and growout techniques for local species has been identified as an impediment to establishment of commercial clam culture industries (Nell et al. 1994). A major purpose of this work was to assess the suitability of current bivalve hatchery protocols for aquaculture production of *R. largillierti* and ascertain the performance of hatchery-produced juveniles in the wild.

Ruditapes (= Venerupis) largillierti is endemic to New Zealand but its range has extended to Tasmania (although not mainland Australia) during this century where it remains indistinguishable from New Zealand populations, on the basis of allozyme analysis (see Manuscript 16). *R. largillierti* grows to a length of 70 mm and a height of 50 mm and is found subtidally in both muddy and sandy substrates in shallow estuarine waters with high current flow (Cook 1997). It occurs sympatrically in Tasmania with native *Katelysia scalarina*, the latter occurring in the more intertidal or shallow subtidal zone. In Georges Bay, the site of the only commercial

fishery for this species in Tasmania, *R. largillierti* can be collected by superficial digging with a pitch fork in heavily reduced subtidal shell beds. It is not exploited commercially in New Zealand (R. Creese pers. comm.), although its potential is recognised (Gribben 1998). Adult *R. largillierti* can be found in an advanced state of gametogenesis through most of the year in Tasmania (see Manuscript 2), although the largest numbers of ripe clams have been observed to occur between late winter and early spring (Paturusi 1994). In response to the heterogeneous gonad maturation of individual *R. largillierti* and to difficulties encountered in spawning other clam species (Kent et al. 1998), several methods to artificially induce spawning were examined as part of this study.

5.4.3 MATERIALS AND METHODS

Spawning

All spawning and larval rearing trials with the exception of one trial to produce clams for growout, were conducted at the School of Aquaculture, University of Tasmania (UTAS) facilities at Launceston Tasmania. One trial on the production of clam seed for growout was done at Geordy River Aquaculture, a commercial bivalve hatchery at Georges Bay, St. Helens, Tasmania (Fig. 1). Broodstock for all spawning trials were collected from Georges Bay. Before spawning, clams were held out of water for 12 h (Manzi & Castagna 1989) typically at c. 14°C. Spawnings were conducted in a dark flat fibreglass trough (100 x 600 x 900 mm) filled with recirculating filtered seawater. Clams were held corporately in the trough and ripe clams were encouraged to spawn by cycling water temperatures (Loosanoff & Davis 1963) and the addition of stripped gametes (Castagna & Kraeuter 1981; Utting & Spencer 1991). In some spawning events a UV light was also included in the recirculating system to help maintain low bacterial levels and stimulate spawning (Bourne et al. 1989). Spawning clams were removed from the tray and placed in individual 1 litre beakers with filtered seawater (FSW) (Utting & Spencer 1991) or allowed to spawn out collectively in the tray (Table 1).

Clams which did not spawn after temperature cycling (13 - 20°C, at 30 min intervals), had a small notch filed in the valve margin adjacent to the anterior adductor muscle and injected with various concentrations and volumes of serotonin (5-HT) (Table 2) as described by Gibbons & Castagna (1984, 1985), Heasman et al. (1994), and O'Connor & Heasman (1995), and placed in 1 µm FSW at 20°C.

Larval rearing (UTAS)

All sea water (FSW) for spawning and larval rearing was 1 µm filtered (cartridge). Disodium ethylenediaminetetra-acetic acid (Na_2EDTA) was added to water at 1 $mg.L^{-1}$ during egg incubation (Day 0), as per Utting & Helm (1985). Fertilised eggs were stocked at between 10 - 36 $eggs.mL^{-1}$, and larval densities ranged from 1 to 5 $larvae.mL^{-1}$. Larvae were cultured in 300 - litre conical bottom, and 1000 - litre flat bottom fibreglass tanks. For all cultures, larvae were retained on submerged nylon mesh screens, of appropriate mesh size, before being transferred to a clean tank every second day. Rearing temperatures were maintained at 20°C ± 2°C after preliminary trials at temperatures of c. 25°C (which is commonly used for rearing larvae of the Pacific oyster, *Crassostrea gigas*)resulted in high mortality and large numbers of deformed D larvae by 48 h. Larvae were fed a 1:1 (by cell number) mixture of *Pavlova lutheri* (Droop) and *Isochrysis galbana* (Tahitian strain) at c. 10 000 cells/larvae (Toba et al. 1992)

Induction of metamorphosis

In June 1996, the efficacy of the catecholamines, epinephrine and nor-epinephrine in inducing metamorphosis ("set") was assessed using Day 16 pediveligers (PVs) with a mean shell length

of 178 μm. The experimental design consisted of triplicate 1 - litre plastic beakers at 20°C for each treatment. FSW (1 μm) in the beakers contained $10^{-3}M$ epinephrine, or $10^{-3}M$ arterenol (norepinephrine), both predissolved in a small volume of 0.05*N* HCl (Coon et al. 1986), or no catecholamine (controls). 11 x 10^3 PVs were added to each of the nine beakers and gently aerated. Treatments and controls were screened, rinsed and replaced with FSW 18 h after initiation, and thereafter daily. Counts were made of competent larvae and metamorphosed animals, as defined by Coon et al. (1985) 3 and 6 days post treatment (Day 19 and 22 respectively). Separate, single fixed factor ANOVAs were conducted on Day 19 and Day 22 percentage settlement data in relation to catecholamine treatments after arcsin square root transformation and confirmation of homogeneity of variance with Cochran's test (Sokal & Rohlf 1995).

Growout

In April 1995, 72 clams were spawned by elevating water temperature from ambient (13°C) to 16°C at a commercial bivalve hatchery at St Helens, resulting in 18 x 10^6 eggs. Eggs were stocked in 2000 - litre flat bottom fibreglass tanks at 9 eggs mlitre^{-1}. Resultant larvae were reared at 19-20°C and fed a combination of *P. lutheri*, *I. galbana*, and *Chaetoceros calcitrans* (Paulsen) (equal cell numbers) at 25 000-30 000 cells mlitre^{-1} (equivalent to 5 000 cells larvae^{-1} at Day 2 to 30 000 cells larvae^{-1} at Day 9 through to settlement). As a result of mortality, larval densities decreased from 2.5 to 0.45 larvae mlitre^{-1} between Day 2 and Day 18 at which time survivors were placed into a 2,000 - litre settlement tank as PVs at 190-220 μm shell length. The tank contained lengths of curved Poly Vinyl Chloride (PVC) "slats" (see Holliday 1996) previously used for oyster settlement. Slats were conditioned in sea water with clam broodstock for 1 week before settlement. Spat were subsequently placed into outdoor tanks at ambient temperature (13°C) and then reared in oyster trays with a 1.7 mm mesh, without slats, in an intertidal nursery pond. At Day 182, clams were placed in 1.7 mm plastic mesh trays and transported out into the bay. Trays were located subtidally and manually worked into the substrate to encourage some sediment to be maintained in the enclosures. Trays were held directly on the substrate by means of wooden stakes. Clams were progressively moved to 3 and 6 mm mesh trays and 6 mm baskets (same methods as for 1.7 mm trays) and were periodically sampled to assess growth and survivorship. At Day 618 the clams were moved to a more sheltered site and transferred to 12 mm baskets into which surrounding substrate was placed to a depth of c. 60 mm (Fig. 2).

5.4.4 RESULTS

Ripe *R. largillierti* were induced to spawn by thermal stimulus (6-10°C above ambient). Fertilised eggs were 63.8 ± 2.5 μm in diameter (mean ± SD), with mean fecundity ranging from 0.5 to 0.9 x 10^6 eggs female^{-1} (Table 1).

Intramuscular injections of serotonin, at various doses and concentrations, failed to induce spawning in female clams, although some males did spawn (Table 2). Male and female clams that were not injected did not spawn

Fertilised eggs developed into trochophore larvae by 24 h at 20°C and D veligers with a mean shell length of 85.3 ± 4.7 μm within 48 h (Fig. 3). Deformed larvae with fouled velums or unusual shell shape were observed in nearly all larval trials. In the 1995 larval trial at Geordy River Aquaculture about half of 2 day old larvae were identified as deformed. Development to pediveliger stage (mean shell length 200.3 ± 7.3 μm) took between 11 and 16 days at 20°C, and metamorphosis to spat (mean shell length 240 μm) occurred between Days 16 and 19 (Fig. 3).

Settlement in treatments with epinephrine, norepinephrine or no catecholamines did not differ significantly after 19 or 22 days (P>0.05). However, the percentage of settled *R. largillierti* significantly increased over time and by Day 22 exceeded 75 % (Fig. 4).

In all larval trials, survival to set was low, falling to <3% before metamorphosis (Fig. 5) with high mortality occurring during early D veliger development (Days 2-4). These results agree with observations of deformities and mutations in a large percentage of newly developing larvae. In larval trials conducted at UTAS, survival through settlement was also compromised by the presence of a marine ciliate (resembling *Uronema nigricans*, see Munday et al. 1997) which were observed invading larvae.

Growth of hatchery-produced clam seed raised in trays and baskets on the substrate was modest (32 mm shell length in 28 months from spawning, an average of 1.3 mm month^{-1} over the growout period) (Fig. 2). This was slower than for *R. largillierti* seed, observed following a major natural settlement event, on the adjacent seabed (A. Flintoff pers. comm.).

5.4.5 DISCUSSION

In a list of desirable biological factors to be considered when selecting suitable clam candidates for aquaculture, Kraeuter & Castagna (1989) identified ease of spawning over a substantial period, ease of larval culture, shallow burial depth, rapid growth to market size and tolerance to wide variations in environmental conditions. We have demonstrated that ripe *R. largillierti* can be readily induced to spawn with thermal stimulation. However, mean fecundities of between 0.5 x 10^6 and 0.9 x 10^6 eggs female^{-1} were lower than that for the stepped venerid *Katelysia scalarina* (0.7 x 10^6 - 2.4 x 10^6 eggs female^{-1}) (Kent et al. unpubl. data) and the Manila clam *Ruditapes philippinarum* (0.9 x 10^6 - 2.7 x 10^6 eggs female^{-1}) (Laing & Lopez-Alvarado 1994) and 1.5 x 10^6 eggs female^{-1} (Utting et al. 1996). Further, the survival of larvae to Day 5 of between 2 and 10 % (Fig. 5), was considerably lower than Day 5 survival of 30 - 50% for *K. scalarina* cultured under similar conditions (Kent et al. unpubl. data). In addition, whereas ripe *R. largillierti* could be spawned using thermal stimulation, moderately developed broodstock (++ as defined by Garland et al. 1993) failed to spawn after repeated exposure to temperature cycling or the addition of gametes. Intramuscular injection of serotonin at various doses into these clams also failed to stimulate spawning in any female stock, although some males did spawn. Many studies have reported ditypic spawning activity of male and female bivalves in response to serotonin injection (Gibbons & Castagna 1985; Ram & Nichols 1993; Monsalvo-Spencer et al. 1997). In contrast, an Australian venerid, *Tapes dorsatus*, can be induced to spawn when females are injected with serotonin (J. Nell pers. comm.). In addition, Martinez et al. (1996) have reported successful spawning induction of both male and female gametes in the scallop *Argopecten purpuratus* by gonadal injection of a combination of serotonin and prostaglandin E_2 (PGE_2) or dopamine and PGE_2. Given the poor survival of the larvae of other clam species produced from stripped eggs (Kent et al. unpubl. data), and the ineffectiveness of serotonin injections on female clam stock, combination injections of serotonin and PGE_2 or dopamine and PGE_2 should be investigated for this species. In addition, Martinez et al. (1996) also demonstrated significantly higher percent fertilisation and survival of D larvae using combination injection of dopamine and PGE_2 as against temperature induced spawning, possibly as a result of only optimum quality eggs being released. These potential benefits may be important for culture of *R. largillierti* , given the heterogeneous gonad development of this species (Gribben 1998; Maguire & Kent unpubl. data) and the poor survival of D larvae we observed.

As a result of gametogenic variation in *R. largillierti*, development of appropriate broodstock conditioning protocols for this species may prove beneficial, as they have for many clam species (Castagna & Manzi 1989; Ruiz-Azcona et al. 1996; Numaguchi 1997) including other venerids (Kent et al. unpubl. data). Aquaculturalists may find broodstock conditioning particularly useful

for *R. largillierti* which appears to only partially spawn in any given event (Paturusi 1994; Gribben 1998; Maguire et al. unpubl. data), thus allowing for year-round supply of hatchery seed.

Average growth of juvenile clams of 1.3 mm month^{-1} over the experimental growout period was comparable to juvenile (12 mm) *R. philippinarum* seeded in sand in Bouin, France (de Valence & Peyre 1990), but lower than that for *K. rhytiphora* (2.6 mm month^{-1}) cultured in 6 mm plastic mesh baskets buried in a medium sand substrate in Port Stephens, Australia (Paterson & Nell 1997). In this study growth of clams from 20 mm shell length (1.0 mm month^{-1}) was also lower than that recorded by Paturusi (1994) for similar sized *R. largillierti* (27.4 mm initial length) of 1.4 mm month^{-1}. Observation of clams from the major natural settlement noted previously suggest that growth of *R. largillierti* in Tasmania can exceed 2 mm month^{-1} (A. Flintoff pers. comm.). In our growout trial, growth was probably compromised by difficulties with maintaining substrate in the trays and baskets plus reduced water flow over clam seed caused by the observed macroalgal fouling of the culture units. Peterson & Black (1993) also reported that growth in cages was significantly slower than that for a fenced stock of *Katelysia* spp., although fenced stock grew better than those in enclosure-free plots. While growth of clams in this study was less than optimum we observed uniform growth rates within samples (Fig. 2). These observations are similar to other growth data for *R. largillierti* (Paturusi 1994), and *K. rhytiphora* and*Tapes dorsatus* (Paterson & Nell 1997) which are also characterised by low sample variance. Presence of shell debris in the spat transferred to 1.7 mm trays within Georges Bay precluded accurate estimate of stock numbers and hence survival during growout. However little mortality has been evident while spat have been in 6-12 mm baskets.

The aquaculture potential of this species warrants further investigation as spat become available from commercial hatcheries. Unfortunately, farming subtidal clams is less convenient than intertidal clams which can be "planted" on beaches under protective netting (Toba et al. 1992). It would be useful to reduce initial larval mortality, and use of serotonin/PGE_2 and further investigation into optimum water temperature for larval rearing (Heasman et al. 1996) maybe worthwhile in this regard. Given the presence of the European shore crab *Carcinus maenus* in Tasmanian waterways (Gardner et al. 1994), some predator protection will probably be required. However alternatives to mesh cages and trays, for example shell beds, should be assessed.

5.4.6 ACKNOWLEDGMENTS

We are grateful to Elizabeth Cox for technical assistance and to Alan Flintoff for supplying broodstock and observations from daily clam fishing activities in St Helens. Marianne Watts kindly provided the ciliate identification.

5.4.7 REFERENCES

Bourne, N.; Hodgson, C. A.; Whyte, J. N. C. 1989: A manual for scallop culture in British Columbia. *Canadian technical report of fisheries and aquatic sciences no. 1694*. Canadian Department of Fisheries (Aquatic Sciences Branch).

Castagna, M.; Kraeuter, J. N. 1981: Manual for growing the hard clam *Mercenaria*. *Special report in Applied Marine Science and Ocean Engineering no. 249*. Gloucester Point, VA, Virginia Institute of Marine Science. 110 p.

Castagna, M.; Manzi, J. J. 1989: Clam culture in North America: hatchery production of nursery stock clams. *In*: Manzi, J. J.; Castagna M. *ed*. Clam mariculture in North America. Developments in Aquaculture and Fisheries Science. Vol 19. Amsterdam, Elsevier. pp. 111-125.

Cook, S. (*ed*.) 1997: Coastal marine invertebrates of New Zealand. Christchurch, New Zealand, Canterbury University Press.

Coon, S. L.; Bonar, D. B.; Weiner, R. M. 1985: Induction of settlement and metamorphosis of the Pacific oyster, *Crassostrea gigas* (Thunberg), by L-DOPA and catecholamines. *Journal of experimental marine biology and ecology 94*: 211-221.

Coon, S. L.; Bonar, D. B.; Weiner, R. M. 1986: Chemical production of cultchless oyster spat using epinephrine and nor epinephrine. *Aquaculture 58*: 255-262.

de Valence, P.; Peyre, R. 1990: Clam culture. *In*: G. Barnabe *ed.* Aquaculture. Vol. 1. New York, Ellis Horwood. Pp. 388-415.

FAO/FIDO 1997: FAO Web site.

Gardner, N. C.; Kwa, S.; Paturusi, A. 1994: First recording of the European shore crab *Carcinus maenas* in Tasmania. *Tasmanian naturalist 116*: 26-28.

Garland, C. D.; O'Brien, D. T.; Fraser, M. I.; Tong, P. E.; Ward, C. 1993: Rapid induction of spawning by Pacific oysters *Crassostrea gigas* in still seawater containing H_2O_2. *Tasmanian fisheries research 28*: 20-27.

Gibbons, M. C.; Castagna, M. 1984: Serotonin as an inducer of spawning in six bivalve species. *Aquaculture 40*: 189-191.

Gibbons, M. C.; Castagna, M. 1985: Responses of the hard clam *Mercenaria mercenaria* (Linne) to induction of spawning by serotonin. *Journal of shellfish research 5*: 65-67.

Gribben, P. 1998: Demography and life history characteristics of the venus clam - *Ruditapes largillierti*. Unpublished MSc thesis, University of Auckland, New Zealand. 147 p.

Heasman, M. P.; O'Connor, W. A.; Frazer, A. W. 1994: Improved hatchery and nursery rearing techniques for *Pecten fumatus* Reeve. *Memoirs of the Queensland Museum 36*: 351-356.

Heasman, M. P.; O'Connor, W. A.; Frazer, A. W. 1996: Ontogenetic changes in the optimal rearing temperatures for the commercial scallop *Pectin fumatus* (Reeve). *Journal of shellfish res. 15*: 627-634.

Holliday, J. E. 1996: Effects of surface orientation and slurry coating on settlement of Sydney rock, *Saccostrea commercialis*, oysters on PVC slats in a hatchery. *Aquaculture engineering 15*: 159-168.

Kent, G. N., Maguire, G. B., John, M., Cropp, M. and Frankish, K., 1998 Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina* (Lamarck 1818). *J. shellfish res.,* 17(4): 1065-1070

Kraeuter, J. N.; Castagna, M. 1989: Factors affecting the growth and survival of clam seed planted in the natural environment. *In*: Manzi J. J.; Castagna, M. *ed*. Clam mariculture in North America. Developments in Aquaculture and Fisheries Science. Vol. 19. Amsterdam, Elsevier. Pp. 149-165.

Laing, I.; Lopez-Alvarado, J. 1994: Effect of dried algae diets on conditioning and fecundity of Manila clam, *Tapes philippinarum* (Adams and Reeve). *Aquaculture and fisheries management 25*: 157-166.

Loosanoff, V. L.; Davis, H. C. 1963: Rearing of bivalve molluscs. *Advances in marine biology 1*: 1-136.

Manzi, J. J.; Castagna, M. 1989: Clam mariculture in North America. Developments in aquaculture and fisheries science. Vol. 19. Amsterdam, Elsevier.

Martinez, G.; Garrote, C.; Mettifogo, L.; Perez, H.; Uribe, E. 1996: Monoamines and prostaglandin E_2 as inducers of the spawning of the scallop, *Argopecten purpuratus* Lamarck. *J. shellfish res., 15*: 245-249.

Monsalvo-Spencer, P.; Maeda-Martinez, A. N.; Reyneso-Granados, T. 1997: Reproductive maturity and spawning induction in the Catarina scallop *Argopecten ventricosus* (=*circularis*) (Sowerby II, 1842). *J. shellfish res., 16*: 67-70.

Munday, B. L.; O'Donoghue, P. J.; Watts, M.; Rough, K.; Hawkesford, T. 1997: Fatal encephalitis due to the sctoicociliate *Uronema nigricans* in sea-caged, southern bluefin tuna *Thunnus maccoyii. Diseases of aquatic organisms 30*: 17-25.

Nell, J. A.; O'Connor, W. A.; Hand, R. E.; McAdam, S. P. 1995: Hatchery production of diploid and triploid clams, *Tapes dorsatus* (Lamarck 1818): A potential new species for aquaculture. *Aquaculture 130*: 389-394.

Nell, J. A.; O' Connor, W. A.; Heasman, M. P.; Goard, L. J. 1994: Hatchery production for the venerid clam *Katelysia rhytiphora* (Lamy) and the Sydney cockle *Anadara trapezia* (Deshayes). *Aquaculture 119*: 149-156.

Numaguchi, K. 1997: A preliminary trial to induce maturity and spawning of the common oriental clam *Meretrix lusoria* out of spawning season. *Journal of the World Aquaculture Society 28*: 118-120.

O'Connor, W. A.; Heasman, M. P. 1995: Spawning induction and fertilisation in the doughboy scallop *Chlamys* (*Mimachlamys*) *asperrima*. *Aquaculture 136*: 117-129.

Paterson, K. J.; Nell, J. A. 1997: Effect of different growing techniques and substrate types on the growth and survival of the clams *Tapes dorsatus* (Lamarck) and *Katelysia rhytiphora* (Lamy). *Aquaculture research 28*: 707-715.

Paturusi, A. 1994: Effects of stocking density and initial size on the performance of the clam *Venerupis largillierti* (Phillipi, 1849) in experimental cages in Georges Bay, Tasmania. Unpublished thesis, University of Tasmania, Launceston, Australia.

Peterson, C. H.; Black, R. 1993: Experimental tests of the advantages and disadvantages of high density for two coexisting cockles in a Southern Ocean lagoon. *Journal of animal ecology 62*: 614-633.

Ram, J. L.; Nichols, S. J. 1993: Chemical regulation of spawning in the zebra mussel (*Dreissena polymorpha*). *In*: Nalepa T. F.; Schloesser D. W. *ed*. Zebra mussels: biology, impacts, and control. Boca Raton, Florida, Lewis Publishers. Pp. 307-314.

Ruiz-Azcona, P.; Rodriguez-Sierra, R.; Martin, J. B. 1996: Culture of coquina clam, *Donax trunculus*, larvae. *Aquaculture 139*: 151-155

Sokal, R. R.; Rohlf, J. F. 1995: Biometry: The principles and practice of statistics in biological research. New York, W. H. Freeman. 887 p.

Toba, D. R.; Thompson, D. S.; Chew, K. K.; Anderson, G. J.; Miller, M. B. 1992: Guide to Manila clam culture in Washington. Washington Sea Grant Program, University of Washington.

Treadwell, R.; McKelvie, L.; Maguire, G. B. 1992: Potential for Australian aquaculture. Australian Bureau of Agricultural and Resource Economics. *Research report 92.2*. Canberra. 81 p.

Utting, S. D.; Helm, M. M. 1985: Improvement of seawater quality by physical and chemical pre-treatment in a bivalve hatchery. *Aquaculture 44*: 133-144.

Utting, S. D.; Millican, P. F.; Laing, I. 1996: The breeding potential and biochemical composition of triploid Manila clams, *Tapes philippinarum* Adams and Reeve. *Aquaculture research 27*: 573-580.

Utting, S. D.; Spencer, B. E. 1991: The hatchery culture of bivalve mollusc larvae and juveniles. Laboratory leaflet no. 68. Ministry of Agriculture, Fisheries and Food, Fisheries Research. Lowestoft, United Kingdom.

5.4.8 TABLES AND FIGURES

Table 1. Mean fecundity, and numbers of male and female *Ruditapes largillierti* induced to spawn collectively by thermal stimulation.

Spawning date	No. of broodstock	Thermal stimulus (°C)	No. spawned		Total no. of eggs (x10^6)	Mean fecundity (eggs x 10^6 $clam^{-1}$)
			Male	Female		
19 May 1994	50	13 - 23 E	15	11	5.2	0.5
6 Feb 1996	54	19 - 25 Cy	5	9	3.6	0.4
3 Jun 1996	67	13 - 20 Cy	25	40	36	0.9
4 Jul 1996	86	14 - 19 Cy	31	55	43.2	0.8

Cy = 30 min cycling, E = single temperature elevation.

Table 2. Numbers of male and female *Ruditapes largillierti* induced to spawn by injection of serotonin at different volumes and concentrations. Range of total amount of serotonin injected was (3 x 10^{-7} to 1.5 x 10^{-6} moles $clam^{-1}$).

Number of broodstock	Injection volume (µl)	Concentration (m*M*)	Number spawned	
			Male	Female
38	0	0	0	0
10	100	15	0	0
25	40	20	2	0
20	100	10	2	0
20	30	10	3	0

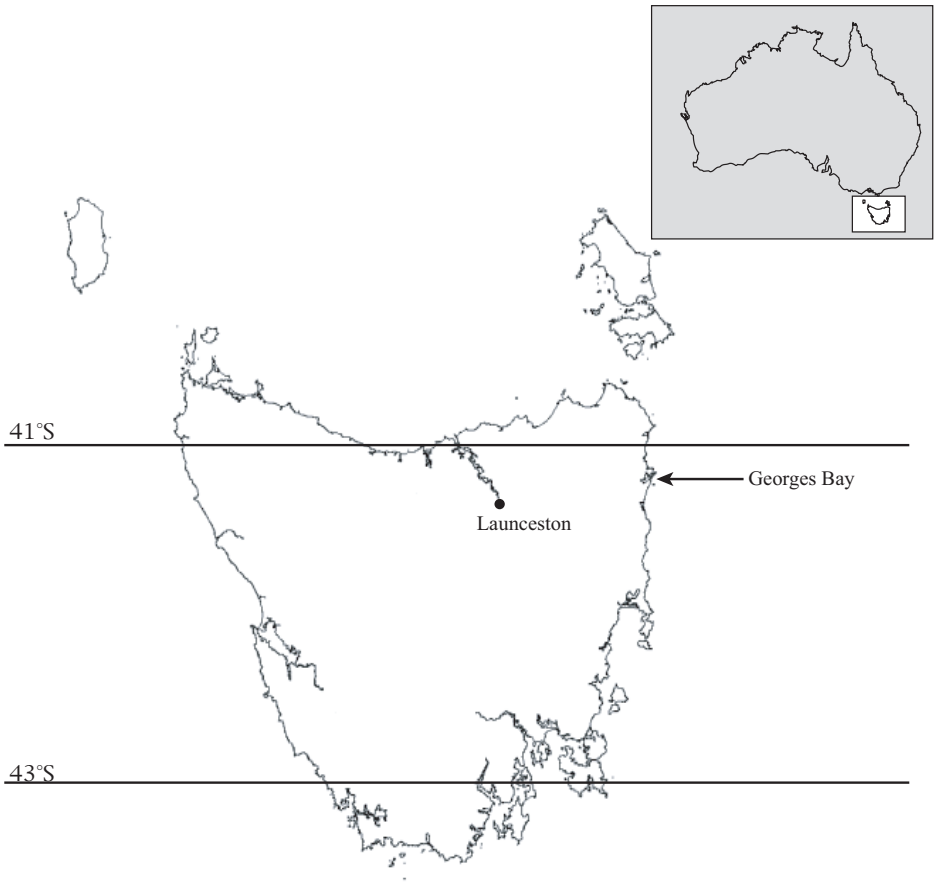

Figure 1. Collection, larval rearing, and growout sites for *Ruditapes largillierti* in Tasmania, Australia.

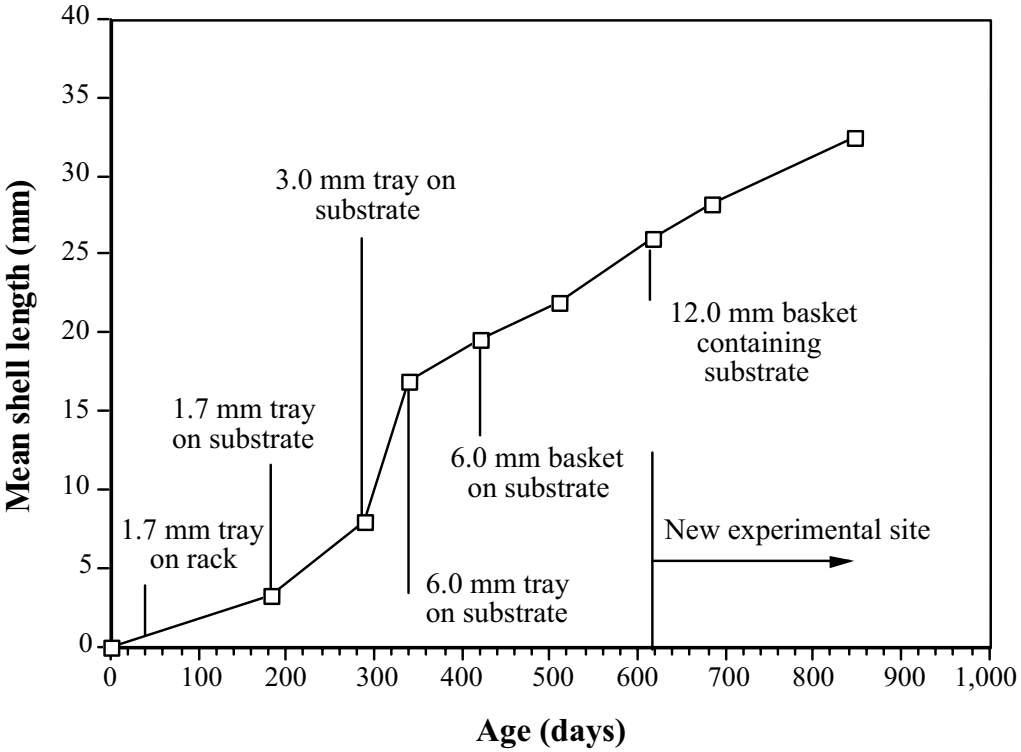

Figure 2. Growth (expressed as shell length) of *Ruditapes largillierti* in Georges Bay, Tasmania, Australia. Points are mean ± SE) (n = 10 - 40). Where not visible, SE range is 0.1-0.8 mm.

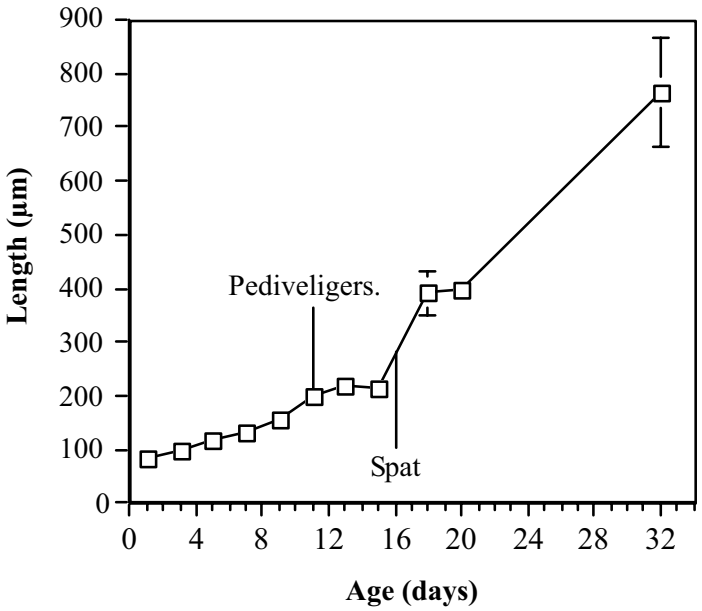

Figure 3. Larval and early juvenile growth (expressed as shell length) of *Ruditapes largillierti.* Points are mean ± SE (*n* = 6 - 37).

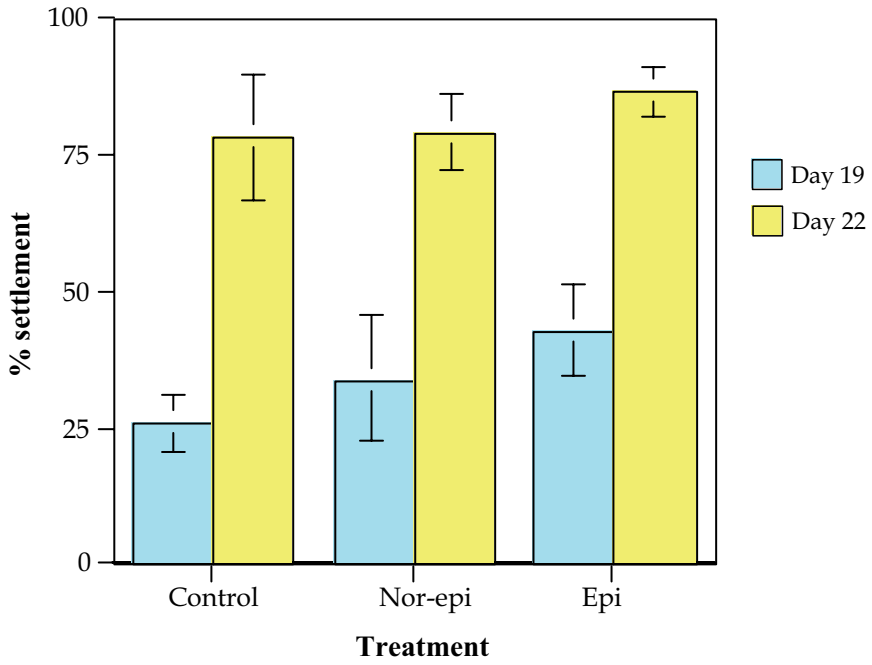

Figure 4. Comparison of efficacy of the catecholamines, epinephrine, and nor-epinephrine in inducing metamorphosis in *Ruditapes largillierti* pediveliger larvae. Error bars ± SE (*n* = 3). (Points sharing a common superscript are not significantly different (P>0.05.)

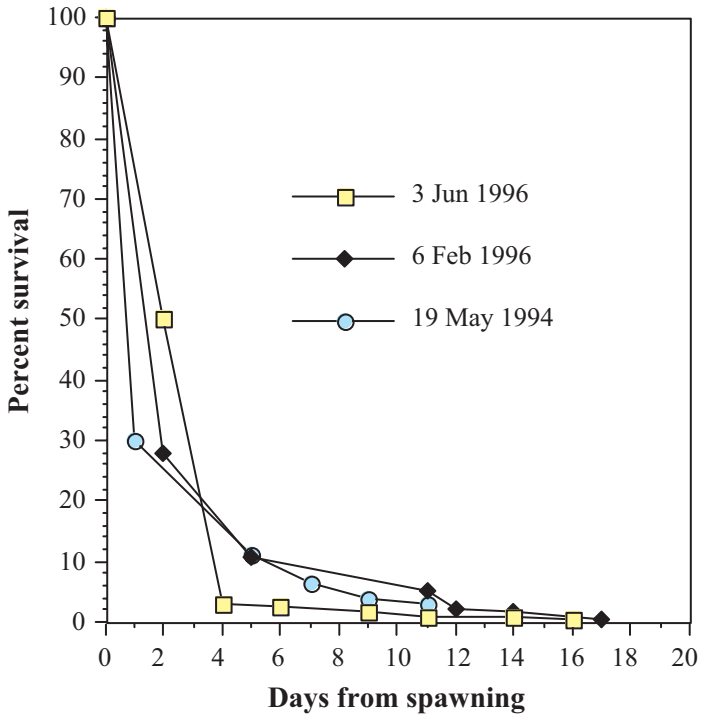

Figure 5. Survival of three batches of *Ruditapes largillierti* larvae to metamorphosis, expressed as a percentage of total number of eggs obtained at spawning.

5.5 MANUSCRIPT 5

The effect of salinity on an estuarine clam; *Katelysia scalarina* (Lamarck, 1818) (Bivalvia: Veneridae). I. Osmo- and ionic regulation

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5.5.1 ABSTRACT

Katelysia scalarina is a small intertidal venerid clam that inhabits fine to medium grain sand, and often comprises the major faunal component of many sheltered bays and estuaries on the east coast of Tasmania as well as being distributed along the southern mainland coastline of Australia. The Tasmanian populations are genetically distinct from these mainland populations (Soh et al., 1998). The bays and estuaries of Tasmania's east coast are prone to large freshwater influxes, subjecting estuarine fauna to unpredictable salinity regimes.

The tolerance of adult (35.0 - 40.0 mm shell length) and juvenile (20.0 - 25.0 mm) *K. scalarina* was investigated using a series of 21 d acute toxicity trials over a salinity range 5-55. Adults were intolerant of low and very high salinities (<25 and 55), while juveniles had a wider salinity tolerance range (5-45). Osmotic analysis of mantle fluid and haemolymph indicated that the two body fluids respond similarly to salinity stress, closely following the external medium at salinities ≥25 and approximating body fluid osmotic pressure at 25 for salinities of 5-25. Despite differences in the salinity tolerance of adults and juveniles, there were no significant differences in their ability to osmoregulate.

Haemolymph levels of Na^+, Cl^-, and Mg^{2+} in adults closely followed the external medium, with haemolymph hyperionic to the external medium at low salinities and slightly hypoionic at high salinities (isionic point in range 25-30). K^+ in adults was constantly hyperionic to the external medium and displayed minimal fluctuations in concentration, indicating some degree of regulation. Calcium and the incidence of valve closure displayed inverse relationships with salinity except at 55. Elevated Ca^{2+} in adults is indicative of shell closure when calcium in the form of $CaCO_3$ is mobilised from the shell to buffer the by - products of anaerobic respiration.

K. scalarina is essentially an osmo- and ionic conformer, particularly at salinities >25, that relies on the mechanism of shell valve closure to isolate the body tissues from unfavourable salinities, for example, 15 and 55. However, the degree to which valve closure totally isolates mantle fluid and haemolymph from the external medium is not clear.

5.5.2 INTRODUCTION

Estuarine organisms exist under the stress of frequent and large variations in ambient salinity, caused by tidal fluctuations, periods of heavy rain and freshwater run-off (Hoyaux *et al*., 1976; Shumway, 1977a; Djangmah *et al.,* 1979; Posey, 1987). Despite the variability within the estuaries in which some bivalve molluscs live, they appear to possess no specialised osmoregulatory organs and most marine molluscs have little, if any, ability for extracellular osmotic regulation (Hoyaux *et al.,* 1976; Burton, 1983).

Under normal salinity conditions most species of molluscs tend to maintain a high degree of stability in their internal ionic composition (Mahasneh & Pora, 1981). The maintenance of optimal ionic composition is necessary for the efficient working of the cell, particularly when environmental salinity is fluctuating. Molluscs vary greatly in their ability to regulate the ions in their internal media; some marine and brackish water species have limited regulatory abilities, while some freshwater species closely regulate ions (Burton, 1983).

Katelysia scalarina is a small (40 mm shell length), intertidal suspension filter feeder found in sheltered bays and estuaries along the southern coast of Australia (Wells & Roberts, 1980; Coleman, 1982; Roberts, 1983). *K. scalarina* inhabits fine to medium grain sand approximately 2-4 cm below the surface and comprises the dominant macrofaunal component of many bays and estuaries on Tasmania's east coast (Bellchambers and Richardson, 1995). Because they are typically sheltered and often partially closed to the sea by bayhead spits, these areas are prone to periods of reduced flushing and large, sporadic fresh water influxes from periods of high rainfall and freshwater runoff. The region's annual rainfall does not display consistent seasonality in distribution (Figure 1A) and tends, on a monthly basis to fall in intense bursts, followed by long dry spells (Figure 1B). These large scale salinity fluxes represent a major environmental stress for the estuarine fauna not only in terms of survival but also in relation to other physiological processes. *K. scalarina* occurs sympatrically with a closely related species *Katelysia rhytiphora* for which salinity tolerance data exist (Nell and Patterson, 1997). However, *K. scalarina* tends to occur higher in the intertidal zone (Nielsen, 1963).

A small commercial fishery for *K. scalarina* exists in several of the larger bays and estuaries of Tasmania's east coast. Established in 1987, the fishery now has an annual value of approximately $Aus 200, 000 depending on market value (DPIF - Sea Fisheries pers. comm). Little is known of the ability of *K. scalarina* to tolerate massive freshwater influxes, however observations suggest that large scale mortality occurs periodically (Bellchambers pers. obs.). The same phenomenon has been reported by numerous authors for *Crassostrea virginica* (see Shumway, 1996). Laboratory experiments were conducted to investigate the effect of hypo- and

hypersaline conditions on the survival, osmoregulation and ionic concentration of *K. scalarina*. The ability of *K. scalarina* to survive large scale salinity fluxes is a critical factor in determining the position and success of any marine farming venture. Knowledge of the salinity tolerance range of *K. scalarina* may also provide an explanation for distribution and abundance of natural populations.

5.5.3 METHODS

Salinity tolerance trials

Adult (35.0-40.0 mm shell length) and juvenile (20.0-25.0 mm shell length) *K. scalarina* were collected from Moulting Lagoon, Coles Bay (42°05'S, 148°10'E) (Figure 2) and transported to Hobart out of water in shaded, moist plastic containers. Experimental treatments commenced the day after collection. Clams were transferred directly from seawater (35) to one of the experimental salinities (5-55 with 5 increments). Four aerated plastic aquaria (3 L) were used for each salinity, each containing twelve clams. Approximately 4 cm of washed sieved sand was placed on the bottom of each aquarium to allow clams to bury. Salinity treatments in all trials were established by adding distilled water or an artificial salt mixture (Coral Reef Red Sea Salt ®) to sand-filtered seawater with a salinity of 35. Water was exchanged every two days and clams were not fed during the experimental period. Temperature was maintained at 15°C throughout the experimental period. Salinities were measured daily using a Wissenschaftlich-Technische-Werksttaten conductivity meter, calibrated using standard saline solution (Ocean Scientific International® K_{15} =0.99982; Salinity=34.993). Dead bivalves were removed and mortalities recorded daily until day 21, the conclusion of the experimental period. Animals were considered dead when gaping widely, or when the foot or siphons failed to contract in response to mechanical stimulation

Osmotic Concentration of Body Fluids

Mantle fluid and haemolymph samples were collected from the salinity tolerance trials after 48 h, as this is the period over which bivalve molluscs adapt to variations in external salinity (Pierce, 1971). Samples were obtained by blotting the shell dry, prising the valves open and inserting a hypodermic syringe. Approximately 50 µL of mantle fluid was obtained. Haemolymph samples for osmotic analyses (adults and juveniles) and ionic analyses (adults only) were obtained by inserting a syringe into the pericardial cavity. Samples were then pooled to provide sufficient volume for analysis (50 µL, 5 clams).

Fluid samples for osmolarity determination were centrifuged, the supernatant removed and stored frozen in capped vials until the end of the experimental period. Samples can be stored for at least a week without a change in osmolarity (Nell & Dunkley, 1984). The osmolarity of body fluids and seawater was determined using a vapour pressure osmometer (Wescor 5100B) with an accuracy of ± 0.1 mOsm.

Ionic Concentration of Body Fluids

The quantities of Na^+, Cl^-, Mg^{2+}, Ca^{2+} and K^+ in the haemolymph were analysed using a Kodak Ektachem 250 Analyser.

Shell Closure

Adult (35.0 - 40.0 mm) and juvenile (20.0 - 25.0 mm) *K. scalarina* were collected from Moulting Lagoon, Coles Bay and transported as above to Hobart. Experiments commenced three days after collection and no mortality occur during transportation or prior to the experimental period.

Clams were transferred directly from seawater (35) to one of the experimental salinities. Duplicate aerated plastic aquaria (2 L) were used for each salinity (15, 25, 35, 45 & 55). Each aquarium contained ten clams with approximately 4 cm of 500 µm sieved sand.

Filtering activity was recorded at 30 minute intervals in 4 hour blocks every 12 hours for a total of 48 hours from commencement of the experiment. In each aquarium the number of clams with siphons protruding from between the shell valves was counted to give an indication of shell closure in different salinities.

Data from all observation periods were averaged to eradicate discrepancies between observation periods and the results expressed as mean percentage shell closure. Data were arcsin $x^{0.5}$ transformed to meet assumptions of normality and homogeneity of variance (Bartlett's test)and analysed using one way ANOVA in SYSTAT for Macintosh. Means were compared using Tukey's HSD test (Sokal & Rohlf, 1981).

5.5.4 RESULTS

Salinity tolerance trials

Adult *K. scalarina* displayed a salinity tolerance range of 25-50 (Figure 3A). Salinities outside this range were lethal by day 21 of the experimental period. There was no obvious difference in the survival of clams within the salinity tolerance range, however 100% survival on day 21 was evident only in the 35-40 treatments. In contrast, juvenile *K. scalarina* displayed a wide salinity tolerance (5-45) with substantial mortality occurring only in the 50-55 treatments (Figure 3B). However 100% survival was evident only in the 30-35 treatments.

Probit analysis was used to calculate LT 50 values from the survival data, using PROBIT module in BIOSTAT I (Pimentel & Smith, 1990). LT 50 data indicate that adult *K. scalarina* survive approximately 10 days at low salinities ($\leq$15) (Table 1). At salinities greater than seawater (35) survival times were longer, with adult and juvenile *K. scalarina* in high salinities (55) surviving 14 and 15 days respectively. Due to the high survival of juveniles in salinities below 50, LT 50 values could not be calculated.

Osmotic concentration of body fluids

The osmotic concentration of adult mantle fluid and haemolymph increased in higher salinity treatments (>25) and approximated body fluid osmotic pressure at 25 for salinities of 5-25 (Figure 4A). At salinities of 25-55 the body fluids were isosmotic or slightly hypo-osmotic to the external medium. However, at low salinities the concentrations of haemolymph and mantle fluid were hyperosmotic to seawater (5-20). Differences in the osmotic concentration of haemolymph and mantle fluid were evident at low salinities (5-30), with the haemolymph maintaining osmotic concentrations similar to normal seawater.

There was no major difference between the osmotic concentration of adult and juvenile haemolymph (Figure 4B) although at most salinities juveniles exhibited slightly higher haemolymph osmolarity.

Ionic Concentration

Na^+, (Figure 5A), Cl^- (Figure 5B) and Mg^{2+} (Figure 5C) concentrations in the haemolymph of adults displayed similar patterns. The concentration of the ions increased with salinity from 30. At low salinities haemolymph composition was relatively constant and was hyperionic to the external medium (5-25), and at high salinities slightly hypoionic (40-55) (isionic point in range 25-30).

Ca^{2+} (Figure 5D) differed in that it was the only ion to display an inverse relationship with salinity. As salinity increased Ca^{2+} concentration displayed a corresponding decrease. Ca^{2+} was initially high at low salinities (5-25), decreased markedly at salinities approaching normal seawater and increased again at 50.

In contrast to the other ions, K^+ (Figure 5E) varied little over a wide salinity range and was always hyperionic to the external medium.

Shell Closure

Salinity had a significant effect on the shell closure of both adult (P = 0.003) and juvenile *K. scalarina* (P = 0.001). Shell closure of adult and juvenile *K. scalarina* was highest in salinities outside the salinity tolerance range (15 & 55) (Figure 6). Clams in 25 also displayed a high percentage of shell closure (adults, 88%; juveniles, 76%) although this salinity is within the established salinity tolerance range. However, a lower percentage of juveniles were closed in 25 treatments. Overall juveniles were more active than adults and were less likely to have their valves closed during the observation periods.

5.5.5 DISCUSSION

Salinity Tolerance

Adult *K. scalarina* displayed a salinity tolerance range of 25-50 and treatments outside this range resulted in significant mortality over 21 d at 15°C. For juvenile substantial mortality was only evident at salinities of 50-55 under these conditions. This compares well with the reported salinity tolerance range of juvenile *K. rhytiphora*, a closely related species (Soh et al., 1998), of 20-45 over 9 d at 25°C with substantial mortality evident at a salinity of 20 (Nell & Patterson, 1997). The greater salinity tolerance of *K. scalarina* may reflect its tendency to colonise areas higher in the tidal range than *K. rhytiphora*. Adult *K. scalarina* have the wide salinity tolerance range typical of many estuarine bivalves which are subjected to a large tidal range, but are generally intolerant of low salinity regimes. Juvenile *K. scalarina* displayed a wider salinity tolerance range, with significant mortality occurring only in 50-55. Previous authors have suggested that different stages of a clam's life cycle may display different tolerances and optima (see Malouf and Bricelj, 1989). However, reported accounts are primarily a comparison of early embryos, larvae and adults (Kennedy *et al.,* 1974), rather than adults and juveniles as in this study. The differences observed here may in part reflect habitat differences of the two stages. Juveniles are frequently found higher on the shore than adults at Moulting Lagoon and are therefore exposed to greater variation in environmental conditions (Bellchambers, 1993). The different survival time of adults and juveniles may also be due to differences in their ability to isolate the body tissues or withstand the buildup of excretory products. However, results from shell closure experiments indicate that, in comparison to adults, juvenile *K. scalarina* display lower rates of closure. Therefore the exact mechanism which allows juveniles to survive extremes in salinity for longer periods remains unknown.

Adult *K. scalarina* exposed to low salinities (5-15) survived for approximately 10 days, while low salinities appeared to have little effect on the mortality of juveniles. Nossier (1986) showed that adult *Cerastoderma edule* and *C. glaucum* exposed to a comparably reduced salinity (from 32 - 5) survived for six days, while *K. rhytiphora* spat survived at low salinities (10-15) for only 3 days (Nell and Patterson, 1997). In contrast, the effect of hypersaline conditions on most molluscs is not well known (Singnoret-Brailovsky *et al*., 1996). Both adult and juvenile *K. scalarina* exposed to high salinities (55) survived for 14 and 15 days respectively. Nell

& Gibbs (1986) reported survival times for five bivalve species, exposed to both hyper- and hyposaline conditions, ranging from 2 to 11 days depending on the species' natural habitat. *Pecten fumatus*, a deep water scallop, survived 2 days in salinities outside its tolerance range, while *Anadara trapezia*, a cockle that inhabits seagrass beds and is prone to periodic aerial exposure, survived 11 days. In comparison, *K. rhytiphora* exposed to high salinity (50) showed greater than 50% mortality on day 5, with a gradual increase until day 9 (Nell and Patterson, 1997).

Osmotic Concentration of Body Fluids

There were no obvious differences in the osmotic concentration of haemolymph and mantle fluid of *K. scalarina* at high salinities, however at low salinities differences were evident. Under hyposaline conditions the haemolymph is hyperosmotic to mantle fluid and reflects the osmotic concentration of normal seawater. Clams were transferred directly from 35 to the experimental treatments, therefore differences in the two fluids may be due to shell valve closure. As reported by other authors, the mantle fluid appears to buffer the internal body fluids from unfavourable salinities (Hoyaux *et al.,* 1976).

Despite the differences in their salinity tolerance ranges, the haemolymph of adults and juveniles displayed no major difference in osmolarity. Both adults and juveniles were hyperosmotic to the external medium in salinities less than 25, probably due to shell closure, isosmotic between 25-45 and hypo-osmotic at 55. In comparison, *Saccostrea glomerata* (Sydney rock oyster) held at salinities outside their tolerance range display a similar osmolarity pattern, hyperosmotic at 5 and hypo-osmotic and dying at 55 (Nell & Dunkley, 1984).

Nell & Gibbs (1986) conducted salinity tolerance trials on five species of bivalves, ranging from an oceanic scallop (*Pecten fumatus*) to an intertidal cockle (*Anadara trapezia*), and their results suggest that the bivalves were unable to remain totally inactive and keep the valves tightly closed. The animals were as active as necessary to provide minimal water exchange, thereby lowering their tissue fluid osmolarity and water balance levels until they became critical and the animals died. Bivalves, especially those from intertidal habitats, need not exchange water over short periods of time to maintain oxygen levels in their tissue fluids. Instead they can close the shell valves and revert to anaerobic metabolism (Bayne *et al*., 1976; De Zwaan, 1983). However, water exchange is required to reduce the concentration of excretory products such as ammonia in the tissue fluids. This buildup of excretory products may explain the higher mortality of *K. scalarina* at salinities less than 25 when the valves remained closed for an extended period particularly adults which displayed increased rates of shell closure.

Ionic Concentration of Body Fluids

There is no evidence of ionic regulation of Na^+ or Cl^- in *K. scalarina*. Ion concentrations displayed the typical marine bivalve trend, and the concentrations of Na^+ and Cl^- in the haemolymph and seawater were almost equal above 30. At salinities below 30 the concentrations of Na^+ and Cl^- were hyperionic to the external medium as are the concentrations at 55, which can be attributed to shell closure. Similar patterns for Na^+ have been reported for *Mytilus edulis* and *Crassostrea gigas* (Shumway, 1977a).

In this study, Mg^{2+} in the haemolymph was hyperionic to the external medium at low salinities, but remains at a similar concentration to normal seawater indicating shell closure. The haemolymph was isoionic at normal salinities and hypoionic at high salinities. *Mytilus galloprovincialis* displays a similar pattern of haemolymph Mg^{2+} (Mahasneh and Pora, 1981). It

has been suggested that Mg^{2+} plays a protective role similar to Ca^{2+}, in that it acts as a buffering agent (Burton, 1983). Mg^{2+} can be synergistic with Ca^{2+} in antagonising K^{+} (Burton, 1983).

Ca^{2+} appears to behave in a more regulated way than the other ions, and was the only ion that displayed an inverse relationship with salinity. Mahasneh and Pora (1981) reported a similar pattern of calcium regulation in *Mytilus galloprovincialis* and suggested the trend may be due to the role of calcium in the composition of cell membrane structure. Shell closure may be another explanation for the patterns in Ca^{2+} regulation observed. The initial response of many bivalves to sudden change in ambient osmolarity is adduction of the valves (Deaton, 1992). If prolonged, decreased ventilation will result in the activation of facultative anaerobic pathways and the accumulation of organic acids in the tissues (De Zwaan, 1983). In the clam *Scrobicularia plana,* these acidic products are buffered by the mobilisation of Ca^{2+} as $CaCO_3$ from the shell (Akberali *et al*., 1977). The hyperionic regulation of Ca^{2+} in low salinities supports the hypothesis that to avoid unfavourable salinities *K. scalarina* relies on the behavioural mechanism of shell valve closure.

The concentration of K^{+} in the haemolymph of *K. scalarina* was constantly hyperosmotic to the external medium regardless of the salinity treatment. K^{+} concentration varied much less than the other ions examined, perhaps suggesting regulation. However, it is not known whether the maintenance of K^{+} concentration at a given level is important in the osmoregulation process (Shumway, 1977a). The effects of marginally raised levels of K^{+} may have less physiological significance than the processes that are responsible for them, such as the absorbance of food (Burton, 1983). However, this explanation is unlikely in the present case as the experimental animals were not fed during the trials.

Burton (1983) suggests that the survival of euryhaline species in different salinities shows the minor importance of absolute concentrations in the haemolymph. It may be more important that the relative concentrations of the different ions remain constant. Previous studies have suggested a correlation between the ionic concentration of Ca^{2+} and K^{+} (Burton, 1983), with the two ions having an antagonistic effect on each other. The haemolymph of *K. scalarina* shows that an increase in one ion corresponds to a decrease in the other, but whether this is due to an antagonistic effect is unclear.

Shell Closure

The initial response of many bivalves to a salinity which is potentially lethal is shell valve closure (Deaton, 1992). This delays equilibration with the environment, not only of their internal body fluids, but of the water in their mantle cavity which acts as a buffer to the external environment (Hoyaux *et al*., 1976). Shumway (1977b) suggested that in bivalves the regulation of cell volume by solute extrusion is a long term emergency phenomenon that periodic valve closure renders unnecessary under conditions of fluctuating salinity. Several steady state experiments have suggested that ventilation in *Mytilus edulis* and *Crassostrea gigas* stops and shell valves close at approximately 50% seawater (Shumway, 1977a), while the critical level for *Modiolus demissus* is much lower (35%). Both adult and juvenile *K. scalarina* displayed increased shell closure in salinities < 25, with shell closure greatest outside the salinity tolerance range. However, juveniles displayed a wider salinity tolerance range than adults coupled with a decreased rate of shell closure. However, Davenport (1979a) has shown for *Mytilus* sp. that exchanges with dilute seawater may still be slow in the absence of artificial irrigation, due to closure of the exhalant siphons and slowing of heart and ciliary beats. Similarly this phenomenon may provide an explanation for the increased salinity tolerance range for juvenile *K. scalarina*, independent of shell closure.

The observations on shell closure do not preclude the possibility of some water and solute exchange even though siphons were not protruding in closed individuals. When adult *K. scalarina* were transferred from 35 to 15, the osmotic pressure of the mantle fluid after 48 h was equivalent to a salinity of 30 not 35 although response times to a salinity challenge and excretory waste contributions to osmotic pressure are potential confounding factors.

5.5.6 CONCLUSION

This study assessed survival of clams over an extended period (21 d) and described physiological responses, as body fluid osmotic pressure, haemolymph ionic composition and shell closure, over 48 h. As such it represents a study of the clam's attempts to respond to salinity challenges rather than describing blood physiology as the point of death is approached and the animal's strategies fail. *K. scalarina* is essentially an osmo- and ionic conformer, particularly at salinities >25, and generally adults are intolerant of low salinity regimes. It is unlikely that it is an osmoregulator and an ionoregulator at low salinities but rather, *K. scalarina* appears to rely on its ability to isolate the body tissues by closing the shell valves. By this means *K. scalarina* can survive unfavourable salinities for extended periods. However, the difference in the salinity tolerance range of adult and juvenile *K. scalarina* may not be related to their ability to isolate the body mass from unfavourable salinities but rather the result of a buildup of metabolic wastes due to prolonged periods of shell closure.

The sporadic and extended salinity fluxes experienced by the sheltered estuaries and lagoons of Tasmania's east coast and the inability of *K. scalarina* to withstand low salinity regimes for extended periods suggests that two of the critical factors for aquaculture site selection will be the duration and periodicity of salinity fluxes.

5.5.7 ACKNOWLEDGMENTS

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5.5.8 REFERENCES

Akberali, H. B., K. R. M. Marriott and E. R. Trueman, (1977). Calcium utilisation during anaerobiosis induced by osmotic shock in a bivalve mollusc. *Nature* **266**, 852-853.

Bayne, B. L., R. J. Thomson and J. Widdows (1976). Physiology: I. In *Marine Mussels: their Ecology and Physiology*. Ed. by B. L. Bayne. Cambridge University Press, London. pp 1231-206.

Bellchambers, L. M. (1993). Aspects of the ecology of *Katelysia scalarina* (Bivalvia: Veneridae), a commercially exploited cockle. Unpublished Honours Thesis. University of Tasmania.

Bellchambers, L. M. and Richardson, A. M. M. (1995). The effect of substrate disturbance and burial depth on the venerid clam, *Katelysia scalarina* (Lamarck, 1818). *Journal of Shellfish Research* **14**, 41-44.

Burton, R. F. (1983). Ionic Regulation and Water Balance. In *The Mollusca, Physiology Part 2*. Ed. by K. M. Wilbur, pp 291-352. Academic Press, New York.

Coleman, N. (1982). Population density and biomass of the bivalves *Tellina mariae* and *Katelysia rhytiphora* from a seagrass bed in Western Port, Victoria. *Journal of the Malacological Society of Australia* **5**, 141-149.

Davenport, J. (1979). The isolation response of mussels (*Mytilis edulis* L.) exposed to rising seawater concentrations. *Journal of the Marine Biological Association of the United Kingdom* **59**, 123-132.

De Zwaan, A. (1983). Carbohydrate metabolism in bivalves. In *Metabolic Biochemistry and Molecular Biomechanics*. Ed. by P. Hochachka, Academic Press, New York.

Deaton, L. (1992). Osmoregulation and epithelial permeability in two euryhaline bivalve molluscs: *Mya arenaria* and *Geukensia demissa*. *Journal of Experimental Marine Biology and Ecology* **158**, 167-177.

Djangmah, J. S., S. E. Shumway, and J. Davenport. (1979). Effects of fluctuating salinity on the behaviour of the West African blood clam *Anadara senilis* and on the osmotic pressure and ionic concentrations of the haemolymph. *Marine Biology* **50**, 209-213.

Hoyaux, J., R. Gilles and C. Jeuniaux. (1976). Osmoregulation in molluscs of the intertidal zone. *Comparative Biochemistry and Physiology*. **53A** 361-365

Kennedy, V. S., W. H. Rosenberg, M. Castagna and J. A. Mihursky. (1974). *Mercenaria mercenaria* (Mollusca: Bivalvia): Temperature-time relationships for survival of embryos and larvae. *Fisheries Bulletin*. **74** 1160-1166.

Mahasneh, D. and E. Pora. (1981). The effect of salinity on the osmotic and ionic regulation in *Mytilus galloprovincialis* and *Modiolus auriculatus*. *Tethys* **10**, 129-134.

Malouf, R. E. and V. M. Bricelj. (1989). Comparative biology of clams: environmental tolerances, feeding, and growth. In *Clam Mariculture in North America*. Ed. by J. J. Manzi and M. Castagna. Elsevier, Amsterdam. pp 23-75.

Nell, J. A. and P. R. Dunkley. (1984). Effects of temperature, nutritional factors and salinity on the uptake of L-Methionine by the Sydney rock oyster *Saccostrea commercialis*. *Marine Biology* **80**, 335-339.

Nell, J. A. and P. J. Gibbs. (1986). Salinity tolerance and absorption of L-Methionine by some Australian bivalve molluscs. *Australian Journal of Marine and Freshwater Research* **37**, 721-727.

Nell, J. A. and K. Patterson. (1997). Salinity studies on the clams *Katelysia rhytiphora* (Lamy) and *Tapes dorsatus* (Lamarck). *Aquaculture Research* **28**, 115-119.

Nielsen, B. J. (1963). Studies of the genus *Katelysia* Romer 1857 (Mollusca, Lamellibranchiata). *Memoirs of the Natural Musuem of Victoria*. **26**, 219-257.

Nossier, M. A. (1986). Ecophysiological responses of *Ceratoderma edule* (L.) and *C. glaucum* (Bruguire) to different salinity regimes and exposure to air. *Journal of Molluscan Studies* **52**, 110-119.

Pierce, S. K. (1971). Volume regulation and valve movements by marine mussels. *Comparative Biochemistry and Physiology A*. **39**, 103-117.

Pimentel, R. A. and J. D. Smith. (1990). BIOSTAT I Ve. 2.0, A Univariate Statistical Toolbox. Sigma Soft, Placentia, CA.

Posey, M. (1987). Effects of lowered salinity on activity of the ghost shrimp, *Callianassa californiensis*. *Northwest Science* **61**, 93-96.

Roberts, D. (1983). The genus *Katelysia* (Bivalvia: Veneridae) in southern Australia. *Journal of the Malacological Society of Australia* **6**, 191 - 204.

Shumway, S. E. (1977a). Effect of salinity fluctuation on the osmotic pressure and Na^{+} Ca^{2+} and Mg^{2+} ion concentrations in the haemolymph of bivalve molluscs. *Marine Biology* **41**, 153-177.

Shumway, S. E. (1977b). The effect of fluctuating salinity on the tissue water content of eight species of bivalve mollusc. *Journal of Comparative Physiology* **116B**, 269-285.

Shumway, S. E. (1996). Natural Environmental Conditions. In *The Eastern Oyster Crassostrea virginica*. Ed. by V. S. Kennedy, I. E. Newell and A. F. Eble. Maryland Sea Grant College, Maryland. pp 467-513.

Singnoret-Brailovsky, G., A, N, Maeda-Martinez, T. Reynoso-Granados, E. Soto-Galera, P. Monsalvo-Spencer and G. Valle-Meza. (1996). Salinity tolerance of the Catarina scallop *Argopecten ventricosus-circularis* (Sowerby II, 1842). *Journal of Shellfish Research* **15**, 623-626.

Soh, S. W. L.; Maguire, G. B.; Ward, R. D.1998: Genetic studies of the venerid clam genus *Katelysia. Journal of Shellfish Research, 17(4):* 1057-1064.

Sokal, R. R. and Rohlf, F. J. (1981). *Biometry*. W. H. Freeman and Company, San Francisco.

Wells, F. E. and D. Roberts. (1980). Molluscan assemblages on an intertidal sandflat in Princess Royal Harbour, Western Australia. *Australian Journal of Marine and Freshwater Research* **31**, 499-507.

5.5.9 TABLES AND FIGURES

Table 1. LT 50 values of adult and juvenile *K. scalarina* in acute toxicity trials. SD = standard deviation, NA = not applicable.

Salinity (‰)	Adult LT50 (days)	SD	Juvenile LT50 (days)	SD
5	9.746	1.221	NA	NA
10	10.217	1.290	NA	NA
15	9.499	1.553	NA	NA
20	23.252	5.946	NA	NA
55	13.906	2.451	14.905	2.880

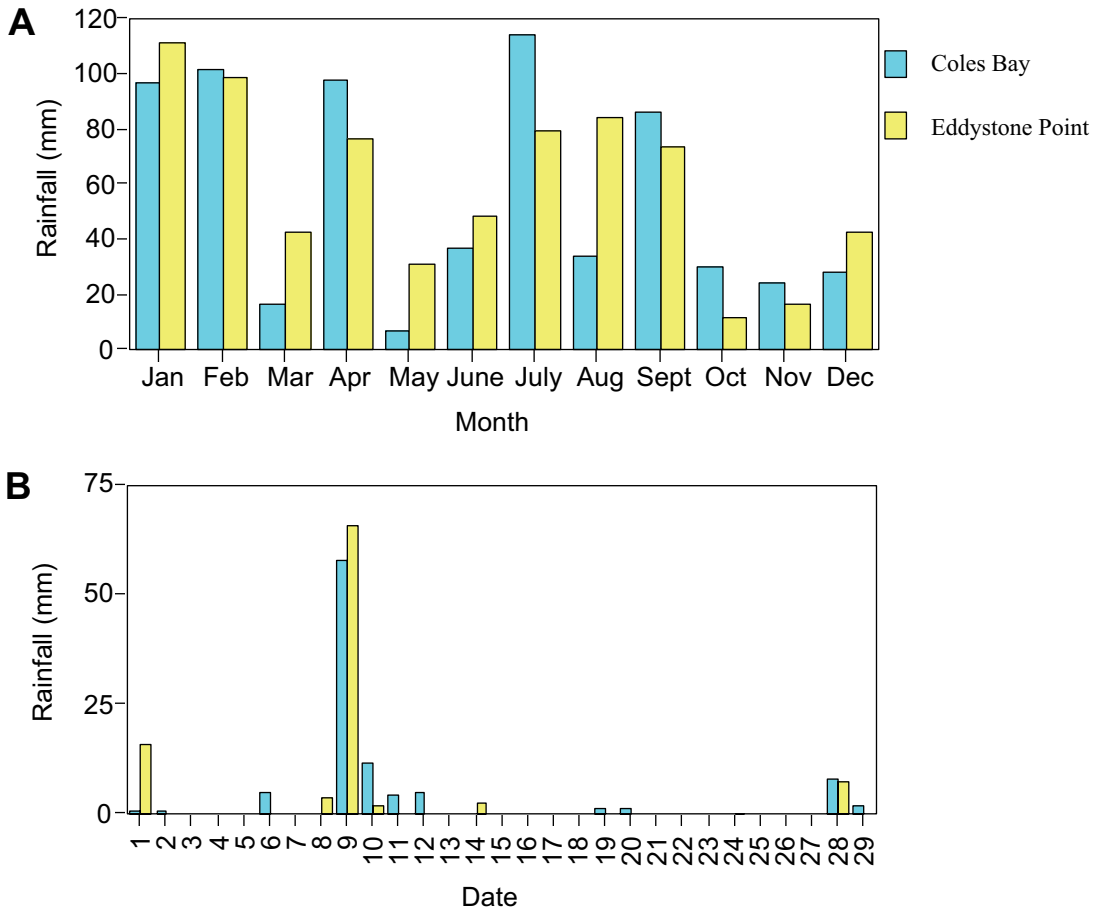

Figure 1. Rainfall data (mm) for the east coast of Tasmania from Coles Bay and Eddystone Point. A) Monthly total of rainfall data for 1996 B) Daily rainfall data for February 1996. Data from the Bureau of Meteorology Tasmania.

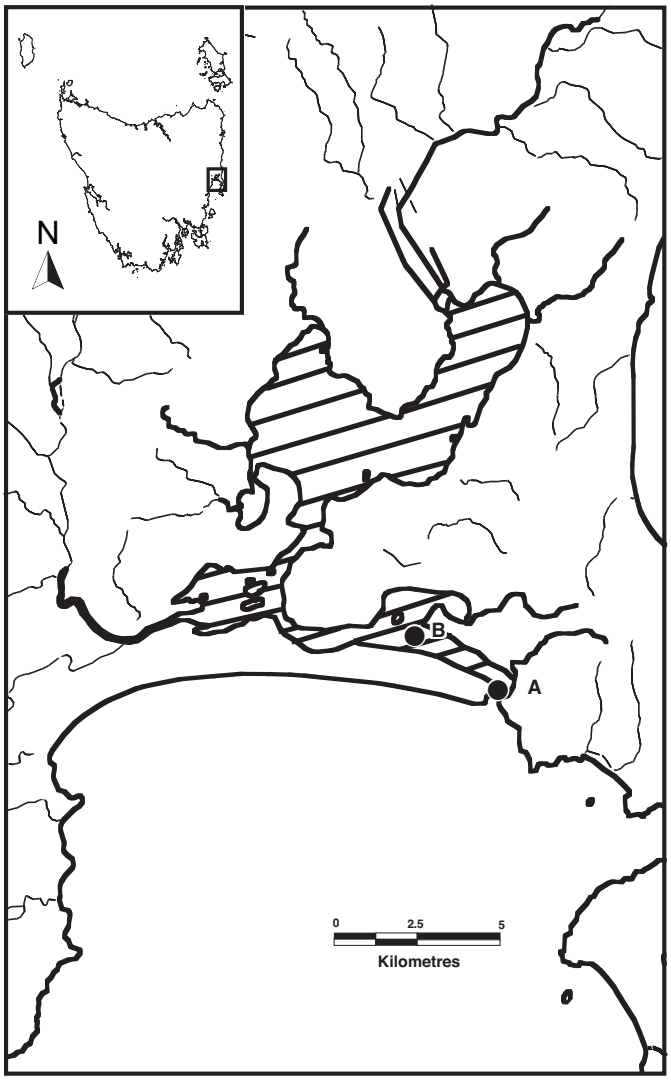

Figure 2. Map of Moulting Lagoon illustrating the collection sites of experimental animals. A) adults B) juveniles.

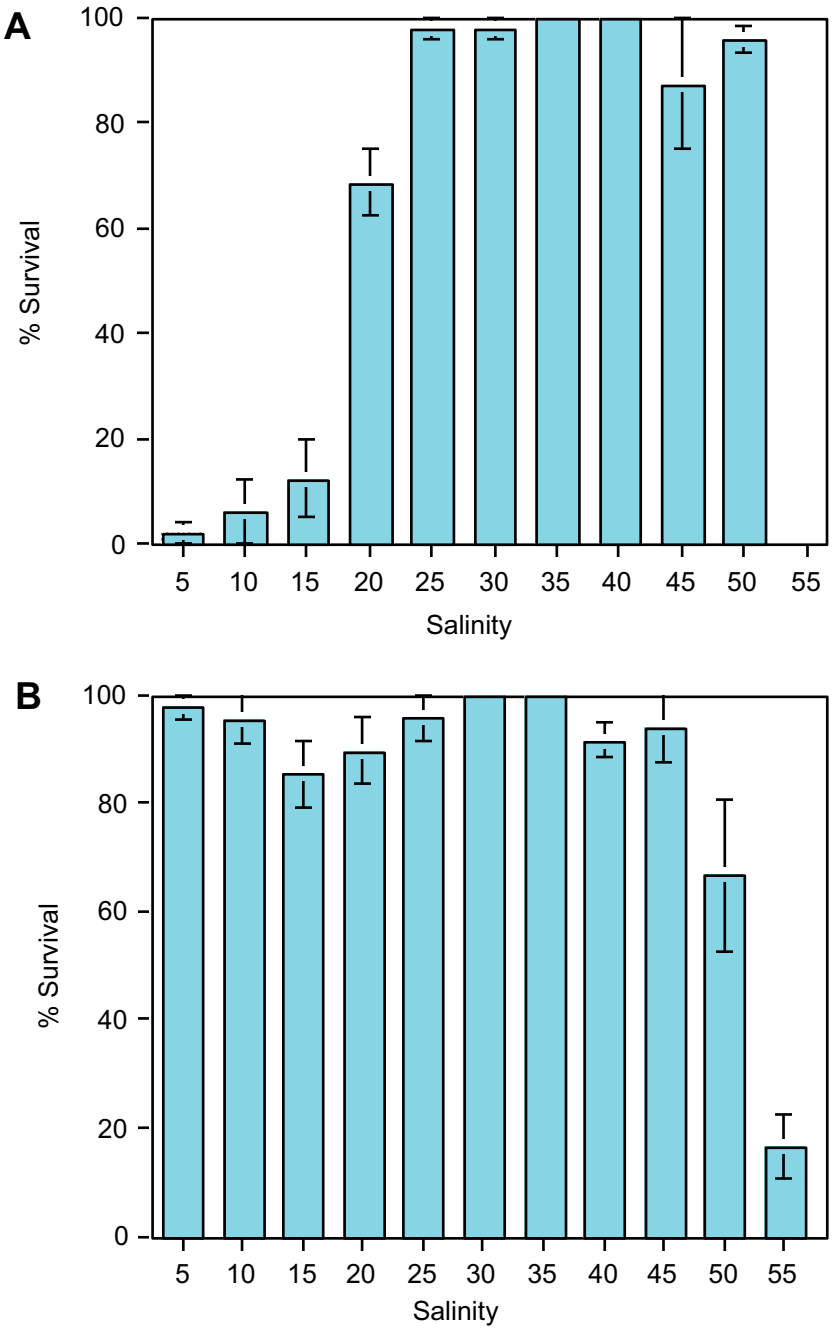

Figure 3. Percentage survival of *K. scalarina* at varying salinities on day 21 of the experimental period. A) adults B) juveniles (mean ± S.E., n=4).

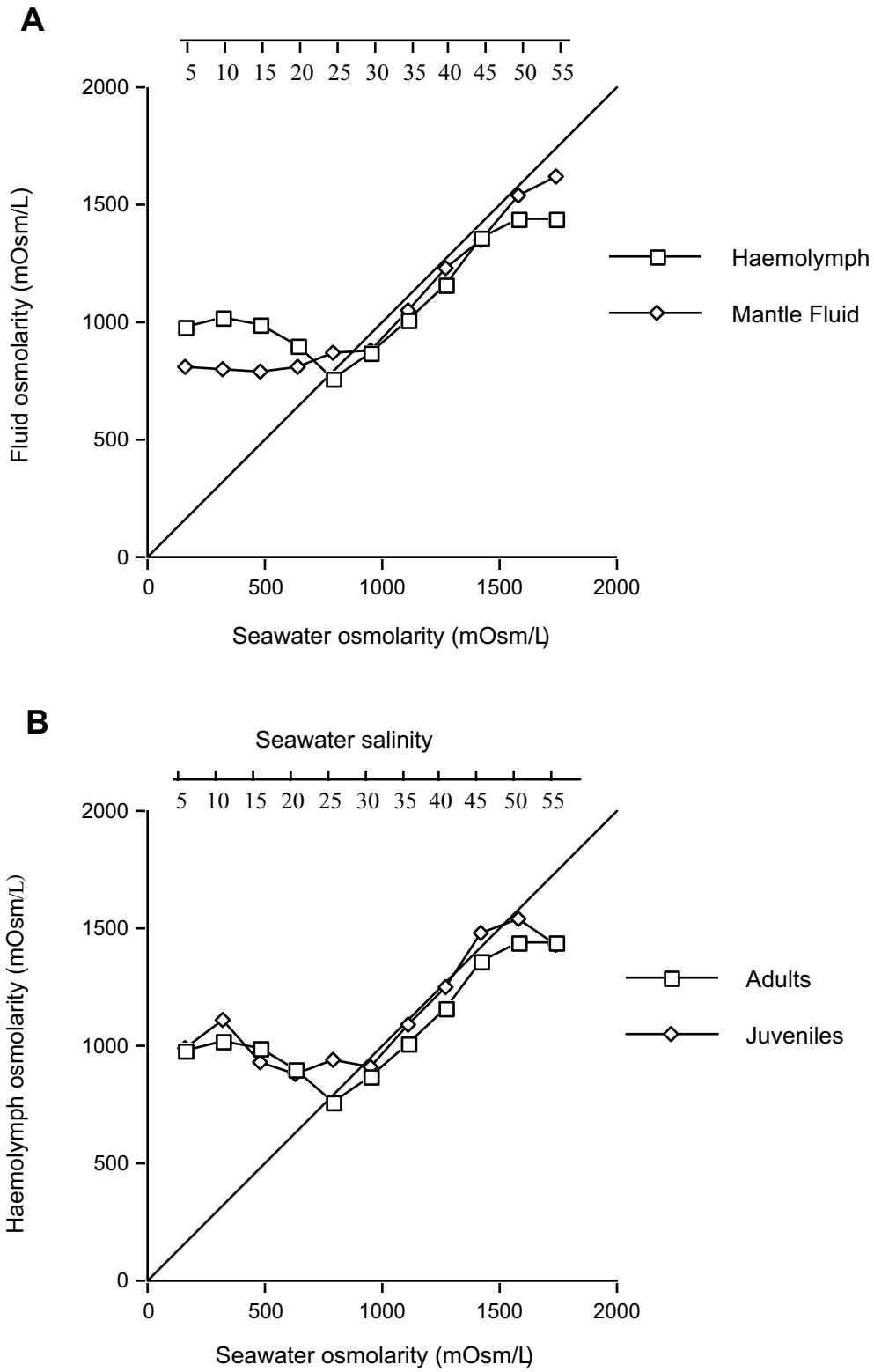

Figure 4. Response of the osmotic concentration of the body fluids of *K. scalarina* to changing external concentrations. A) Adult haemolymph and mantle fluid. B) Adult and juvenile haemolymph (mean ± S.E., n=4). Diagonal line represents the isosmotic line.

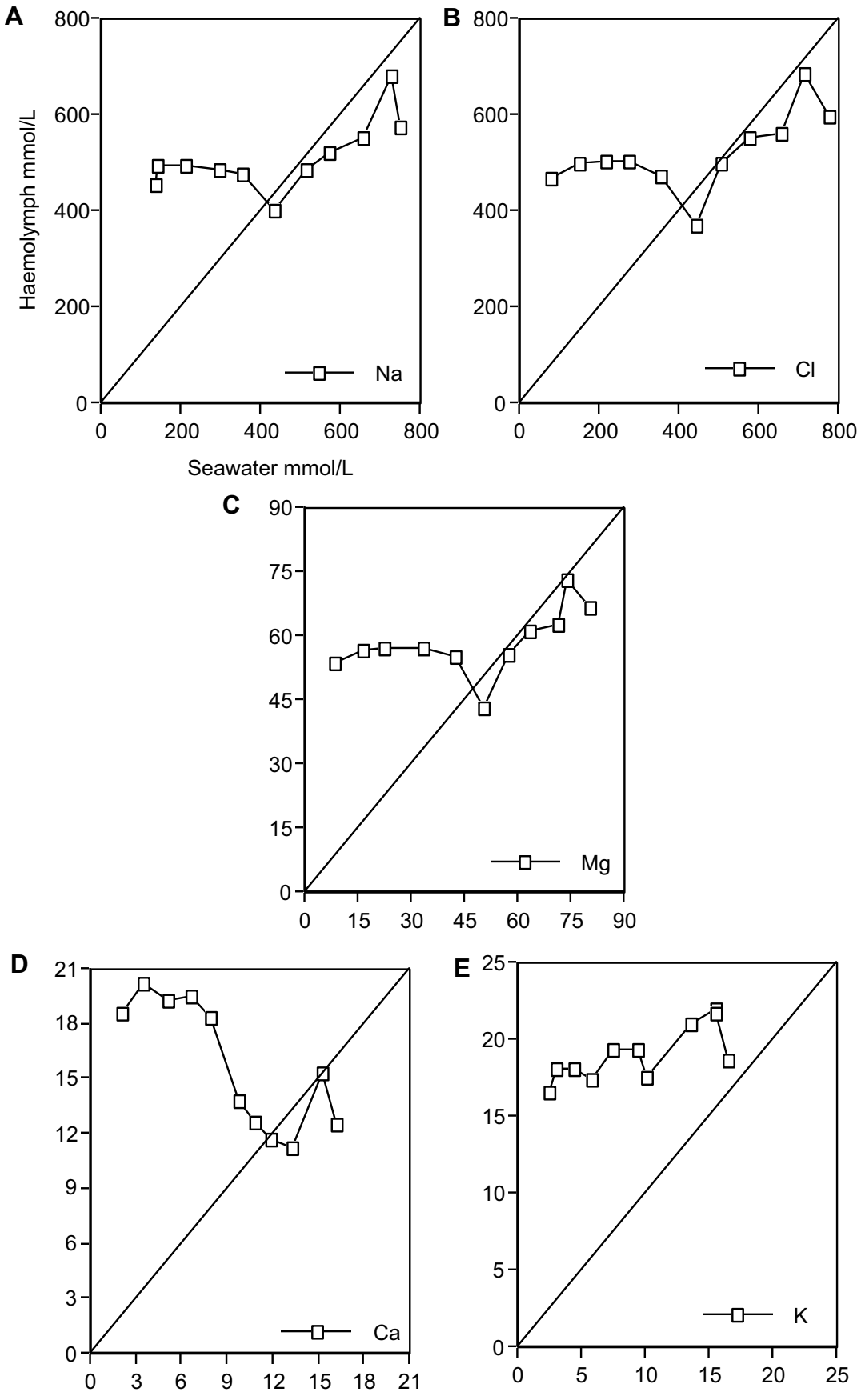

Figure 5. Response of the ionic concentration of adult *K. scalarina* haemolymph to changing external salinity(mean ± S.E., n=4). Diagonal line represents the isoionic line.

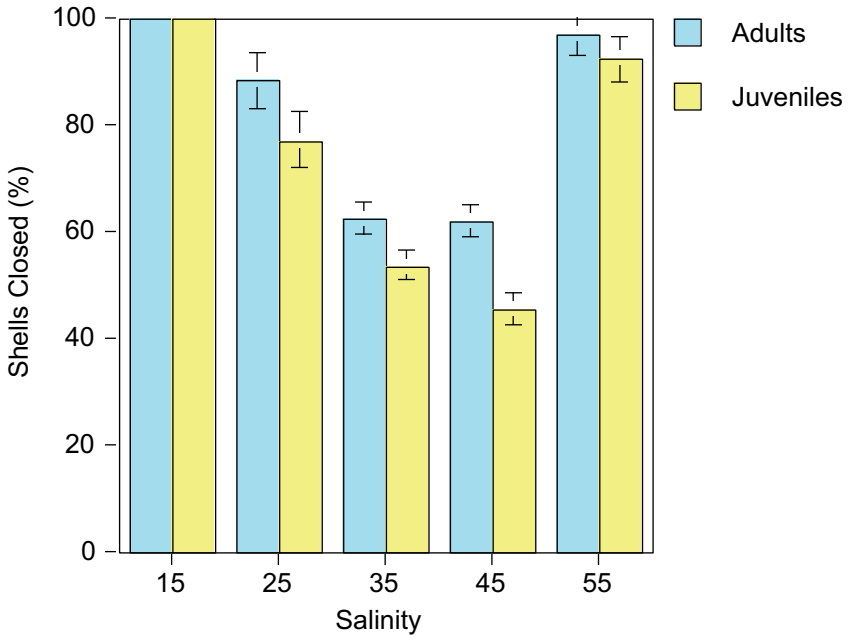

Figure 6. Percentage of adult and juvenile *K. scalarina* with shell valves closed during observation periods (mean±S.E., n=2)

5.6 MANUSCRIPT 6

Effects of salinity on an estuarine clam; *Katelysia scalarina* (Lamarck, 1818) (Bivalvia: Veneridae). II Regulation of free amino acids

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5.6.1 ABSTRACT

Adult *K. scalarina* are essentially an osmo- and ionic conformer with a wide salinity tolerance range (25-50), that may rely on the mechanism of shell valve closure to isolate the body tissues from large fluctuations in external salinity. However, it would appear due to the constant levels of alanine and ammonia + methionine in the haemolymph that prolonged shell valve closure is not utilised by *K. scalarina* over the tolerated salinity range. Cell volume regulation over the tolerated salinity range appears to be controlled by fluctuations in the free amino acid (FAA) pool. The concentration of FAA displayed an inverse relationship with salinity, decreasing from 2.22 mmol/L in 25 to 1.255 mmol/L in 50. Taurine + arginine comprised approximately 25% of the FAA pool of *K. scalarina*. Taurine + arginine also displayed the largest decrease in concentration with increasing salinity, decreasing from 0.532 mmol/L in 25 to 0.343 mmol/L in 50. However, salinities outside the tolerated salinity range do not fit the general pattern of decreasing FAA with increasing salinity.

In the absence of osmo or ionic regulation, *K. scalarina* displays a wide salinity tolerance due, in part, to its ability to regulate volume during salinity stress by altering the size of the FAA pool.

5.6.2 INTRODUCTION

Many bivalves can survive a wide range of salinities by actively regulating cell volume via control of intracellular nitrogenous osmolyte concentration, in particular free amino acids (FAA) (Henry *et al.*, 1980; Sadok *et al*., 1997) and by associated changes in nitrogen metabolism and excretion (Hawkins & Hilbish, 1992). Physiological adjustments to salinity changes, made via tissue and haemolymph FAA levels, are regulated by enzymatic adaptations such as the salinity-induced change of amino peptidase-1 activity in the cytoplasmic vacuoles and granules of the digestive and intestinal cells (Young *et al*., 1979); these changes are directly related to experimental salinity (Deaton *et al*., 1984).

FAA regulation during salinity stress has been reported for a number of bivalve species e.g. *Mytilus edulis* (Smaal *et al*., 1991; Sadok *et al*., 1997) *Rangia cuneata* (Henry *et al*., 1980), *Modiolus demissus* (Bartberger & Pierce, 1976; Baginski & Pierce, 1978), *Geukensia demissa* (Deaton, 1994) and *Crassostrea virginica* (Pierce *et al*., 1992). Alanine, glycine, glutamate, proline, aspartate and taurine compose the majority of the FAA pool, although the quantities of individual compounds vary significantly between species (Henry *et al.*, 1980). The total size of the FAA pool varies directly with environmental salinity, therefore FAA are believed to function as intracellular osmotic regulators maintaining the isosmotic balance between intracellular and extracellular fluids (Pierce *et al*., 1992).

Adult *K. scalarina* are essentially an osmo- and ionic conformer with a wide salinity tolerance range (25-50), that may rely on the mechanism of shell valve closure to isolate the body tissues from large fluctuations in external salinity. The aims of this research were 1) to determine, in the absence of osmotic and ionic regulation, how *K. scalarina* copes with the osmotic variation, in body fluids, within the wide salinity tolerance range of the species (Manuscript 5) and, 2) to determine whether the accumulation of particular amino acids indicated that shell closure was being utilised by *K. scalarina* as a mechanism of coping with by totally isolating haemolymph from the external environment. Variations in the concentrations of several amino acids, in particular alanine and glutamic acid, aspartic acid and glycine, are indicative of anaerobic metabolism which occurs during prolonged periods of shell closure.

5.6.3 METHODS

Experimental Procedure

Adult *K. scalarina* (35.0 - 40.0 mm shell length) were collected from Moulting Lagoon, Coles Bay, Tasmania (42°05'S, 148°10'E) and transported out of water to Hobart; no mortality occurred during or after transportation. Experimental treatments commenced the day after collection. Clams were transferred directly from seawater of 35 to one of the experimental salinities which ranged from 20-55 with 5 increments, representing five salinities in which mortality over 21 d is negligible (salinity range 25-50) and two more marginal salinities at which significant mortality (about 30%) or total mortality occurs (at 20 and 55 respectively) over that period (Manuscript 5). For each salinity two aerated aquaria (3 L) were used, containing ten clams. Approximately 4 cm of washed sieved sand was placed on the bottom of each aquarium to allow clams to bury, thereby maintaining experimental conditions as close as possible to the natural habitat. Salinity treatments were established by adding distilled water or an artificial salt mixture (Coral Reef Red Sea Salt ®) to sand-filtered seawater with a salinity of 35. Temperature was maintained at 15°C throughout the experimental period. Salinities were measured daily using a Wissenschaftlich-Technische-Werksttaten conductivity meter, calibrated using standard saline solution (Ocean Scientific International® K_{15} =0.99982; Salinity=34.993).

Samples of adult haemolymph for amino acid analysis were collected after 48 h, as this is the time period in which adjustments to the FAA pool occur (deVooys, 1991). By pooling across the two replicate aquaria, three samples of haemolymph were collected from each salinity treatment and involved six clams per sample. Samples were centrifuged, the supernatant removed and stored in eppendorf tubes at -80°C until amino acid analysis was conducted.

Amino Acid Analysis

Thawed serum samples (20 µl) were deproteinised with ethanol (400 µl), centrifuged (8000 rpm X 10 min) and the supernatant transferred to an eppendorf tube. The supernatant was dried under a stream of nitrogen on a heated dri-block (40°C, 15 min) and the solid residue dissolved in 400 µl of buffer (0.1M $NaHC0_3$ & 0.1M boric acid at pH 8.5 "Aminotag") and centrifuged (8000 rpm x 10 min). The supernatant was removed and added to 400 µl of 1.5 mM derivatising agent (9-fluorenylmethyl chloroformate, FMOC) in acetone. After 10 minutes reaction time the mixture was extracted with pentane/ethyl acetate (80:20, 1.2 mL) and the lower layer transferred to a sample vial. FMOC derivatised amino acids were assayed using a Varian Amino Acid Analyser comprising of a HPLC ternary gradient pump (model 9010), an autosampler (model 9100), a fluorometer (model 9070, set at 270 emission, 340 detection) and UV-Vis diode array (model 9065) detectors. An Alltech Alltima C18 5µ (250 x 4.56 mm), column maintained at 32°C was used with a Waters TCM heating unit.

The standard buffers were:

A) 0.015M sodium citrate, 0.010M tetramethyl ammonium chloride (pH 2.85) with H_3PO_4.

B) 90%A at pH 4.5, and 10% methanol.

C) Acetonitrile.

The injection volume of each sample was 20 µl and a run lasted 33 minutes, with a 10 minute equilibration time between runs. The gradient profile, maintained at a constant flow rate of 1.4 mL/min, involved linear transformations between a series of compositions (Table 1).

The standard used was a mixture of Pierce type amino acid standard (SIGMA Co.) with added taurine (SIGMA Co.) and asparagine (Boehringer-Mannheim) such that the total amine concentration approximated concentrations in the serum samples. Processing of the standard followed the same derivatisation and extraction procedures as the serum samples. Duplicates of each replicate were analysed and the average used for quantification. Quantification was conducted using the fluorescence trace on an IBM compatible computer with Varian software for integration.

Final amino acid results were analysed using a one way ANOVA in SYSTAT. Homogeneity of variances was checked using Bartlett's test of homogeneity of variances.

5.6.4 RESULTS

Salinity of the external medium had a significant effect on the concentration of FAA in the haemolymph of adult *K. scalarina* (P=0.0001), with FAA concentration displaying an inverse relationship with salinity (Figure 1). There was a distinct decrease in the concentration of FAA over the tolerated salinity range, from 2.22 mmol/L in 25‰ to 1.255 mmol/L in 50‰. Salinities outside the tolerated salinity range do not fit the general pattern of decreasing FAA with increasing salinity.

Taurine + arginine composed the majority of the haemolymph FAA pool of adult *K. scalarina* (Figure 2). These two amino acids are grouped as they could not always be separated, however, taurine comprised the majority (>90%) of this group in samples that resolved. Arginine and taurine reflected the general pattern of decreasing concentration with increasing salinity, ranging from 0.532 mmol/L at 25 to 0.343 mmol/L at 50. The second most abundant FAA were glycine and alanine. Alanine appears to remain at a relatively constant level throughout the salinity range, while glycine displayed an inverse relationship with salinity, ranging from 0.266 mmol/L in 25 to 0.195 mmol/L in 50. The remainder of FAA in the haemolymph were composed of twelve identifiable FAA or groups all of which displayed a slight decrease in concentration over the salinity range with the exception of aspartate, glutamate, lysine and serine. Aspartate (0.138 mmol/L - 0.026 mmol/L), glutamate (0.154 mmol/L - 0.055 mmol/L), lysine (0.106 mmol/L - 0.034 mmol/L) and serine (0.107 mmol/L- 0.0356 mmol/L) displayed a more substantial decrease in concentration over the salinity range. Tyrosine, phenylalanine, threonine and valine remained at low but relatively constant levels despite variations in the external salinity. Proline did not resolve from the derivatising agent and so is not reported. Methionine and ammonia could not be separated and are therefore reported together. Similarly, cysteine, isoleucine and leucine are also reported as a group.

Variations in individual amino acids are more evident when the concentration at each salinity treatment is expressed as a percentage of the highest concentration for that particular amino acid within the tolerated range (Figure 3). Serine, aspartate, glutamate, threonine, valine, phenylalanine, cysteine/isoleucine/leucine and lysine all display a decrease of greater than 50% over the experimental range. This represents a substantial decrease despite the fact that these amino acids did not comprise a significant component of the FAA pool in terms of concentration.

5.6.5 DISCUSSION

Salinity had a significant effect on the concentration of FAA in the haemolymph of adult *K. scalarina*. The concentration of FAA displayed an inverse relationship with salinity, decreasing from 2.22 mmol/L in low salinities (25) to 1.255 mmol/L in high salinities (50) after 48 h exposure. The extracellular FAA pool of *K. scalarina* displays a step-wise increase in FAA in salinities less than 35, probably because of an influx of FAA from the tissues. Conversely, in salinities greater than 35, the haemolymph of *K. scalarina* displays a decrease in FAA concentration that is presumably due to an efflux of FAA from haemolymph into the intracellular fluid. Similarly, Bartberger & Pierce (1976) suggest that a decrease in external salinity is reflected by an increase in haemolymph amino acid concentrations which is in turn reflected by a decrease in the tissue FAA pool. However, Matsushima *et al.* (1987) states that the intracellular FAA pool of the bivalve *Corbicula japonica* increases during the initial stage of hyperosmotic stress (short term), plateaus and then continues to increase over a long period (long term).

The majority of the FAA pool of *K. scalarina* was composed of taurine + arginine, these two amino acids comprising approximately a quarter of the total FAA pool. Taurine and arginine are grouped together as in the majority of samples these two amino acids could not be separated, although taurine composed the majority of this group in samples that did resolve. As taurine and arginine and reported together it is difficult to comment on the individual trends of these amino acids.

Previous studies indicate that taurine is frequently present in significantly higher concentrations than arginine (see Baginski & Pierce, 1977; Shumway *et al*., 1977; Livingstone *et al*., 1979). The results of Shumway *et al*. (1977) from the adductor muscle of 8 species of bivalve molluscs

indicate that taurine comprised between 10.78% - 50.09% of the FAA pool while arginine composed between 1.49 - 7.30%. In most of the species studied taurine levels were approximately ten times higher than arginine. Therefore, although arginine and taurine could not be separated in this study results from other studies suggest that taurine composes the majority of the FAA pool and it is likely that this is also the case for *K. scalarina*.

While comprising the majority of FAA in the haemolymph of *K. scalarina,* taurine + arginine also displayed the largest decrease in concentration with increasing salinity, from 0.532 mmol/L (25) to 0.343 mmol/L (50). Taurine is thought to have an important role in osmoregulation (Lange, 1963) and declines in intracellular fluids with an abrupt decrease in salinity (Livingstone *et al*., 1979). Baginski & Pierce (1977) working on *Modiolus demissus demissus* suggest that taurine comprises the majority of FAA pool and is used for long term salinity acclimation. Long term taurine acclimation probably relieves the requirement for utilisation of other essential amino acids for intracellular regulation (Lange, 1963). Patterns of taurine accumulation in the haemolymph of adult *K. scalarina* reflect previous studies and indicate that taurine may be the major amino acid used in maintaining intracellular volume under salinity stress.

While taurine + arginine displayed the largest decrease in terms of mmol/L and comprised the majority of the FAA pool, when decreases in FAA concentration are expressed as a percentage of the total concentration of each amino acid. Several other amino acids display much larger decreases in overall concentration than taurine. Aspartate (80%), glutamate (65%), serine (65%), threonine (60%), valine (60%), phenylalanine (65%), cysteine/isoleucine/leucine (65%) and lysine (70%) all displayed decreases substantial decreases in the percentage total amino acid in comparison to taurine (35%). However, as these amino acids comprised only a small proportion of the total FAA pool decreases in their overall concentration may not be significant in coping with osmotic stress.

Over the salinity range 25-50 the changes in haemolymph concentration for taurine + arginine are indicative of an intracellular increase in arginine, a nitrogen bearing precursor to urea. This is consistent with a detoxification process under conditions which prevent the normal excretion of ammonia i.e. shell closure due to salinity stress (Ivanovinci *et al*., 1981). *K. scalarina* certainly uses the behavioural response of shell closure to minimise the effects of unfavourable salinities (Bellchambers, 1998).

Alanine was the second most abundant FAA in the haemolymph of *K. scalarina*. In bivalves, alanine is usually rapidly synthesised by the mitochondria following the onset of salinity stress (Baginski & Pierce, 1977, 1978; Bishop *et al*., 1981). Intracellular free alanine also accumulates under anaerobic conditions (Baginski & Pierce, 1978; Henry *et al*., 1980; Ivanovinci *et al*., 1981). In this study alanine concentrations remained constant across all salinity treatments. Coupled with the fact that haemolymph ammonia concentration displayed only a slight increase in low salinities, it is suggested that anaerobic metabolism due to shell closure during the experiment was minimal. Alternatively, anaerobic metabolism may have occurred but was interspersed with periods of aerobic metabolism allowing adequate flushing of nitrogenous wastes. In Manuscript 5, it was indicated that even at a salinity of 15, mantle fluid osmotic pressure readings were not consistent with absolute separation of the clams from the medium.

Anaerobic metabolism in many invertebrate species is characterised by higher levels of alanine and glutamic acid and lower level of aspartic acid and glycine. A marked decrease in glycine concentration with a concomitant increase in the ratio of taurine to glycine has been associated with stress conditions in a variety of molluscan species. However, while taurine and arginine decreased, glycine was consistent after an initial drop from 0.266 mmol/L (25) to 0.227 mmol/L (30) and remained relatively constant. Therefore it is unlikely that *K. scalarina* responded to

salinity stress by reverting to a continuous combination of anaerobic metabolism and total shell closure.

5.6.6 CONCLUSION

In the absence of osmo or ionic regulation, adult *K. scalarina* display a wide salinity tolerance range due their ability to regulate volume during salinity stress by altering the size and composition of the FAA pool. The majority of the FAA pool of *K. scalarina* is composed of taurine, which has been reported as the major osmotic effector in several other bivalve species. It would appear due to the constant levels of alanine, ammonia + methionine and glycine in the haemolymph of *K. scalarina*, that prolonged shell valve closure is not utilised by adult *K. scalarina* over a salinity range of 30-50, that is, over most of the tolerated salinity range (30-50). Salinity stress is adequately compensated for at least over the duration of this experiment, by the variations in the size and composition of the FAA pool. Because of uncertainty over the efficacy of shell closure in totally isolating *K. scalarina* from its environment, it is not possible to make a firm conclusion about the accumulation of FAA at salinities, outside of the tolerated range, where virtually all individuals seem to remain closed (Manuscript 5).

It has been suggested that the limit of euyhalinity in osmoconformers depend on the ability of the cells to regulate volume in a varying salinity regime which in turn depends on the ability to which the size of the FAA pool can be regulated (Pierce, 1971; Henry *et al*., 1980). Gainey & Greenburg (1977) suggest that the penetration of freshwater species into the marine environment and marine species into the freshwater environment seems to be more limited by their ability to regulate ion concentration than to mobilise amino acids for cellular osmoregulation. Similarly, the limited ion regulation capabilities of *K. scalarina* , as indicated by the high incidence of valve closure at low salinities, (Manuscript 5) may be a factor which prevents their longer term survival in reduced salinities.

5.6.7 ACKNOWLEDGMENTS

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5.6.8 REFERENCES

Baginski, R. M. and Pierce, S. K. (1977). The time course of intracellular free amino acid accumulation in tissues of *Modiolus demissus* during high salinity adaption. *Comparative Biochemistry and Physiology* **57A**, 407-412.

Baginski, R. M. and Pierce, S. K. (1978). A comparison of amino acid accumulation during high salinity adaption with anaerobic metabolism in the ribbed mussel, *Modiolus demissus demissus*. *Journal of Experimental Zoology* **203**, 419-428.

Bartberger, C. A. and Pierce Jr., S. K. (1976). Relationship between ammonia excretion rates and haemolymph nitrogenous compounds of a euryhaline bivalve during low salinity acclimation. *Biological Bulletin* **150**, 1-14.

Bellchambers, L. M. (1998). The ecology and ecophysiology of *Katelysia scalarina*: a commercially exploited clam. Unpublished PhD Thesis University of Tasmania, Launceston.

Bishop, S. H., Ellis, L. L. and Burcham, J. M. (1983). Amino acid metabolism in molluscs. In *The Mollusca, Metabolic Biochemistry and Molecular Biomechanics*. Ed. by P. Hochachka, pp 243-327. Academic Press, New York.

Bishop, S. H., Greenwalt, D. E. and Burcham, J. M. (1981). Amino acid cycling in ribbed mussel tissues subjected to hyperosmotic shock. *Journal of Experimental Zoology* **215**, 277-287.

Deaton, L. E. (1994). Hypo-osmotic volume regulation in bivalves: protein kinase C and amino acid release. *Journal of Experimental Zoology* **268**, 145-150.

Deaton, L. E., Hilbish, T. L. and Koehn, R. K. (1984). Protein as a source of nitrogen during hyperosmotic volume regulation in the mussel *Mytilus edulis. Physiological Zoology* **56**, 609-619.

de Vooys, C. G. N. (1991). Anaerobic metabolism in sublittoral living *Mytilus galloprovincialis* in the Mediterranean - IV. role of amino acids in adaption to low salinities during anaerobiosis and aerobiosis. *Comparative Biochemistry and Physiology* **100A**, 423 - 431.

Gainey, L. F. and Greenburg, M. J. (1977). Physiological basis of the species abundance-salinity relationship in molluscs: A speculation. *Marine Biology* **40**, 41-49.

Hawkins, A. J. S. and Hilbish, T. J. (1992). The cost of cell volume regulation: protein metabolism during hyperosmotic adjustment. *Journal of the Marine Biological Association of the U.K.* **72**, 569-578.

Henry, R., Mangum, C. and Webb, K. (1980). Salt and water balance in the oligohaline clam, *Rangia cuneata*. *Journal of Experimental Zoology* **211**, 11-24.

Ivanovici, A. M., Rainer, S. F. and Wadley, V. A. (1981). Free amino acids in three species of mollusc: responses to factors associated with reduced salinity. *Comparative Biochemistry and Physiology* **70A**, 17-22.

Lange, R. (1963). The osmotic function of amino acids and taurine in the mussel, *Mytilus edulis*. *Comparative Biochemistry and Physiology* **10**, 173-179.

Livingstone, D. R., Widdows, J. and Fieth, P. (1979). Aspects of nitrogen metabolism of the common mussel *Mytilus edulis*: adaption to abrupt and fluctuating changes in salinity. *Marine Biology* **53**, 41-55.

Matsushima, O., Katayama, H. and Yamada, K. (1987). The capacity for intracellular osmoregulation mediated by free amino acids in three bivalve molluscs. *Journal of Experimental Marine Biology and Ecology* **109**, 93-99.

Pierce, S. K. (1971). Volume regulation and valve movements by marine mussels. *Comparative Biochemistry and Physiology* **39A**, 103-117.

Pierce, S. K., Rowland-Faux, L. M. and O'Brien, S. M. (1992). Different salinity tolerance mechanisms in Atlantic and Chesapeake conspecific oysters: glycine betaine and amino acid pool variations. *Marine Biology* **113**, 107-115.

Sadok, S., Uglow, R. F. and Haswell, S. J. (1997). Haemolymph and mantle fluid ammonia and ninhydrin positive substances variations in salinity-challenged mussels (*Mytilus edulis* L.). *Journal of Experimental Biology and Ecology* **211**, 195-212.

Shumway, S. E., Gabbott, P. A. and Youngson, A. (1977). The effect of fluctuating salinity on the concentration of free amino acids and ninhydrin-positive substances in the adductor muscles of eight species of bivalve molluscs. *Journal of Experimental Biology and Ecology*. **29**, 131-150.

Smaal, A. C., Wagenvoort, A., Hemelraad, J. and Akkerman, I. (1991). Response to stress of mussels (*Mytilus edulis*) exposed in Dutch tidal waters. *Comparative Biochemistry and Physiology* **100C**, 197-200.

Young, J. P. W., Koehn, R. K. and Arnhein, N. (1979). Biochemical characterisation of "Lap", a polymorphic aminopeptidase from the mussel *Mytilus edulis*. *Physiological Zoology* **57**, 609-619.

5.6.9 TABLES AND FIGURES

Table 1. Buffer compositions used for amino acid analysis of *K. scalarina* haemolymph.

Time (mins)	%A	%B	%C
0	73	0	27
0.5	68	0	32
11.5	58	0	42
13	58	0	42
13.5	31	30	39
14	0	62	38
18	0	61	39
28	0	28	72
29	0	25	75
30	0	25	75

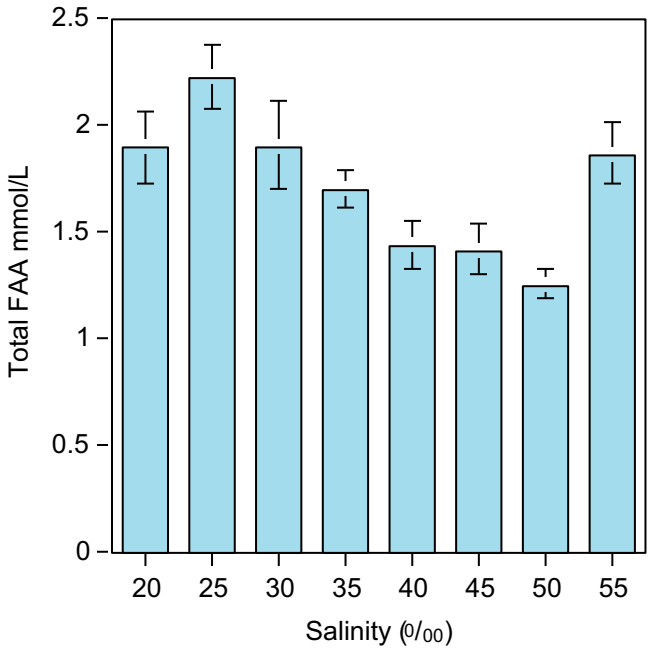

Figure 1. Total FAA in the haemolymph of *K. scalarina* (mean±S.E., n=3 replicate samples). * salinities outside salinity tolerance range.

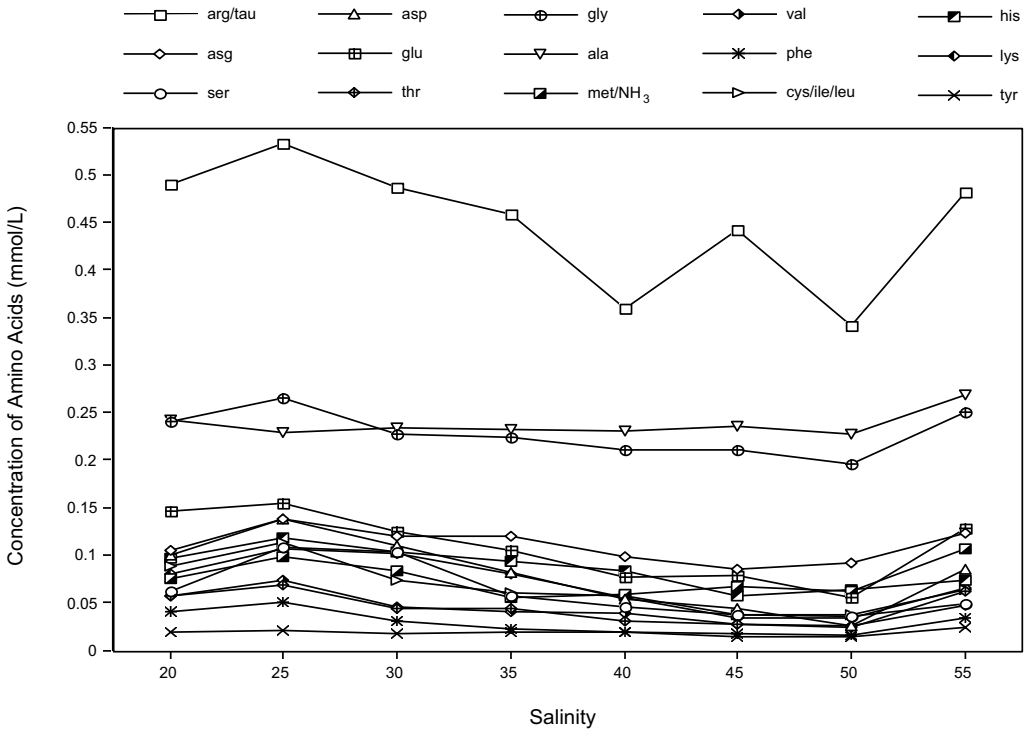

Figure 2. Concentration of individual amino acids in the haemolymph of adult *K. scalarina.*

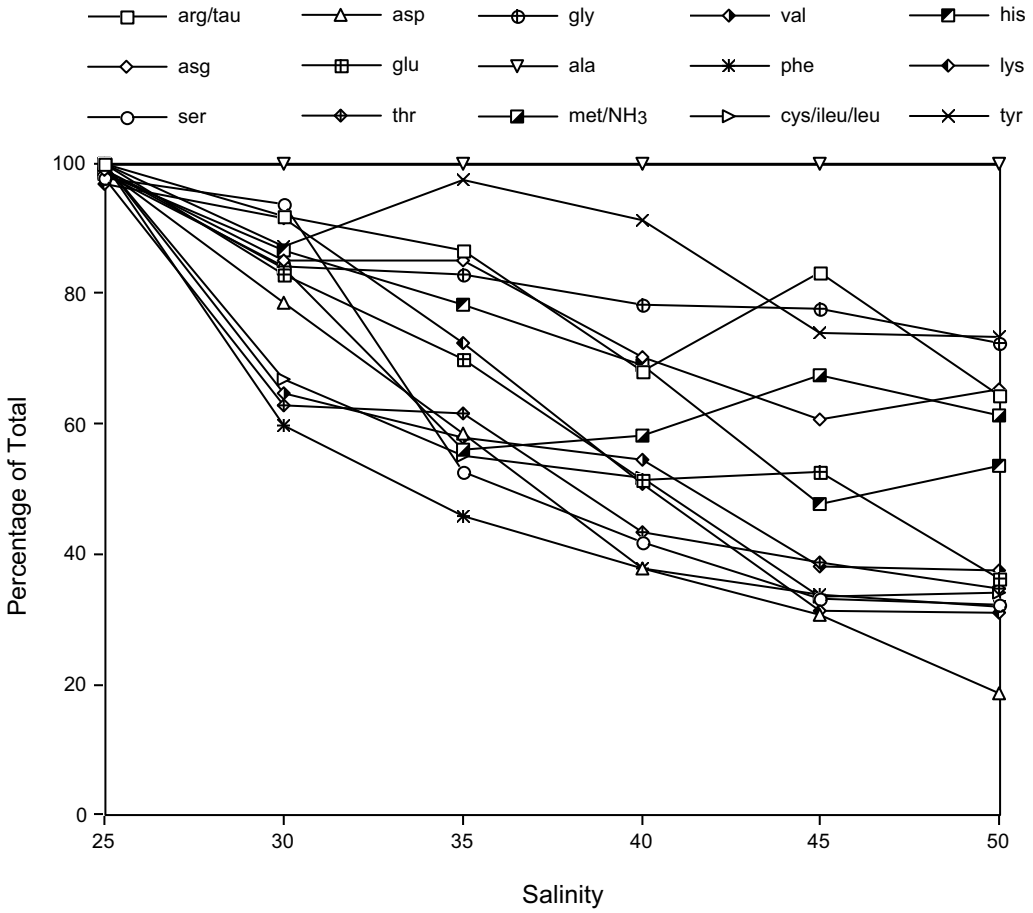

Figure 3. Individual amino acids expressed as a percentage of the highest value, for that amino acid, recorded across all salinity treatments (data for salinities in the range 25-50).

5.7 MANUSCRIPT 7

Effects of salinity on an estuarine clam; *Katelysia scalarina* (Lamarck, 1818) (Bivalvia: Veneridae). III Respiration and algal clearance

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5.7.1 ABSTRACT

Although adult *Katelysia scalarina* are capable of surviving for extended periods ($\geq$ 21 d) in a salinity range of 25-50, optimal growth may be restricted to a narrower band. Respiration and algal clearance trials were conducted to determine whether irregular valve closure patterns limit oxygen consumption and algal clearance, which may in turn limit growth potential.

Salinity has a significant effect on the oxygen consumption of adult *K. scalarina* (35 - 40 mm shell length). Oxygen consumption was depressed in salinities outside 35 even for salinities within the tolerance range. Oxygen consumption was highest in the salinity range 35-45 (maximum of 10.78 mg/g whole weight/h at 35) and declined in salinities either side of this range, even within the tolerated salinity range, but with the lowest rate of consumption being recorded outside of the tolerance range at 15 (3.62 mg/g/h).

Algal clearance rates of adult and juvenile *K. scalarina* (20 - 25 mm shell length) indicated that both groups respond similarly to changes in salinity. Adults displayed a substantial decrease in algal clearance at salinities of 5-20 and 55 which are outside their salinity tolerance range (25-50). Juvenile *K. scalarina* displayed optimal algal clearance rates between 25 and 45, which is within their tolerance range (5-45), whereas it was depressed at the lower end of this range (5-20). It was also depressed at 50-55 which is outside of the juvenile tolerance range.

In general, oxygen consumption data suggested a narrower optimum range for growth than food consumption data, however, the latter indicator has correlated well with growth in another *Katelysia* species.

5.7.2 INTRODUCTION

Respiration represents the energetic cost of maintaining basic metabolic processes (Pamatmat, 1983; Dame, 1996) and is an important indicator of an organism's ability to cope with stress (Vernberg and Vernberg, 1981). Oxygen uptake may indicate an animal's capacity for growth or metabolic adaptation to a variety of environments (Storey and Storey, 1990).

Previous authors have suggested that fluctuations in environmental salinity may alter the metabolism of aquatic invertebrates (Brown and Meredith, 1981; Shumway, 1982; Shumway and Koehn, 1982) and that reductions in respiration rate may conserve metabolic reserves during periods of stress (Newell, 1973). However, the causes of salinity effects on oxygen consumption rates are not clear. Some authors suggest that altered metabolic rates, in response to changing salinity, are the result of stimulation or inhibition of movement (Shumway, 1979; Brown and Meredith, 1981; Stickle and Bayne, 1982), an increase or decrease in the osmotic concentration of body fluids, changes in internal ion ratios, and interference with neuromuscular, hormonal or enzymatic mechanisms (Kinne 1971; Newell, 1973).

In the absence of any extracellular physiological ability to osmoregulate, the initial response of many bivalves to a salinity which may eventually be lethal, is shell closure (Shumway, 1977; Deaton, 1992). Many bivalve molluscs exhibit behavioural periodicity in their valve movements, with periods of activity and quiescence (Higgins, 1980). However, even a slight contraction of the valves will result in reduced water flow which will in turn affect the rate of feeding (Riisgård, 1991) and gas exchange (Shumway, 1996). Similarly, exposure to non-lethal salinities may cause periodic closure or irregular ventilation patterns. Decreasing filtration rates in this manner will reduce the time available for feeding, thus limiting growth potential (Bayne and Newell, 1983; Jørgensen *et al*., 1986). Therefore, salinity is a limiting factor for many marine molluscs and may, when reduced, restrict the potential for energy acquisition, thereby limiting overall energy balance and scope for growth (Stickle and Bayne, 1982; Bayne and Newell, 1983).

Although adult *K. scalarina* are capable of surviving a salinity range of 25-50 (Manuscript 5) optimal growth may be restricted to a narrower range. Experiments were conducted to determine whether irregular shell closure patterns limit oxygen consumption and algal clearance in *K. scalarina*, which would in turn limit growth potential. Nell and Paterson (1997) reported a close correlation between algal consumption and growth of a closely related species *Katelysia rhytiphora*.

5.7.3 METHODS

Respirometry

Respiration and algal clearance trials were conducted at Marine Shellfish Hatcheries, Bicheno, Tasmania, Australia from February to March 1997. Adult *K. scalarina* (35.0 - 40.0 mm shell length) were collected from Moulting Lagoon, Coles Bay, Tasmania, Australia (42°05'S, 148°10'E). Clams were transported for approximately 1 hour out of water, however no mortality occurred during or after transportation. Clams were maintained prior to respirometry in tanks of flowing aerated seawater (35) and fed a mixed algal diet of *Pavlova lutheri* and *Tetraselmis suecica*, (about 4 L every third day). Clams used in the respiration trials were starved for 24 h prior to and during respirometry, to ensure that oxygen consumption was not altered by digestive processes.

Salinity treatments (15 -55 with 10 increments) were established by adding double filtered freshwater (1 µm nominal and activated charcoal) or an artificial salt mixture (Coral Reef Red Sea Salt®) to seawater with a salinity of 35. Seawater was drawn from an exposed section of the coastline unaffected by freshwater runoff. Salinities were measured daily using a WTW (Wissenschaftlich - Technische - Werksttaten) conductivity meter, calibrated using standard saline solution (Ocean Scientific International® K_{15} = 0.99982; Salinity =34.993). Water was exchanged after every trial and clams were only used once.

Respiration trials commenced one week after collection. Clams were transferred directly to the respirometer chambers with the experimental salinities. Pilot trials using sand in the bottom of chambers to allow the clams to bury, thus maintaining conditions as close as possible to field conditions, proved unsuccessful because of blockages in the equipment and hence unreliable results. All subsequent results were conducted without sand in the respirometer chambers. Synchronous salinity trials were conducted as duplicates for each of two salinities and a control (salinity 35 with no clams). The control was included to provide an estimate of microbial oxygen consumption/production so that a correction factor could be applied to the data. Each chamber contained approximately 17-20 clams (approximately 400 g total wet weight). Trials were not

conducted using individual clams as the oxygen consumption rates were beyond the sensitivity of the respirometer. Trials were conducted for a period of 48 h, as it is during this period that stabilisation of oxygen consumption occurs for bivalves following a salinity change (Kinne, 1971; Bayne, 1973).

Respirometer

The respirometer was composed of five elliptical perspex chambers (2.3 L) in a continuous flow open circuit design. Water from each reservoir entered each chamber near the base. The flow was continuous (80 mL/min), maintained by a rotameter, and checked manually twice daily. Water left the respiration chambers from the top where it was diverted by solenoids to either waste or to a flow cell containing an Orion (Model 9708) oxygen electrode. Each of the five chambers was monitored for 10 minutes every hour and the remaining ten minutes in each hour was used to calibrate the oxygen electrode using fully aerated seawater. Data from the oxygen electrode were collected by a data logger (Data Electronics Australia, Model DT600) and downloaded to a computer at the end of each experiment.

Final mV output was converted using a LOTUS spreadsheet. Drift between calibrations was assumed to be linear (for both flow and oxygen calibrations). The oxygen used by each tank was then calculated as a percentage of the full saturation value using the calibration adjusted mV output from the oxygen electrode. Values for tanks containing animals were corrected for the oxygen uptake of the control tank and the final values were divided by the wet weight of the animals (including shell) to express results as oxygen consumption in mg O_2/kg whole wet weight/h.

Data from low salinity treatments (15 and 25) resulted in negative values for oxygen consumption, due to nutrient enrichment of the freshwater used for dilutions, resulting in elevated microbial activity. The microbial population, as indicated by oxygen consumption in the control chamber, was stable after 24 hours. Therefore, the data presented for low salinities are from the second 24 hour period. As the clams in all treatments displayed dormant periods where oxygen consumption approached zero, this number was used to correct for any microbial addition.

Algal Clearance

Two types of algae were used in separate trials to ensure clearance rates were not masked by particle size selection. The prymnesiophyte, *Pavlova lutheri* (4-6 μm) and the green flagellate, *Tetraselmis suecica* (12-14 μm) were used as these two species are common aquaculture species. Adult and juvenile (20.0 - 25.0 cm, shell length) *K. scalarina* were collected from Moulting Lagoon. Experimental treatments commenced the day after collection. Clams were transferred directly from seawater with a salinity of 35 to one of the experimental salinities ranging from 5-55 with 5 increments. For each salinity, three aerated plastic aquaria (500 mL), each containing four pre-weighed clams, were used. A control treatment containing empty shells was used to determine the natural settling rate of the algae, and hence allow correction of the data for the treatments. Salinity treatments were established by adding distilled water or an artificial salt mixture (Coral Reef Red Sea Salt®) to sand-filtered seawater with a salinity of 35. Temperature was maintained at 15°C throughout the experimental period. Water samples containing algae were taken from each of the treatments before addition of the clams and algae and again after 48 h, and then preserved with 2.5% gluteraldehyde for subsequent algal counts. Three replicates of each algal sample were counted using a haemocytometer and algal densities were extrapolated to 1 L. The mean number of algal cells present in each sample were subtracted from the number of cells originally fed to the clams and the results expressed as cells consumed/g/L.

Statistical Analysis

Respiration

Corrected data were analysed using one way ANOVA in JMP for Macintosh. Homogeneity of variances was checked using Bartlett's test of homogeneity of variances.

5.7.4 RESULTS

Respiration

Adult *K. scalarina* did not maintain a constant rate of oxygen consumption throughout the experimental period (Figure 1). Oxygen consumption for clams in 35 illustrates that respiration went through a series of fluxes, or periods of activity and quiescence. Salinity significantly affected oxygen consumption of adult *K. scalarina* ($P = 0.013$). Maximum oxygen consumption was evident in 35-45 and oxygen consumption decreased progressively at salinities either side of this range (15-25 and 55) (Figure 2). However, oxygen consumption decreased most dramatically at salinity 15 (3.62 mg/g whole weight /h), this being substantially different from all other treatments including the maximum of 10.78 mg/g /h at 35.

Algal Clearance

The highest number of *P. lutheri* cells consumed by adult *K. scalarina* was in the 25-50 treatments (Figure 3A). Algal consumption decreased dramatically in salinities outside this range, however, there was no substantial difference in algal consumption between 25-50. A similar pattern of consumption of *P. lutheri* was displayed by juvenile *K. scalarina* (Figure 3B), with high rates between 25-50, however, algal consumption decreased in salinity treatments greater than 45 and less than 25.

Consumption of *T. suecica* by adult *K. scalarina* (Figure 4A) displayed a similar pattern to *P. lutheri*. With high consumption rates between 25-50 and a decrease outside this range. However, the decrease was not as pronounced as was displayed with *P. lutheri*. Juvenile consumption of *T. suecica* was highest between 25-50 (Figure 4B). Again, algal consumption of clams displayed a marked decline in salinities outside this range with the highest algal consumption by juveniles being at 25-40.

5.7.5 DISCUSSION

Salinity had a significant effect on the oxygen consumption of *K. scalarina*. Respiration trials indicate that *K. scalarina* undergoes an initial period of osmotic shock in which the shell valves are closed but that the clams resume a stable pattern of reduced respiration in salinities outside that of normal seawater within 24 h. Previous authors have suggested that following a salinity shock respiration rate is initially reduced, followed by a phase of stabilisation (Kinne, 1971) lasting a few hours, and leading to a new steady state within two days (Kinne, 1971; Bayne, 1973). *K. scalarina* appears to follow this general pattern.

The highest rates of oxygen consumption for adult *K. scalarina* were in the 35 and 45 salinity treatments. Decreases were also evident in 25, within the salinity tolerance range, and at 15 and 55 which are outside the salinity tolerance range. Other authors have reported that marine invertebrates display a range of responses to a change in the ambient salinity. The rate of oxygen consumption by *Amphibola crenata*, a marine gastropod, is unaffected by salinity, in the range 0-125% sea water (Shumway, 1981). However, Shumway and Koehn (1982) found that the oxygen consumption rate of whole oysters, *Crassostrea virginica*, increased with decreasing

salinity at 10 and 20°C. Immediate temporary elevation of oxygen consumption following salinity change may result from an increased overall alertness by the animal to counteract physiological stress (Kinne, 1971).

Similarly, high salinity treatments also resulted in decreased oxygen consumption. *K. scalarina* in 35 treatments had an oxygen uptake of 10.78 mg/g/h while clams in 55 treatments had an oxygen uptake of 7.33 mg/g/h. Brown and Meredith (1981) report that high salinities induced a marked reduction in the rate of oxygen uptake of the whelk, *Bullia digitalis*. At 45 mean transformed oxygen consumption was 388 µg/h and at 51 it was 241 µg/h. The decrease in oxygen uptake in *B. digitalis,* associated with salinity changes, was attributed in part to decreased activity (Brown and Meredith, 1981). In this study *K. scalarina* were held out of the substrate in smooth sided tanks and thus little movement of any kind occurred. *K. scalarina* is a sediment dwelling bivalve that, excluding burrowing behaviour, normally displays limited movement in its natural environment (Bellchambers, 1993). Thus it seems unlikely that inactivity alone explains the reduction in oxygen consumption of *K. scalarina*. *K. scalarina* exposed to salinities outside its natural range may experience a reduction in the respiratory current which may account for the reduced oxygen consumption and algal clearance rates recorded. Shumway (1979), working with 11 species of gastropod molluscs, reported withdrawal into the shell as a primary response to decreased salinity, oxygen uptake ceasing thereafter.

Comparison of the algal clearance rates of adult and juvenile *K. scalarina* suggest that both groups respond to changes in ambient salinity in a similar manner. Both groups display a substantial decrease in algal clearance in salinities outside the salinity tolerance range. Juvenile *K. scalarina* appear to display a zone of optimal algal clearance between 25 and 45, while for adults it was 25-50. The depressed consumption at 50 by juveniles is consistent with differences in survival rates between adults and juveniles at this salinity (Manuscript 5). A notable difference between survival and food consumption patterns was the depressed consumption by juveniles at low salinities (5-20) within their tolerance range (5-45). Nell and Paterson (1997) report that the algal consumption of *K. rhytiphora* spat (34.4±0.5 mg) was highest in salinities between 30-35. Similarly, the growth of *K. rhytiphora* in terms of shell length was significantly higher within this range indicating that there is a strong correlation between algal consumption and growth.

5.7.6 CONCLUSION

In certain bivalves cessation of the respiratory current may protect the pallial organs from osmotic stress and the molluscan gill may be similar in this respect. However, complete and extended cessation of the respiratory current or filtration rate of *K. scalarina* is unlikely and this is also evident in the 24 h pattern for oxygen consumption (Figure 1). The dramatic decrease in oxygen consumption rates and algal clearance in salinities <25 is in accordance with the salinity tolerance range for adults. While juvenile and adult *K. scalarina* displayed a decrease in algal clearance in salinities <25, there is still evidence of clearance at low salinities, suggesting that shell closure cannot be continuously isolating the clams from their external environment. Similarly, adult clams in low salinity treatments (15-25) were still respiring, although at a reduced rate.

Oxygen consumption trials indicate a limitation of oxygen uptake at salinities outside the range 35-45 even if those salinities were within the adult tolerance range. This may in the long term decrease the scope for growth, however evidence from adult algal clearance trials does not support such a narrow optimum range but rather an optimum range of 25-50. Juveniles appear to have some restriction in salinities greater than 45 and less than 25. While *K. scalarina* survives a range of salinities, optimal salinity for growth, as inferred from the physiological

processes investigated, may be a somewhat narrower band. Unless clam farms are located in upper estuarine areas, this should not lead to lengthy periods of depressed growth.

5.7.7 ACKNOWLEDGMENTS

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5.7.8 REFERENCES

Bayne, B. L. (1973). The responses of three species of bivalve mollusc to declining oxygen tension at reduced salinity. *Comparative Biochemistry and Physiology* **45A**, 793-806.

Bayne, B. L. and Newell, R. C. (1983). Physiological energetics of marine molluscs. In *The Mollusca, Physiology Part 1*. Ed. by S. M. Saleudin and K. M. Wilbur, pp 407-515. Academic Press, New York.

Bellchambers, L. M. (1993). Aspects of the ecology of *Katelysia scalarina* (Bivalvia: Veneridae), a commercially exploited cockle. Unpublished Honours Thesis, University of Tasmania, Hobart.

Brown, A. C. and Meredith, F. L. (1981). The effects of salinity changes on respiration in the sandy-beach whelk *Bullia digitalis* (Dillwyn). *Comparative Biochemistry and Physiology* **69A**, 599-601

Dame, R. F. (1996). *Ecology of Marine Bivalves: an Ecosystem Approach*. CRC, Boca Raton, FL.

Deaton, L. E. (1992). Osmoregulation and epithelial permeability in two euryhaline bivalve molluscs: *Mya arenaria* and *Geukensia demissa*. *Journal of Experimental Marine Biology and Ecology* **158**, 167-177.

Higgins, P. J. (1980). Effects of food availability on the valve movements and feeding behaviour of juvenile *Crassostrea virginica* (Gmelin). I. Valve movements and periodic activity. *Journal of Experimental Marine Biology and Ecology* **45**, 229-244.

Jørgensen, C. B., Larsen, P. S., Møhlenberg, F. and Riisgård, H. U. (1986). The bivalve pump. *Marine Ecology Progress Series* **34**, 69-77.

Kinne, O. (1971). Salinity: animals - invertebrates. In *Marine Ecology*. Ed. by O. Kinne, pp 821-995. Wiley Interscience, London.

Nell, J. A. and Patterson, K. J. (1997). Salinity studies on the clams *Katelysia rhytiphora* (Lamy) and *Tapes dorsatus* (Lamarck). *Aquaculture Research* **28**, 115-119.

Newell, R. C. (1973). Factors affecting the respiration of intertidal invertebrates. *American Zoologist* **13**, 513-528.

Pamatmat, M. M. (1993). Measuring aerobic and anaerobic metabolism of benthic infauna under natural conditions. *Journal of Experimental Biology* **228**, 405-413.

Riisgård, H. U. (1991). Filtration rate and growth in the blue mussel, *Mytilus edulis* Linneaus, 1758: dependence on algal concentration. *Journal Shellfish Research* **10**, 29-35.

Shumway, S. E. (1977). Effect of salinity fluctuation on the osmotic pressure and Na^+ Ca^{2+} and Mg^{2+} ion concentrations in the haemolymph of bivalve molluscs. *Marine Biology* **41**, 153-177.

Shumway, S. E. (1979). The effects of fluctuating salinity on respiration in gastropod molluscs. *Comparative Biochemistry and Physiology* **63A**, 279-283.

Shumway, S. E. (1981). Factors affecting oxygen consumption in the marine pulmonate *Amphibola crenata* (Gmelin, 1791). *Biological Bulletin* **160**, 332-347.

Shumway, S. E. (1982). Oxygen consumption in oysters: an overview. *Marine Biological Letters* **3**, 1-23.

Shumway, S. E. (1996). Natural Environmental Conditions. In *The Eastern Oyster Crassostrea virginica*. Ed. by V. S. Kennedy, I. E. Newell and A. F. Eble, pp 467-513. Maryland Sea Grant College, College Park, Maryland, MD.

Shumway, S. E. and Koehn, R. K. (1982). Oxygen consumption in the American oyster *Crassostrea virginica*. *Marine Ecology Progress Series* **9**, 59-68.

Stickle, W. B. and Bayne, B. L. (1982). Effects of temperature and salinity on oxygen consumption and nitrogen excretion in *Thais* (*nacella*) *lapillus* (L.). *Journal of Experimental Marine Biology and Ecology* **58**, 1-17.

Storey, K. B., and Storey, J. M. (1990). Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *Quarterly Review of Biology* **65** (2), 145-174.

Vernberg, F. J. and Vernberg, W. B. (1981). *Functional Adaptions of Marine Organisms*. Academic Press, New York, NY.

5.7.9 FIGURES

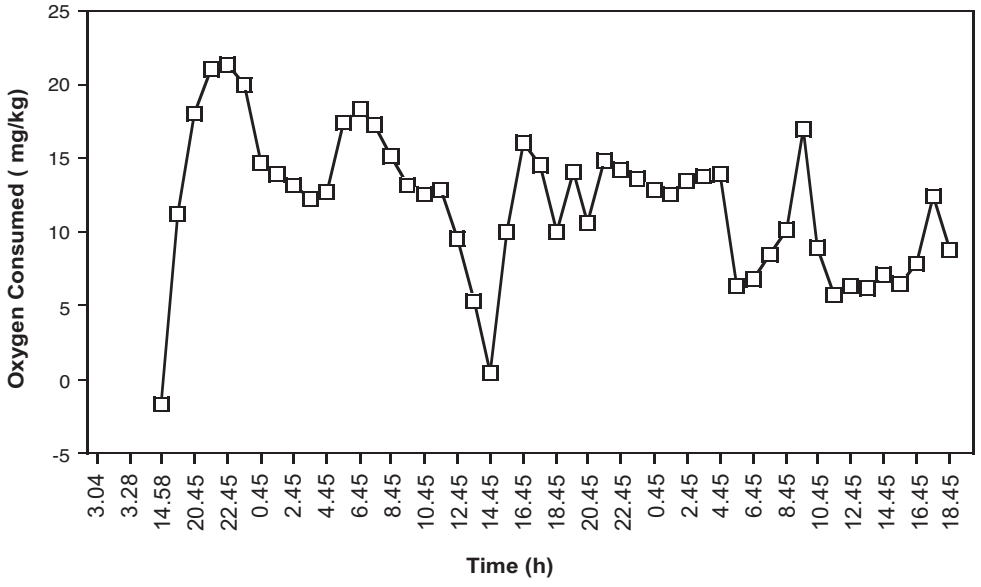

Figure 1. 24 h pattern of oxygen consumption of adult *K. scalarina* in 35 treatment.

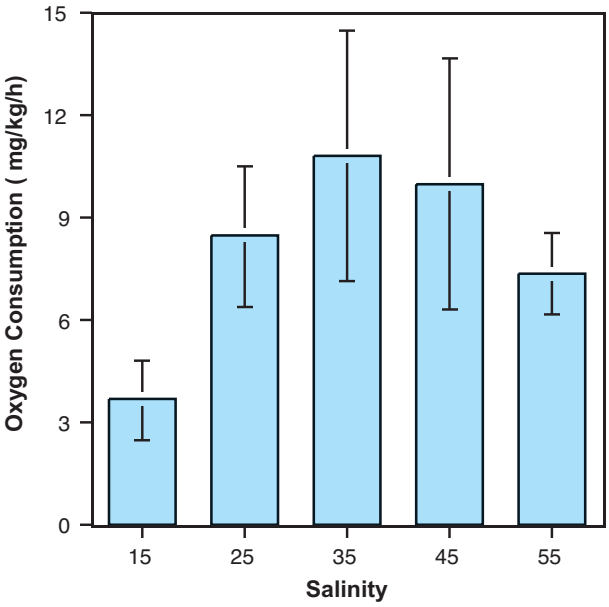

Figure 2. Oxygen consumption of adult *K. scalarina* (mean ± S.E.).

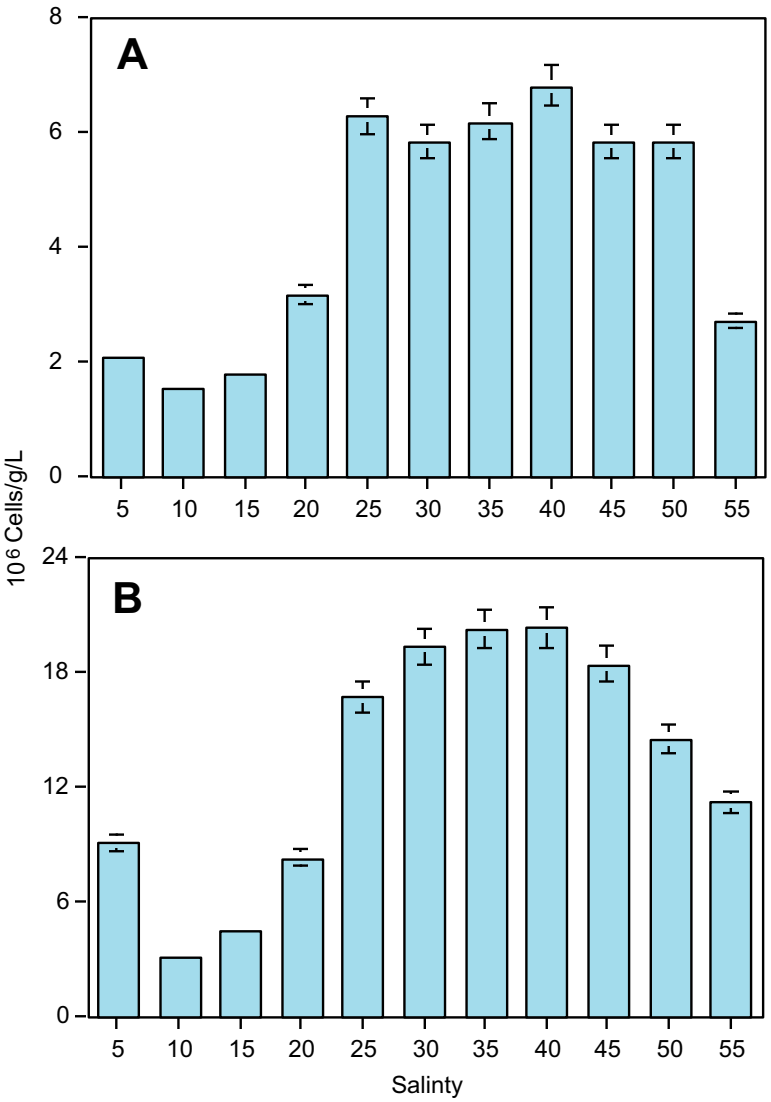

Figure 3. Clearance of *Pavlova lutheri* by *K. scalarina* (mean ± s.e.). A) adults B) juveniles.

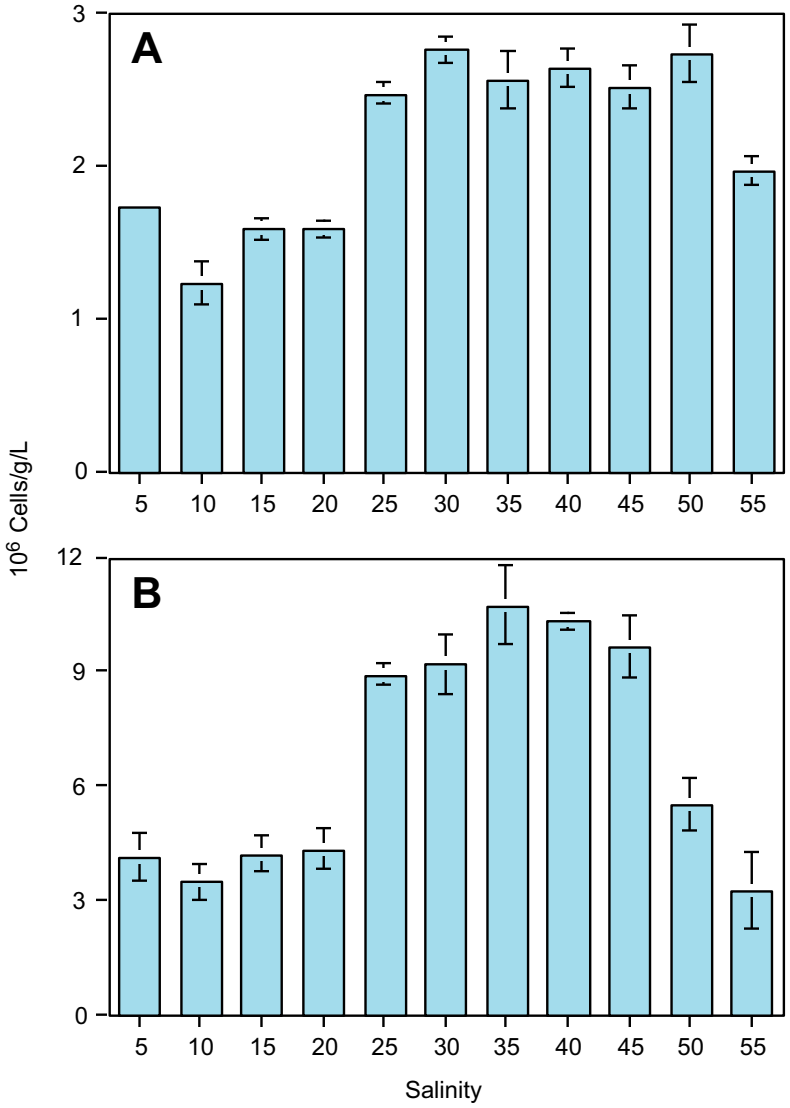

Figure 4. Clearance of *Tetraselmis suecica* by *K. scalarina* (mean ± S.E.). A) adults B) juveniles.

5.8 MANUSCRIPT 8

Absence of effects of intra-specific competition on the growth and survival of *Katelysia scalarina* (Lamarck, 1818) (Bivalvia: Veneridae).

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5.8.1 ABSTRACT

Clam density manipulations were conducted in plastic mesh cages in the intertidal zone of Moulting Lagoon, Coles Bay, Tasmania over 10.5 months. Experiments manipulations utilised a range of artificially increased and decreased densities (57.2 - 1886.9 clams.m^{-2}) to investigate the effect of intra-specific competition on the survival, growth and condition index of the venerid clam *Katelysia scalarina.* The effects of density on juvenile recruitment and macrobenthos were also measured. None of the measured parameters displayed a significant response to density manipulations of *K. scalarina* except for a significant (P=0.04) but slight effect on shell height when sediment pH was included as a covariate. Survival at this site was very high (usually ≥95%).

The failure of *K. scalarina* to respond to density treatments up to five times that of the natural population may in part be due to the location of the experimental treatments. Experimental cages were situated low in the intertidal zone in a location that sustained much faster growth than in an equivalent study in Western Australia. It is possible that the clams had access to an abundant food source, thereby negating the effects of artificially enhanced densities.

These factors are important considerations in the management of natural and enhanced populations and the establishment of any marine farming venture. The finding that growth survival and condition index were density-independent over a wide density range is promising from an aquacultural or fisheries enhancement perspective. Moulting Lagoon would be an attractive location for an aquaculture or fisheries enhancement project, however, as an environmentally sensitive location it is unlikely that such usage would be permitted.

5.8.2 INTRODUCTION

Competitive interactions among invertebrates in soft sediments are attributed to a variety of mechanisms: direct interference (Woodin, 1974; Peterson and Andre, 1980; Wilson, 1983; Weinburg, 1985), indirect interference by alteration of environmental conditions (Rhodes & Young, 1970; Reise, 1985), competitive exclusion (Woodin, 1976), inhibition of larval settlement (Woodin, 1976; Andre & Rosenburg, 1991) and food depletion (Levinton, 1972; Buss & Jackson, 1981; Peterson, 1982b; Olafsson, 1986; Peterson & Black, 1988; Peterson & Beal, 1989) (see Appendix 6 for review). The existence of intra-specific competition has been recognised among suspension feeding bivalves in many soft sediment habitats (Peterson, 1982b; Bertness, 1985; Weinburg, 1985; Olafsson, 1986; Peterson & Black, 1987; Peterson & Beal, 1989; Vincent *et al.*, 1994), however the extent to which density dependent processes influence the life history of suspension feeders is unclear (Jensen, 1993).

Manipulative field experiments have consistently demonstrated that dense assemblages of suspension feeding bivalves do not exhibit density-dependent mortality, even when competition is evidenced by reductions in growth (Peterson & Andre, 1980; Peterson, 1982b; Peterson & Black, 1987; Peterson & Beal, 1989; Peterson, 1992). Several lines of evidence suggest

that food limitation is the cause of growth reductions at high densities (Peterson, 1992). Hydrodynamic studies illustrate the ability of suspension feeders to deplete local supplies of benthic phytoplankton which may result in growth inhibition of individuals (Frechette & Bourget, 1985a; Frechette & Bourget, 1985b; Frechette *et al.*, 1989: Monismith *et al*., 1990). The fact that bivalves grow less in high density patches implies that local resource limitation of some description is a common phenomenon for soft bottom suspension feeders in shallow waters (Peterson & Black, 1993). However, doubts over the prevalence of local food resource limitation among suspension feeders still persist (Levinton, 1972; Olafsson, 1986) due to the variable and unpredictable nature of phytoplankton populations.

Adult clams are presumed to interfere with colonising larvae either through direct predation (Woodin, 1976; Andre & Rosenburg, 1991) or physical disturbance and habitat alteration (Woodin, 1976; Peterson, 1982b; Andre & Rosenburg, 1991; Jensen, 1992). However, it has been suggested that recruitment of *K. scalarina* is increased around high densities of adult bivalves (Peterson & Black, 1993), even though the larvae of some bivalves may be ingested by adults and treated as food particles (Andre & Rosenburg, 1991).

Previous density manipulations have focused on detecting the negative effects of crowding, but it is possible that at high densities the penalties of competition are outweighed by positive effects via mutualistic, commensal, facilitative or promotive interactions (Peterson & Black, 1993) such as predator protection and habitat stability. Some doubt still prevails regarding the effect of high densities on natural populations of bivalves (Jensen, 1993), and what role competition plays in structuring soft sediment habitats, as not all bivalve species respond in the same manner to varying density (Peterson & Black, 1987). This research is also relevant to culture of the species as stocking density can influence profitability of many forms of aquaculture (Treadwell *et al*., 1991, 1992). The effects of clam density in aquaculture trials are reviewed in Manuscript 11.

Katelysia scalarina (Lamarck 1818) is a small intertidal bivalve that occurs in sheltered bays and estuaries where it is frequently the dominant faunal component (Wells & Roberts, 1980; Wells & Threlfall, 1980; Coleman, 1982; Roberts, 1983). In southern Australia *K. scalarina* lives in fine to medium grain sand, two to four centimetres below the surface and grows to approximately 40 mm in size (Nielsen, 1963). A small commercial fishery for *K. scalarina* exists in several of the larger bays and estuaries of Tasmania's east coast. Recently, concern has been centred on sustaining and protecting naturally occurring clam populations by establishing efficient methods for clam mariculture or fisheries enhancement. However, despite its apparent economic value and abundance in soft sediment habitats of southern Australia, little is know about the life history and biology of the species. Therefore before the appropriateness of enhancing a clam fishery can be established, an understanding of the structuring forces on natural populations of *K. scalarina* is essential.

In the present study the effects of intraspecific competition on the mortality, growth and recruitment of *K. scalarina* and the associated macrobenthic community are examined. Experiments included in this study were designed to include not only elevated abundances, but also several artificially reduced abundances to test the positive and negative consequences of density.

Study Site

Moulting Lagoon

Experimental manipulations of *K. scalarina* were conducted in the intertidal zone of Moulting Lagoon, a permanently open lagoon situated on the east coast of Tasmania, near Coles Bay on

the Freycinet Peninsula (42°05'S, 148°10'E) (see Fig. 1, 5.5 Manuscript 5). Partly enclosed by a bayhead spit, the lagoon is generally less than one metre deep and is fed by two rivers and several smaller streams which may cease to flow during summer (Dec.- Feb.) (Blackhall, 1986). The lagoon has a total water surface area of approximately 41.5 km^2 when full.

Moulting Lagoon has a maximum tidal range between 0.8 m at the mouth and 0.3 m in its upper reaches; the tidal rhythm is semi-diurnal with significant diurnal inequality. At low tide large areas of the estuary become exposed as sand or mud flats, these are most extensive in the lower and middle estuary where the tidal range is greatest (Last, 1983). The lagoon sediments are composed of sands derived from Triassic sandstone and mudstone.

5.8.3 METHODS

Initial Sampling

Juvenile *K. scalarina* (20-25 mm shell length) were collected from Meredith Point, Pelican Bay, Moulting Lagoon between 26th Feb-1st March 1996. Experimental manipulations were established in the intertidal zone of Swanwick Bay, Moulting Lagoon. The size frequency distribution of clams used in the experiments were narrow, to minimise differences between experimental treatments, and because insufficient numbers of smaller clams were available. Clams were measured along the longest antero-posterior axis (length) and dorso-ventral margin, the distance from hinge to opposite margin (height) to the nearest 0.1 mm with vernier callipers and randomly allocated to a density treatment.

Density manipulations were conducted inside cages designed to prevent migration, so that densities could be maintained over the experimental period. Cages were also used to prevent the access of large mobile consumers such as crabs and wading birds to the clams. As all density treatments were enclosed identically, any additional effects of enclosures were held constant across the treatments. Cages were constructed of 9 mm^2 Nylex^R mesh (33 cm wide x 53 cm long x 14 cm) and fastened with plastic cable ties to wooden stakes driven into the substrate. Density treatments were established by adding the appropriate number of measured *K. scalarina* to enclosures to achieve a range of densities from 57.18 - 1886.94 clams m^{-2}. Sediment below the enclosures were removed to a depth of 10 cm and returned to the cages by sieving through a 2 mm sieve to remove all potential predators and competitors.

Density treatments were established in a random block design, 90 m from the high tide mark, since this is the zone is where the natural population reaches its highest abundance and greatest shell size (Bellchambers, 1993). Nine densities were used, with four replicates of each treatment across two fully randomised blocks. Densities ranged from 10 to 330 clams per cage with increments of 40. Neighbouring cages were separated by 1 m and blocks were separated by 5 m (Fig. 1).

Final Sampling

Density manipulations were sampled between 13-17th January 1997, approximately one year after establishment. All cages were removed, live clams remeasured and mortalities recorded. Missing clams i.e. shells were a consequence of either removal by scavengers/predators, postmortem transport, predation or sampling error. Dead shells were classified as undamaged, drilled (by gastropods), or chipped (by crabs). Undamaged empty shells are the result of starvation, physiological stress or predation that leaves no evidence on the shell (Peterson & Black, 1993). Live clams were re-measured to determine whether density affected growth. Similarly, recording mortalities and the nature of the mortality allowed estimates of predation

and predation type at various densities. The number and size of new recruits, and the number and species of macrobenthos in each treatment were recorded to examine whether recruitment and predation intensity and type varied with local clam density. Macrobenthos was defined as organisms retained in the cages after removal from the experimental plots and therefore included only larger macrobenthos which may have been competing for food resources or potential predators, such as crabs, gastropods, other bivalves and small fish.

A subsample of 30 clams per cage was measured and weighed for calculation of condition index, to determine body condition (see Chapter 2). Wet shell and meat weights were recorded to the nearest 0.01g after external moisture was removed with absorbent materials.

Four sediment samples, redox and pH measurements were collected from both inside and outside (1m) the a low (50) and high (250) density treatment to determine whether the presence of large aggregations of suspension feeders altered the sediment characteristics. Sediment samples were analysed for organic content and phi size. Samples were sieved through a 500 µm sieve and placed in a drying oven at 80°C for 24 h. To determine organic content, 10 g of dried sample were placed in a muffle furnace for 4 h at 450°C and percentage loss of the fraction recorded (Allen, 1989). Adjustments were made for calcium carbonate by acid digestion (Allen, 1989). The remaining proportion of each sediment sample was sorted into constituent grades through a series (<63 µm, 63 µm, 125 µm, 250 µm and 500 µm) of interlocking Endicott sieves to determine phi size (Gray, 1981).

Statistical analysis

Data for growth (length and height), survival, meat ratio and number of recruits were analysed using two way ANOVA in JMP 3.0 for Macintosh (SAS Institute, 1995) with density and block as fixed factors. Non significant results were re-analysed using one way ANOVA, averaging for block, if block and block x density interactions were non significant. Bartlett's test for homogeneity of variances was applied to all data to check equality of variances (Sokal and Rohlf, 1981). Survival data was transformed as arcsin (% survivalx0.01)$^{0.5}$ and the normality of the data was tested using the Shapiro-Wilk test (Zar, 1984).

Soil data were analysed as a series of covariates. Sediment characteristics were tested as both simple covariates and in a factorial design to determine whether the effect of sediment characteristics depended on the treatment level.

5.8.4 RESULTS

Survival

K. scalarina displayed high survival rates (usually >95%) across all experimental treatments regardless of density (Figure 2), with the lowest survival rate being 81% in the 90 clam treatments in Block B. However, 100 % survival was evident only in the lowest density treatment in Block A. There was no significant effect of density on survival (P = 0.404) and the interaction between density and block was not significant (P = 0.431), but a marginally significant block effect was evident (P = 0.0402). Data were re-analysed as a one way ANOVA, averaging for block, on the basis that there was no significant block x density interaction and only a minor block effect. Again, density had no significant effect on the survival of *K. scalarina* (P = 0.492).

Clams recovered from the experimental treatments were classified into one of four categories: alive, chipped, drilled or unexplained mortality (Figure 3). Empty shells rarely displayed any sign of damage or death due to predation. The majority of dead clams (82.04%, Figure 3D)

displayed no obvious signs of predation and are assumed to have died from starvation or other physiological causes. Dead shells exhibiting predator damage were either chipped by crabs (11.02%, Figure 3C) or drilled by naticid gastropods (6.94%, Figure 3B). The type or frequency of predation did not appear to be vary with density.

Growth

Increases in length over the experimental period were small (Figure 4). The largest average increase in length was in the 210 clams cage^{-1} in both blocks, with an increase of 4.46 mm in block A and 4.44 mm in block B. The smallest increase in length in both block A and B was in the 10 clams cage^{-1} treatment, with increases of 4.03 and 3.89 mm respectively. However, there was no significant effect of density on shell length increase ($P = 0.091$).

Similarly, *K. scalarina* did not display large increases in height during the experimental period (Figure 5), the largest recorded increase was in the 50 clams cage^{-1} for both block A and B, of 4.68 mm and 4.39 mm respectively. There was no significant relationship between height and density ($P = 0.223$).

Meat ratio (% of total weight consisting of meat weight) was very consistent across the experimental treatments. The highest meat ratio was in the 290 clams cage^{-1} treatment in block A, with a value of 40.3%. The lowest meat ratio, 34.8%, was in the 330 clams cage^{-1} treatment in block B. Density did not have a significant effect on meat ratio (Figure 6, $P = 0.353$).

Recruitment

Recruitment of juvenile *K. scalarina* was low in all the experimental treatments and most treatments had no recruits at all (Figure 7). The highest numbers were found in the 170 and 290 clams cage^{-1} treatments with a total of three recruits each. There are no significant differences ($P = 0.855$) across the experimental treatments.

Macrobenthos

Numbers of macrobenthic invertebrates recovered from the treatment cages were relatively consistent over the experimental treatments (Figure 8A), with the highest number in any cage being 10 in the 170 treatment. Overall block B displayed higher numbers of retrieved macrobenthic organisms. Macrobenthic organisms were subdivided into four categories: other bivalves, gastropods, crabs and the introduced European shore crab, *Carcinus maenas*. The division of the macrobenthos into categories allowed estimates of their effects on the experimental treatments in terms of predation potential and competition.

The major macrobenthic category was crabs (61%, Figure 8B), the majority of which were the soldier crab, *Mictyris platycheles* and the shore crab, *Paragrapsus gaimardii. Mictyris platycheles* is a detritivore that processes large amounts of sand to extract unicellular algae (Edgar, 1997). *Paragrapsus gaimardii* is a common estuarine crab around most of Tasmania and Victoria and although it is a small scavenging crab that consumes a range of prey, it is incapable of crushing bivalves of the size used in these experiments. Thus despite the high numbers of these two species of crab in the experimental treatments they pose no predation threat to *K. scalarina* except possibly to juveniles recruiting to the cages.

The next numerically dominant group was gastropods (27.27%, Figure 8D). Gastropods present were primarily *Nassarius pauperatus*, *Cominella lineolata*, and *Poliniices conicus*, all of which are carnivores, drilling gastropods that prey on bivalve molluscs (Peterson, 1982a). *Bembicium auratum*, a small periwinkle which feeds on algae was also present. The abundance

of gastropods appeared to increase with clam density, however the highest frequency observed was three per treatment. Despite the number of gastropods present mortality due to gastropod, as evidenced by dead drilled shells, was low (6.94%, Figure 3B)

Several species of bivalves were also found in the experimental treatments (9.09%, Figure 8C), mainly *Eumarcia fumigata* and *Soletellina biradiata* which may have been competing with *K. scalarina* for food resources. However, as numbers of additional bivalves were low, maximum of two per cage, these additional recruits were very likely to have a significant impact on the experimental treatments.

Carcinus maenas, the European shore crab, (Figure 8E) was placed in a separate category due to its known potential as a major bivalve predator (Gee *et al.*, 1985; McGrorty *et al.*, 1990). *C. maenas* comprised 2.60% of the total macrobenthos, but did not appear to be a major cause of mortality as it contributed to at most 11.02% of the total mortality (as evidenced by chipped shells), which was mainly due to large numbers (12.94%) of *K. scalarina* consumed in one cage.

Sediment Characteristics

Of all the sediment characteristics tested (particle size, organic content, redox, and pH, see Bellchambers, 1998), only the interaction of pH and density within the treatment cages had a significant effect on shell height increase of *K. scalarina.* Inclusion of this covariate led to a significant result (P> 0.0423, Table 1) for the effect of density on shell height. Based on these adjusted treatment means, shell height gain decreased with increasing density.

Table 1. Covariate analysis of the effect of pH inside the treatment cages on the height of *K. scalarina*

Source	DF	Prob >F
Density	1	0.0414
pH	1	0.8762
Density*pH	1	0.0423

5.8.5 DISCUSSION

Survival

Competition among suspension feeding bivalves in soft sediment environments does not often, even under extreme crowding, produce mortality or competitive exclusion (Creese & Underwood, 1982; Peterson, 1982b; Peterson, 1992; Peterson & Black, 1993). In this study *K. scalarina* displayed high survival across all experimental treatments regardless of density, with the lowest survival rate being 81% in any treatment. Survival was also high at all densities assessed in another study at Moulting Lagoon provided that the clams were not in an upper beach position (Manuscript 9). Peterson & Black (1993) reported similar survivorship for *Katelysia* spp. in Princess Royal Harbour, over a period of eight months. Density manipulations conducted in four lagoonal systems in Western Australia and California, suggested that mortality of *Katelysia scalarina*, *K. rhytiphora*, *Protothaca staminea* and *Chione undatella* rarely responded to increases in density even when competition was evidenced by reductions in growth rate in all four systems (Peterson, 1982b; Peterson & Black, 1987; Peterson & Black, 1988; Peterson & Beal, 1989). In this study there was no significant effect of density on survival, however there was a trend of increasing mortality at higher densities. In comparison Peterson & Black (1993)

report that both survivorship and growth of *Katelysia* spp. decreased by 20 - 30 % in high density treatments (320 clams.m^{-2}), as evidenced by a significant increase of dead undamaged shells. In high density treatments at Moulting Lagoon, the absolute number of dead undamaged shells increased, indicating death by starvation or other physiological effects. However, on a percentage basis, density had no significant effect on survival (P=0.492). Predation was a relatively insignificant cause of mortality (16.96%). However, mortality due to predation was closely linked with the presence of *Carcinus maenas* or drilling gastropods in the macrobenthic samples.

Growth

Growth of *K. scalarina* at Moulting Lagoon is apparently insensitive to increases in density as it remained consistent across all density treatments, even when densities were thirty times the natural adult *K. scalarina* density (50-60 clams.m^{-2}). Similarly, density had only a slight effect on growth in another trial at Moulting Lagoon (Manuscript 9). Peterson & Black (1993) report that individual growth of *K. scalarina* in Princess Royal Harbour, Western Australia only rarely varied significantly between density treatments. Previous density manipulations of suspension - feeding bivalves have shown either reductions in growth with density (Peterson, 1982b; Bertness, 1985; Olafsson, 1986; Peterson & Black, 1987; Peterson & Black, 1988; Peterson & Beal, 1989; Jensen, 1992) or failure to respond to density (Peterson & Black, 1993). Density manipulations which have revealed marked declines in growth with increased density are primarily from oligotrophic coastal lagoons (Peterson, 1982b; Peterson & Black, 1987), while examples where crowding induced small reductions in growth (Peterson & Beal, 1989), or failed to result in any detectable effect (Olafsson, 1986) were from nutrient-rich systems. Moulting Lagoon is characterised by persistently clear waters, a large tidal range, regular flushing, and is populated by relatively high densities of suspension feeding bivalves. Natural densities of *Katelysia* spp. populations in Princess Royal Harbour (~160 clams.m^{-2}) are also much higher than Moulting Lagoon populations (~60 clams.m^{-2}).

Moulting Lagoon does not fit clearly into either of the above categories, but the failure of clams to respond to density treatments may be due the position of the experimental treatments within the intertidal zone. Density manipulations were conducted low on the shore, approximately 90 m from the high tide mark, as it is in this region that *K. scalarina* reaches its highest abundance and size (Bellchambers, 1993). Previous authors report no effect of density on shell growth in low shore treatments (Peterson & Black, 1987; Vincent *et al.*, 1994). It has been suggested that the absence of density dependence in low shore treatments is a consequence of longer submergence time, allowing bivalves to obtain organic matter and an undepleted food source as little or no extraction of organic matter by other suspension-feeders has yet occurred (Peterson & Black, 1987). Other explanations may include the high percentage of sand (Olafsson, 1986) and high mobility of sediment, which enhances the resuspension of the benthic diatoms and other deposited particles. Thus, suspension feeders experience an unpredictable and variable food supply during the growing season (Peterson & Black, 1988). Therefore periods of intense competition for food resources are interspersed with periods of abundant food supply, rendering mortality or long term growth restrictions due to inadequate food resources unlikely.

Comparable studies of *K. scalarina* in Princess Royal Harbour reported growth rates of 0.031 mm.month^{-1} (Peterson & Black, 1993), but the lowest increase in this study (0.324 mm.month-^{1}) was approximately ten times higher. The high growth rate in comparison to the work of Peterson & Black (1993) suggests that *K. scalarina* in high density treatments were not suppressing shell growth in order to maintain body tissue and basic metabolic processes. Conversely, the

failure of *K. scalarina* to display a decrease in meat ratio indicates that clams in higher density treatments were not allocating resources to shell growth at the expense of body condition. It would appear that adequate resources were available to maintain both body tissue and shell growth. However, the relationship between the growth rate of enclosed populations and the natural population in this area remains unclear.

Sediment characteristics

The main factor influencing organisms in soft sediment habitats is the nature of the sediment (Raffaelli & Hawkins, 1996). Examination of sediment characteristics, such as phi size, organic content and redox potential, within and outside treatment cages did not significantly affect the growth of *K. scalarina.* However, density and the interaction between density and pH inside the treatment cages displayed a significant effect on the height of *K. scalarina*. These differences may be due to sedimentary modifications created by the cages (Hulberg & Oliver, 1980). Many authors have reported that although soft sediment organisms are intimately associated with and reliant on the sediment for survival, dense aggregations of infauna have the ability to significantly alter the physical and chemical structure of their habitat (Rhodes, 1974; Reise, 1985; Hall *et al.*, 1993; Woodin, 1997). Thus these result should be interpreted with caution, as while pH may be influencing the growth of *K. scalarina*, conversely the dense aggregations may be affecting the pH of sediment within the treatment cages. This conclusion is supported by the fact that pH outside the treatment cages was not a significant covariate.

Predation

The experimental manipulations failed to exclude all potential predators. The cage design prevented access of large mobile consumers such as fish, rays, starfish and wading birds, however a number of small macrobenthic predators were found in the cages. Two main types of predators were found, naticid gastropods and the crab*, Carcinus maenas*. These types of predation leave distinctive signs: naticid gastropods leave characteristically drilled shells behind, whereas crab predation can be similarly inferred from crushed and chipped shells. *C. maenas* is an efficient predator of benthic invertebrates (Jensen & Jensen, 1985; Reise, 1985) and experiments have shown they can greatly decrease prey populations (Gee *et al.*, 1985; McGrorty *et al.*, 1990). Experiments were not specifically designed to test predation, therefore predation was limited to cages which predators were able to penetrate. Despite this, predation was not a major cause of mortality at Moulting Lagoon, with only 16.96% of dead shells displaying signs of predation and, despite previous reports, only 11.02% were attributable to *C. maenas*. Peterson & Black (1993) report that mortality of *Katelysia* spp. due to these categories of predation was also low in Princess Royal Harbour. Macrobenthic predators were present at Moulting Lagoon, however average densities were less than four crabs cage^{-1} which may be insufficient to cause significant mortality. Mackinnon (1997) states that predation of *K. scalarina* by *C. maenas*, in feeding trials, decreases dramatically once clams exceed a preferred size range, with 5.25 clams (5-15 mm shell length) consumed per hour while only 0.5 clams (16-29 mm shell length) were consumed in the same time period. Clams used at Moulting Lagoon were in excess of 20 mm suggesting that the absence of predation by *C. maenas* may be due to the clams exceeding the preferred prey size range. Previous authors have suggested that high densities of suspension feeders act in a positive manner to prevent predation, as the structural complexity of dense shell assemblages may prevent the access of predators (Peterson & Black, 1993) thereby indirectly affecting survivorship.

Recruitment

The majority of previous studies have detected negative influences of adult density on larval settlement or juvenile recruitment (Woodin, 1976; Peterson & Andre, 1980; Peterson, 1982b;

Andre & Rosenberg, 1991; Jensen, 1992; Olafsson *et al.*, 1994; Thrush *et al.*, 1996). Larvae that respond positively to the presence of adult suspension feeders may be subjected to greater risk of cannibalism immediately prior to settlement (Woodin, 1976; Andre & Rosenberg, 1991) but this risk appears to be small (Ertman & Jumars, 1988). Experimental manipulations at Moulting Lagoon displayed no significant effect of adult density on juvenile *K. scalarina* recruitment, however as the total number of juveniles present was low it is difficult to infer trends. Previous studies have suggested that *K. scalarina* demonstrate higher rates of recruitment where adults are most dense, however this trend was detectable in only one of the two experimental years (Peterson & Black, 1993). The failure of juvenile recruitment of *K. scalarina* at Moulting Lagoon to respond to adult densities may be due to the sporadic and infrequent spawning of adults in the lagoon (pers. obs.) rather than a reflection of negative cues, density induced responses or confounding effects of the mesh cages on recruitment.

Macrobenthos

Previous studies have reported a failure of benthic invertebrates to respond to manipulations of suspension feeding bivalves (Peterson, 1982b; Hunt *et al.*, 1987) and imply that competitors do not increase at low densities. Density manipulations of *K. scalarina* at Moulting Lagoon displayed no apparent effect on the macrobenthic abundance. Macrobenthic invertebrates collected in the cages were low, with a total of 154 individuals present across all density treatments. *Mictyris platycheles* and *Paragrapsus gaimardii* composed 61.04% of the collected macrobenthic assemblage. Both these species are common estuarine crabs and are abundant in Moulting Lagoon. Bivalves composed 9.09% of the cage macrobenthos, and as total numbers were so low it is unlikely that they are competing with *K. scalarina* for food. The other two groups of macrobenthic invertebrates, drilling gastropods and *C. maenas*, represent potential predators of *K. scalarina*, however predation was a minor cause of total mortality. Peterson & Black (1988) also demonstrated that total densities of smaller invertebrates failed to respond to changes in *Katelysia* spp. density. A growing body of evidence that suggests that increased densities of benthic suspension feeders in soft sediments do not cause significant reductions in recruitment of benthos (Peterson, 1982b; Hunt *et al.*, 1987; Ertman & Jumars, 1988 ; Peterson & Black, 1988). Peterson (1979) suggests this is because competition is relatively ineffective in structuring communities of benthic infauna in soft substrate.

5.8.6 CONCLUSION

The failure of *K. scalarina* populations at Moulting Lagoon to display density dependent responses supports previous suggestions that competition plays a relatively insignificant role among suspension-feeding invertebrates in soft sediments (Peterson & Black, 1993). However, the physiological stress caused by prolonged exposure to high densities may manifest itself in ways not examined in the scope of this study. *Katelysia* spp. with a history of high density may be physiologically stressed from crowding in some way that enhances their susceptibility to other stresses (Peterson & Black, 1988). Clams exposed to high densities for prolonged periods may alter their ability to withstand or survive environmental fluxes (Peterson & Black, 1988) such as temperature and salinity fluctuations which prevail in estuarine systems. Peterson & Black (1988) suggested that prolonged physiological stress caused by density manipulations may also make clams more prone to pollution, disease and harmful algal blooms.

The absence of density dependent competition in *K. scalarina* at Moulting Lagoon may be due to abundant resources, both food and space, to support a higher population density than currently exists. However, this raises the question of what prevents natural populations from achieving their optimal density. Peterson & Andre (1980) suggest that the potential for competition

may exist but is not realised because of infrequent physical and biological disturbances which maintain low infaunal densities. This is certainly not the case with *K. scalarina* in caged experiments but may partially explain the patterns of abundance and distribution in natural populations. Alternatively, populations in these systems may be limited by some other factor such as high predation of pre-recruits or erratic recruitment patterns.

The very high survival rates and relatively fast growth, at least for this species, recorded at Moulting Lagoon, along with the apparent variability in recruitment and the demonstrated density-independence for survival, growth and condition index, suggest that this site has potential for fisheries enhancement or aquaculture. However, environmental concerns, including impacts on birds that prey on *K. scalarina*, would probably preclude such initiatives particularly if predator exclusion netting was needed (see Manuscript 14).

5.8.7 ACKNOWLEDGMENTS

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5.8.8 REFERENCES

Allen, S. E. (1989). Chemical Analysis of Ecological Materials. Oxford, Blackwell Scientific Publications. 368.

Andre, C. and Rosenberg, R. 1991**.** Adult-larval interactions in the suspension-feeding bivalves *Cerastoderma edule* and *Mya arenaria*. *Mar. Ecol.- Prog. Ser.* **71**, 227 - 234.

Bellchambers, L. M. 1993. Aspects of the ecology of Katelysia scalarina (Bivalvia: Veneridae), a commercially exploited cockle. Honours Thesis, University of Tasmania, Hobart.

Bellchambers, L. M. 1998. Ecology and ecophysiology of Katelysia scalarina, (Bivalvia: Veneridae), a commercially important clam. Unpublished PhD Thesis, University of Tasmania, Launceston.

Bertness, M. D. 1985. Population dynamics of the ribbed mussel, *Geukensia demissa*: the costs and benefits of an aggregated distribution. *Oecologia* **67**, 192-204.

Blackhall, S. A. 1986. Moulting Lagoon, Proposed Game Reserve. National Parks and Wildlife Service Tasmania.

Buss, L. W. and Jackson, J. B. C. 1981**.** Planktonic food availability and suspension-feeder abundance: evidence of in situ depletion. *J.. Exper. Mar. Biol. Ecol.* **49**, 151-162.

Coleman, N. 1982**.** Population density and biomass of the bivalves *Tellina mariae* and *Katelysia rhytiphora* from a seagrass bed in Western Port, Victoria. *J. Malacol. Soc. Aust.* **5**, 141-149.

Creese, R. G. and Underwood, A. J. 1982**.** Analysis of inter- and intra- specific competition amongst intertidal limpets with different methods of feeding. *Oecologia* **53**, 337 - 346.

Edgar, G. J. 1997. *Australian Marine Life*. Reed Books, Kew.

Ertman, S. C. and Jumars, P. A. 1988**.** Effects of bivalve siphonal currents on the settlement of inert particles and larvae. *J. Mar. Res.* **46**, 797-813.

Frechette, M. and Bourget, E. 1985a**.** Energy flow between the pelagic and benthic zones; factors controlling particulate organic matter available to an intertidal mussel bed. *Can. J. Fish. Aquat. Sci.* **42**, 1158 - 1165.

Frechette, M. and Bourget, E. 1985b**.** Food-limited growth of *Mytilus edulis* in relation to the benthic boundary layer. *Can. J. Fish. Aquat. Sci.* **42**, 1166 - 1170.

Frechette, M., Bulman, C. A. and Geyer, W. R. 1989**.** The importance of boundary-layer flow processes in supplying phytoplankton to the benthic suspension-feeder *Mytilus edulis*. *Limnol. Oceanogr* **34**, 19 - 36.

Gee, J. M., Warwick, R. M., Davey, J. T. and George, C. L. 1985**.** Field experiments on the role of epibenthic predators in determining prey densities in an estuarine mudflat. *Est. Coast. Shelf Sci.* **21**, 429-448.

Gray, J. S. 1981. *The Ecology Of Marine Sediments*. Cambridge University Press, Cambridge.

Hall, S. J., Robertson, M. R., Basford, D. J. and Fryer, R. 1993. Pit-digging by the crab *Cancer pagurus*: a test for long term, large-scale effects on infaunal community structure. *J. Anim. Ecol.* **62**, 59 - 66.

Hulberg, L. W. and Oliver, J. S. 1980. Caging manipulations in marine soft-bottom communities: Importance of animal interactions or sedimentary habitat modifications. *Can. J. Fish. Aquat. Sci.* **37**, 1130-1139.

Hunt, J. H., Ambrose, W. G. and Peterson, C. H. 1987**.** Effects of the gastropod, *Ilyanassa obsoleta* (Say), and the bivalve, *Mercenaria mercenaria* (L.), on larval settlement and juvenile recruitment of infauna. *J. Exper. Mar. Biol. Ecol.* **108**, 229-240.

Jensen, K. T. 1992**.** Dynamics and growth of the cockle, *Cerastoderma edule*, on a intertidal mud flat in the Danish Wadden Sea: effects of submersion time and density. *Neth. J. Sea Res.* **28**, 335-345.

Jensen, K. T. 1993**.** Density-dependent growth in cockles (*Cerastoderma edule*): evidence from interannual comparisons. *J. Mar. Biol. Ass. U.K.* **73**, 333 - 342.

Jensen, K. T. and Jensen, J. N. 1985. The importance of some epibenthic predators on the density of juvenile benthic macrofauna in the Danish Wadden Sea. *J. Exper. Mar. Biol. Ecol.* **89**, 157-174.

Last, P. 1983. Ecology and zoogeography of Tasmania shore fishes. Unpublished PhD Thesis, University of Tasmania, Hobart.

Levinton, J. 1972**.** Stability and trophic structure in deposit - feeding suspension - feeding communities. *Amer. Nat.* **106**, 472 - 486.

McGrorty, S., Clarke, R. T., Reading, C. J. and Goss-Custard, J. D. 1990**.** Population dynamics of the mussel *Mytilus edulis*: density changes and regulation of the population in the Exe estuary, Devon. *Mar. Ecol. Prog. Ser.* **67**, 157-169.

Mackinnon, C. 1997. Impact of the Introduced European Green Crab, *Carcinus maenas* on Tasmanian Bivalve Populations. Unpublished Honours thesis, University of Tasmania.

Monismith, S. G., Koseff, J. R., Thompson, J. K., O'Riordon, C. A. and Nepf, H. M. 1990**.** A study of model bivalve siphonal currents. *Limnol. Oceanogr* **5**, 680 - 696.

Nielsen, B. J. 1963**.** Studies of the genus *Katelysia* Romer 1857 (Mollusca, Lamellibranchiata). *Mem. Nat. Mus. Vict.* **26**, 219 - 257.

Olafsson, E. B. 1986**.** Density dependence in suspension-feeding and deposit-feeding populations of the bivalve *Macoma balthica*: a field experiment. *J. Animal Ecol.* **5** , 517 - 526.

Olafsson, E. B., Peterson, C. H. and Ambrose, W. G. 1994**.** Does recruitment limitation structure populations and communities of macro-invertebrates in marine soft sediments: the relative significance of pre- and post-settlement processes. *Oceanogr Mar. Biol. Ann. Rev.* **2**, 65-109.

Peterson, C. H. 1979**.** The importance of predation and competition in organising the intertidal epifaunal communities of Barnegat Inlet, New Jersey. *Oecologia* **39**, 1-24.

Peterson, C. H. 1982a**.** Clam predation by whelks (*Busycon* spp.): Experimental tests of the importance of prey size, pry density, and seagrass cover. *Mar. Biol.* **66**, 159-170.

Peterson, C. H. 1982b**.** The importance of predation and intra- and interspecific competition in the population biology of two infaunal suspension feeding bivalves, *Protothaca staminea* and *Chione undatella*. *Ecol. Monogr.* **52**, 437 - 475.

Peterson, C. H. 1992**.** Competition for food and its community-level implications. *Benthos Research* **42**, 1-11.

Peterson, C. H. and Andre, S. V. 1980**.** An experimental analysis of interspecific competition among marine filter feeders in a soft-sediment environment. *Ecol.* **61**, 129-139.

Peterson, C. H. and Beal, B. F. 1989**.** Bivalve growth and higher order interactions: importance of density, site and time. *Ecol.* **70**, 1390 - 1404.

Peterson, C. H. and Black, R. 1987**.** Resource depletion by active suspension feeders on tidal flats: Influence of local density and tidal elevation. *Limnol. Oceanogr.* **32**, 143-166.

Peterson, C. H. and Black, R. 1988**.** Density- dependent mortality caused by physical stress interacting with biotic history. *Amer. Nat.* **31**, 257 - 270.

Peterson, C. H. and Black, R. 1993**.** Experimental tests of the advantages and disadvantages of high density for two coexisting cockles in a southern ocean lagoon. *J. Anim. Ecol.* **62**, 614 - 633.

Raffaelli, D. and Hawkins, S. 1996. *Intertidal Ecology*. Chapman & Hall, London.

Reise, K. 1985. *Tidal Flat Ecology : An Experimental Approach to Species Interactions*. Springer - Verlag, Berlin.

Rhodes, D. C. 1974. Organism-sediment relations on the muddy sea floor. *Oceanogr. Mar. Biol. Annu. Rev.* **12**, 263-300.

Rhodes, D. C. and Young, D. K. 1970**.** The influence of deposit feeding organisms on sediment stability and community trophic structure. *J. Mar. Res.* **28**, 150 - 178.

Roberts, D. 1983**.** The genus *Katelysia* (Bivalvia: Veneridae) in southern Australia. *J. Malacol. Soc. Aust.* **6**, 191 - 204.

SAS Institute 1995. JMP for Macintosh Ver 3.1

Sokal, R. R. and Rohlf, F. J. 1981. *Biometry*. W. H. Freeman and Company, San Francisco.

Thrush, S. F., Hewitt, J. E., Pridmore, R. D. and Cummings, V. J. 1996**.** Adult/juvenile interactions of infaunal bivalves: contrasting outcomes in different habitats. *Mar. Ecol. Prog. Ser.* **132**, 83-92.

Treadwell, R., McKelvie, L. and Maguire, G. B. 1991. *Profitability of Selected Aquacultural Species*. Australian Bureau of Agricultural and Resource Economics Discussion Paper 91.11, ABARE, Canberra, 85 pp.

Treadwell, R., McKelvie, L. and Maguire, G. B. 1992. *Potential for Aquaculture in Australia.* Australian Bureau of Agricultural and Resource Economics Research Report 92.2, ABARE, Canberra, 81 pp.

Vincent, B., Joly, D. and Harvey, M. 1994**.** Spatial variation in growth of the bivalve *Macoma balthica* (L.) on a tidal flat : effects of environmental factors and intraspecific competition. *J. Exp. Mar. Biol. Ecol.* **181**, 223-238.

Weinburg, J. R. 1985**.** Factors regulating population dynamics of the marine bivalve *Gemma gemma*: intraspecific competition and salinity. *Mar. Biol.* **86**, 173-182.

Wells, F. E. and Roberts, D. 1980**.** Molluscan assemblages on an intertidal sandflat in Princess Royal Harbour, Western Australia. *Aust. J. Mar. Freshwater Res.* **31**, 499-507.

Wells, F. E. and Threlfall, T. J. 1980**.** A comparison of the molluscan communities on intertidal sand flats in Oyster Harbour and Peel Inlet, Western Australia. *J. Moll. Stud.* **46**, 300-311.

Wilson, W. H. J. 1983**.** The role of density dependence in a marine infaunal community. *Ecol.* **64**, 295-306.

Woodin, S. A. 1974**.** Polychaete abundance patterns in a marine soft-sediment environment: the importance of biological interactions. *Ecol. Monogr.* **4**, 171-187.

Woodin, S. A. 1976**.** Adult-larval interactions in dense infaunal assemblages; patterns of abundance. *J. Mar. Res.* **34**, 25 - 41.

Woodin, S. A. 1997. Shallow Water Benthic Ecology: A North American perspective of sedimentary habitats. Ecology of Estuaries and Soft Sediment Habitats. Deakin University, Warrnambool, Australia, *Aust. J. Ecol*.

Zar, J. H., 1984. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J., 718 p.

5.8.9 FIGURES

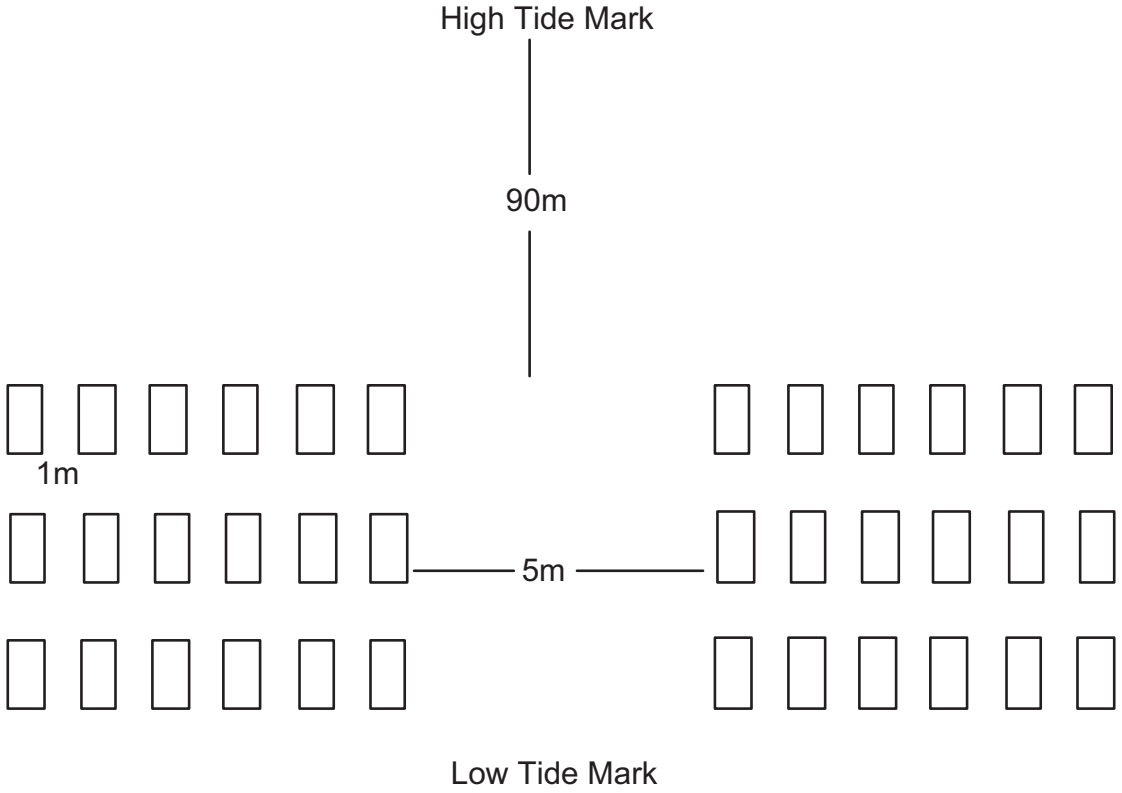

Figure 1. Representation of experimental design at Moulting Lagoon, Coles Bay.

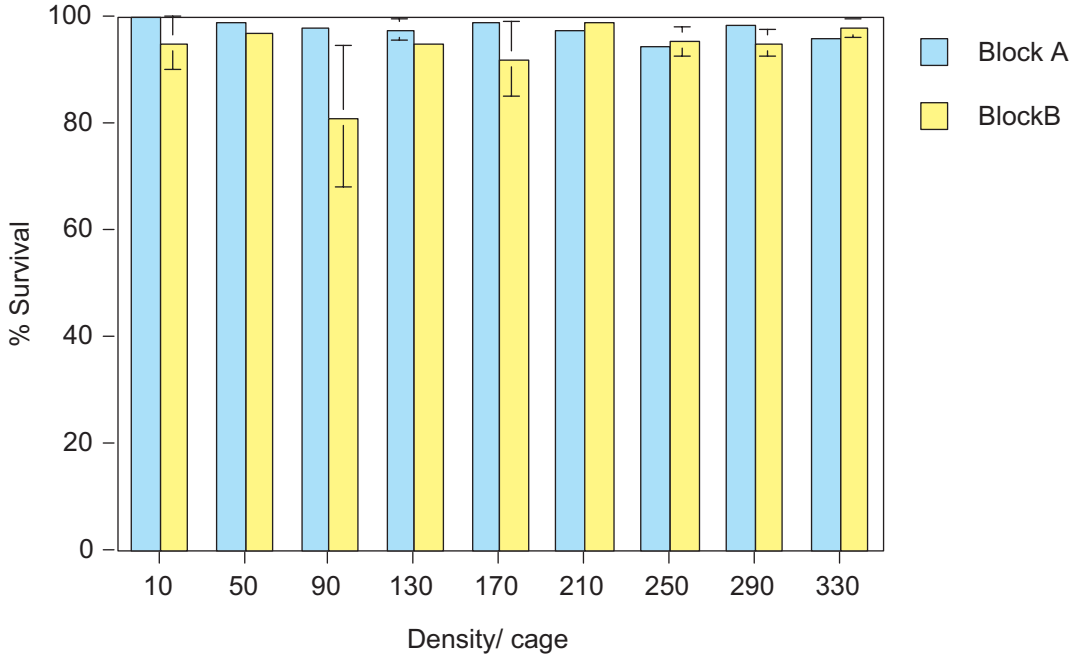

Figure 2. Percentage survival of *K. scalarina* in experimental density manipulations over 1 year (mean±S.E., n=4 replicate baskets).

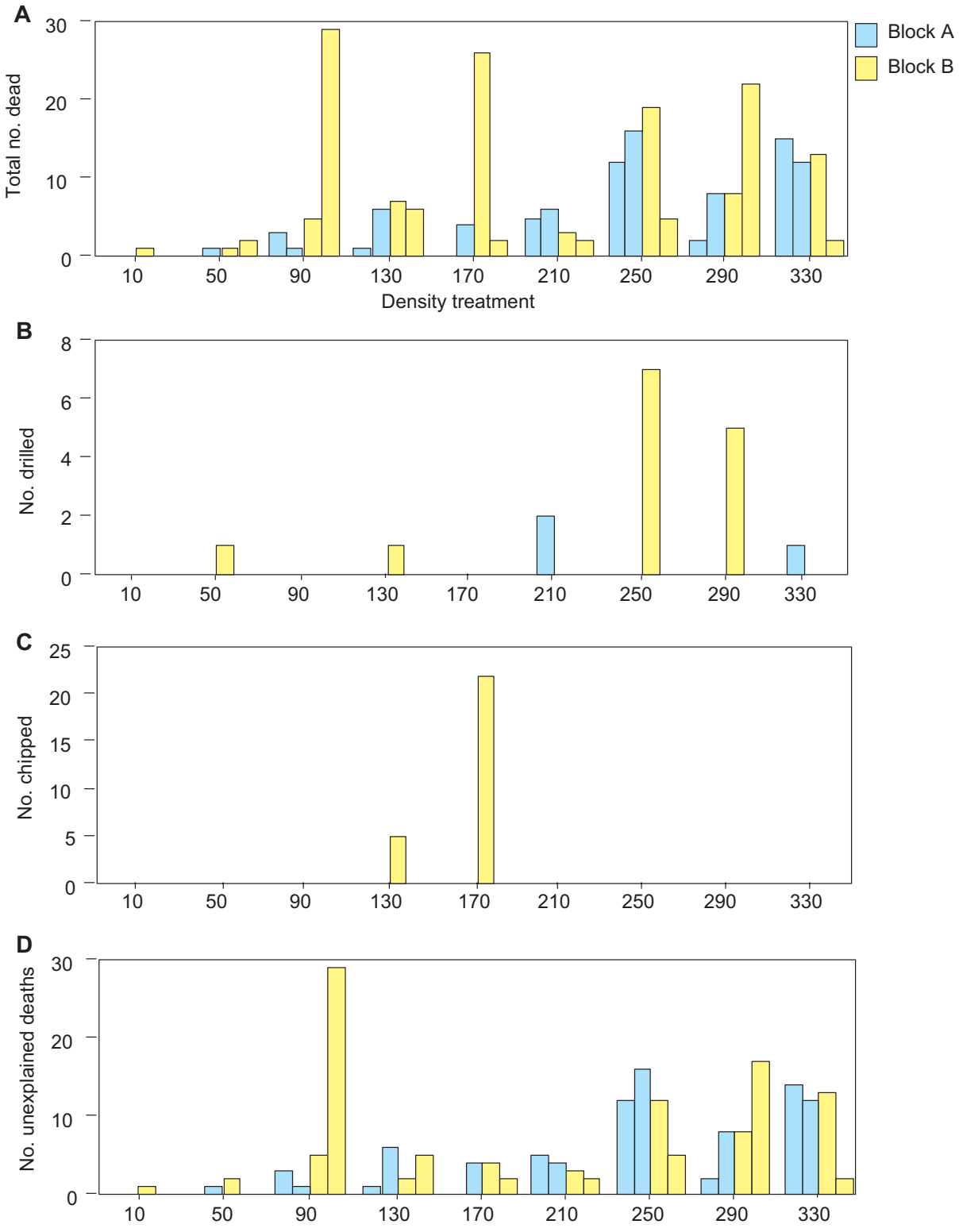

Figure 3. Survival of *K. scalarina* in density treatments, each column represents one Treatment basket A) Total mortality of *K. scalarina* (n=245); B) Total number of drilled *K. scalarina* (n=17); C) Total number of chipped *K. scalarina* (n= 27); D) Total number of unexplained deaths (n=201).

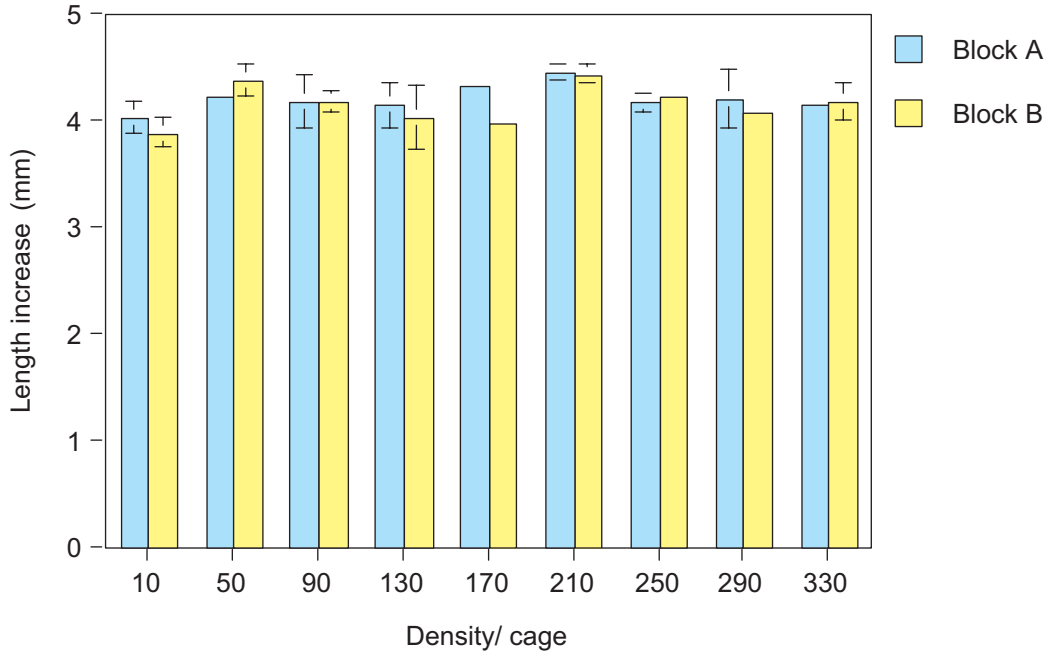

Figure 4. Growth of *K. scalarina* in experimental treatments measured as increase in length (mm) over one year (mean±S.E., n=4 replicate baskets).

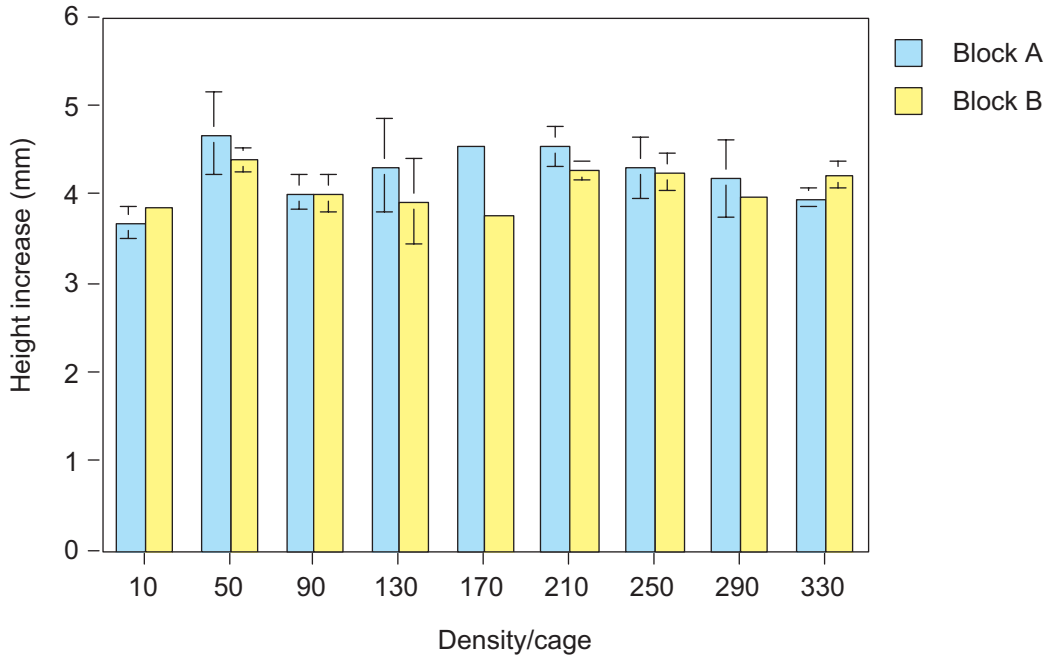

Figure 5. Growth of *K. scalarina* in experimental treatments measured as increase in height (mm) over one year (mean±S.E., n=4 replicate baskets).

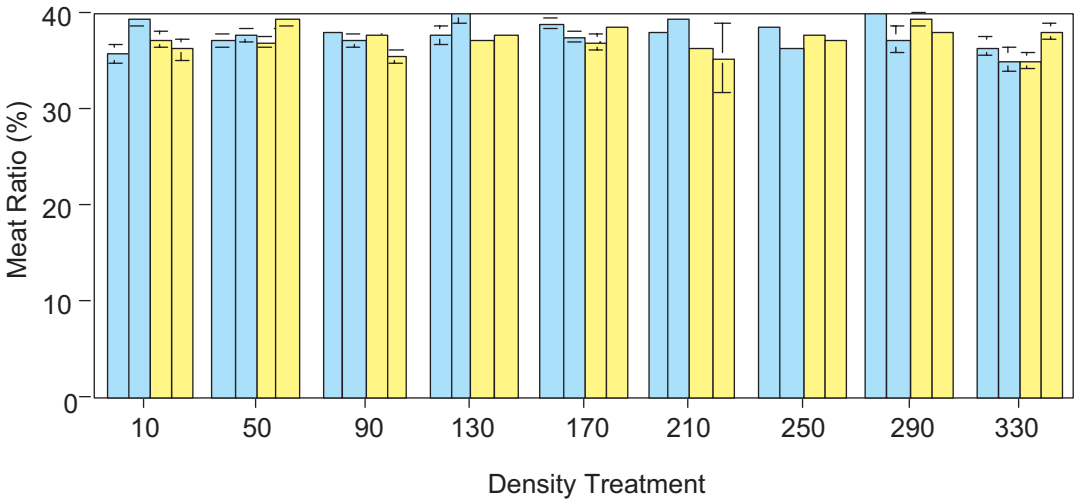

Figure 6. Percentage meat weight of total weight of *K. scalarina* in experimental treatments. (mean±S.E., n=30 clams).

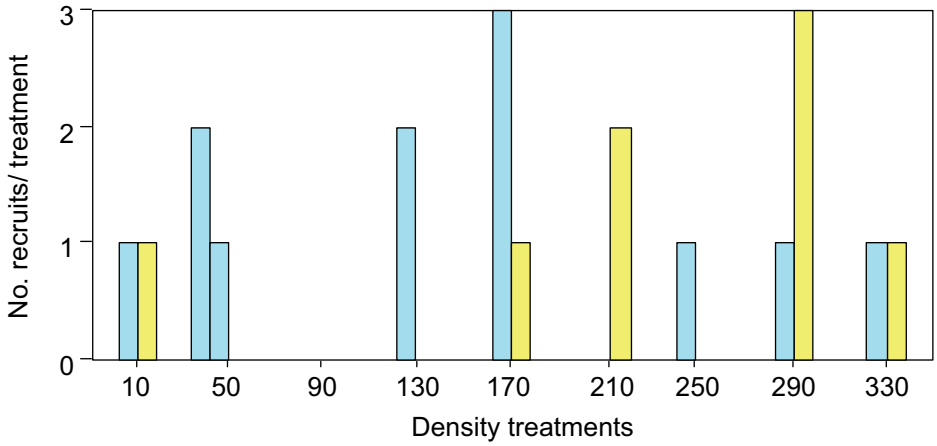

Figure 7. Recruitment of juvenile *K. scalarina* to experimental treatments (n=20).

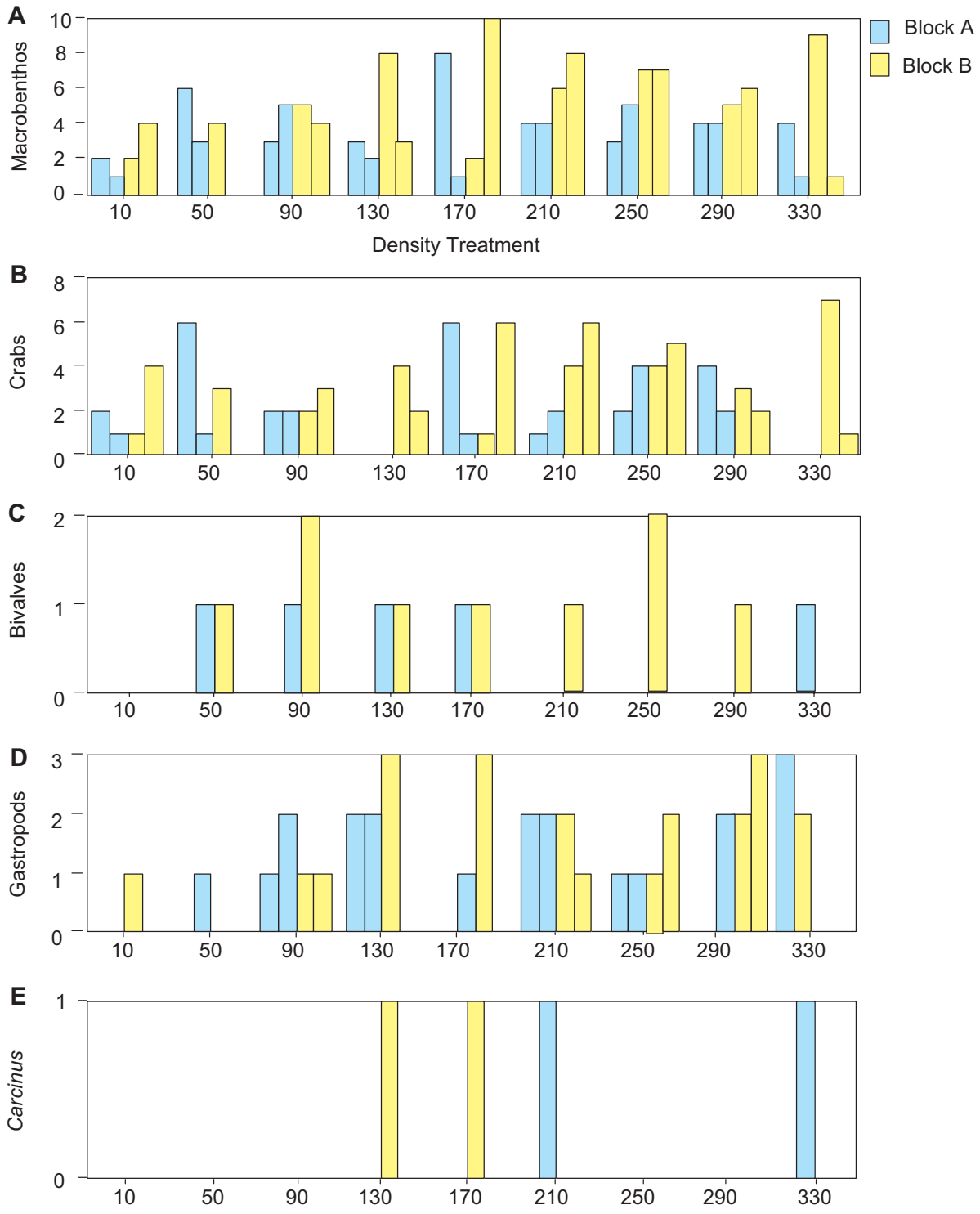

Figure 8. Numbers of macrobenthos present in experimental treatments. A) Total numbers of macrobenthos (n=154) B) Number of crabs (n=94) C) Number gastropods (n=42) D) Number of bivalves (n=14) E) Number of *Carcinus maenas* (n=4).

5.9 MANUSCRIPT 9

Effect of tidal position and density on *Katelysia scalarina* (Lamarck 1818) (Bivalvia: Veneridae)

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5.9.1 ABSTRACT

Density and tidal positions manipulations were conducted over 12 months in intertidal cages at Moulting Lagoon, Coles Bay, Australia. Experimental manipulations involved 15 combinations with three density treatments (171.5-686.1 clams.m^{-2}) and five tidal positions (low to high tide levels in 30 m horizontal intervals). Density (P=0.007) and tidal position (P=0.007) had significant effects on the survival of *K. scalarina* which was ≥90% except for the combination of highest shore position and the two highest densities. Density (P=0.043) and tidal position (P<0.000) had significant effects on growth of *K. scalarina* (shell length or height) with growth decreasing as the distance from the low tide mark increased. Shell growth at high tidal positions was approximately half of that lower on the shore whereas growth at the highest density was usually only reduced by <10% compared to the lowest density. In contrast, condition index increased with distance from the low tide mark (P<0.01) largely due to suppressed shell growth at high tidal positions.

The response of *K. scalarina* to density and tidal positions may explain distribution and abundance patterns of natural populations and is an important consideration in the management and establishment of any marine farming endeavour.

5.9.2 INTRODUCTION

The major role of intraspecific competition has been recognised in many soft bottom bivalves, however, while competition for food may cause reduced growth in suspension feeders, it does not usually cause increased mortality or competitive exclusion (see Manuscript 8).

Other researchers have suggested that while there are strong spatial variations in growth patterns for suspension feeding bivalves, these variations can mainly be explained by tidal level and local population density (Vincent *et al.*, 1994). Tidal gradient can be considered a strong environmental stress gradient, as physical and physiological disturbances increase with tidal height (Menge and Sutherland, 1987). Suspension feeders living at higher elevations may also encounter water masses already partial cleared of suspended food (Peterson & Black, 1987; Peterson & Black, 1988; Peterson, 1992). Submersion period also determines the foraging time available to intertidal suspension feeders and in turn their supply of food. A decrease in submersion time may also have adverse physiological effects, as bivalves may be exposed to harmful salinities and temperatures (Newell, 1979; Vincent et al., 1994; Roegner & Mann, 1995). Higher growth rates at lower elevation have been observed in numerous bivalve populations (Bertness & Grosholz, 1985; Peterson & Black, 1987; Peterson & Black, 1988; Jensen, 1992; Vincent et al., 1994).

The relevant biology of *K. scalarina* was described in Manuscript 8. A small commercial fishery for *K. scalarina* exists in several of the larger bays and estuaries of Tasmania's east coast and its aquaculture potential is being evaluated (Kent *et al.*, 1998). Both beach position and stocking density are important management variables in clam culture (Toba *et al.*, 1992).

The aim of the study was to examine the changes in growth and survival of *K. scalarina* at three population densities along an environmental gradient based on tidal height and immersion time.

5.9.3 METHODS

Study Site

Experimental manipulations of *K. scalarina* were conducted in the intertidal zone of Moulting Lagoon which is a permanently open lagoon situated on the east coast of Tasmania, Australia near Coles Bay on the Freycinet Peninsula (42°05'S, 148°10'E). See Manuscript 8 for a description of the physical characteristics of this lagoonal system.

Initial sampling

Sampling and allocation of clams were as for Manuscript 8 except that the initial collection period was 16th - 23rd Feb 1996. Experimental treatments were established in a random block design at five tidal positions, ranging from the low tide mark to the high tide mark at 30 m intervals. Three densities were used (30, 60 and 120 clams.basket^{-1}; 171.5-686.1 clams.m^{-2}) with four replicates of each treatment at every tidal position. Neighbouring cages were separated by 1m.

Final Sampling and statistical analysis

Experimental manipulations were sampled between 6-10 Jan 1997 approximately a year after the establishment of the experiment. Other procedures were as described in Manuscript 8.

5.9.4 RESULTS

Survival and growth

Tidal height ($P = 0.0065$) and density ($P = 0.0065$) had a significant effect on the survival of *K. scalarina*. The major effect occurred through depressed survival at the highest beach position and, at this position, mortality was exacerbated by increasing density (Figure 1). In other treatment combinations, survival was >90%. The lowest mortality recorded (3.13%) was in the high shore, 30 clam.basket^{-1} treatment whereas the highest mortality recorded (41.55%) was in the high shore, 120 clam treatment.

Density ($P= 0.043$) and tidal position ($P< 0.000$), but not their interaction ($P=0.967$), had significant effects on growth of *K. scalarina* (shell length) with growth, decreasing as the distance from the low tide mark increased. At high tidal positions shell growth, measured as length or height, was approximately half of that lower on the shore whereas growth at the highest density was usually only reduced by <10% compared to the lowest density (Figures 2-3). The largest length increase (4.21 mm) was in the 30 clam Mid/Low tidal treatment. The smallest length increase (2.08 mm) was in the 120 clam high tidal treatment.

Meat Ratio

Meat ratio (% of total weight consisting of meat weight) displayed a significant response to tidal position ($P= 0.008$) (Figure 4). The highest meat ratio (39.32%) was present in the 60 clam Mid/High treatment, while the lowest meat ratio (34.47%) was in the 120 clam Mid /Low treatment. At higher tidal heights shell growth was more depressed than meat growth and hence the meat to shell ratio improved. Neither density ($P= 0.062$) nor the interaction between tidal height and density ($P=0.062$) had significant effects on the meat ratio of *K. scalarina*.

Recruitment

Recruitment of juvenile *K. scalarina* was low in all the experimental treatments and most treatments had no recruits present (Figure 5). The majority of recruits were found in the High and Mid/high treatments, with a total of 8 & 7 respectively. In general, higher numbers of recruits were present in the 30 and 60 clam treatments rather than 120 clam treatment.

Macrobenthos

Numbers of macrobenthic invertebrates recovered from the treatment cages varied over the experimental treatments, with a total of 159 invertebrates collected (range 0-20 animals. $cage^{-1}$) (Figure 6A). Although most cages had some macrobenthos present there was high variability between replicates at the same density and tidal position. The highest total number of macrobenthic invertebrates collected at a tidal height was 39 in the low shore treatment. Macrobenthic organisms were divided into four categories; other bivalves, gastropods, crabs and the introduced European shore crab *Carcinus* maenas, and this allowed assessment of their effects on clams as predators and competitors.

The major macrobenthic category was crabs (39.62%, Figure 6B), the majority of which were the soldier crab, *Mictyris platycheles* and the shore crab, *Paragrapsus gaimardii.* Crab numbers were highest in the mid/low to mid/high treatments and, within this range, the highest abundance of crabs was in the 60 and 120 clam treatments. These crabs posed no predation threat to *K. scalarina* stocked into the cages (Manuscript 8).

The next numerically dominant group was bivalves (Figure 6C, 32.07%). Bivalves present were mainly *Eumarcia fumigata* and *Soletellina biradiata* which may have been competing with *K. scalarina* for food resources. Unlike the crabs the bivalve number reached their highest density at the low shore treatment. As numbers of additional bivalves were low it is unlikely these additional recruits would have a significant impact on resources.

Gastropods also comprised a major part of the macrobenthic invertebrates collected (Figure 6D, 20.12%). Gastropods present were primarily *Nassarius pauperatus*, *Cominella lineolata*, and *Poliniies conicus*, all of which are carnivorous, drilling gastropods that prey on bivalve molluscs (Peterson 1982a). *Bembicium auratum*, a small periwinkle which feeds on algae was also present. The total abundance of gastropods at each tidal height appeared consistent, although the distribution of gastropods within a tidal position was variable. Drilling gastropods were the only group of predators which caused mortality of *K. scalarina* in the experimental treatments, but this occurred in only one basket.

Carcinus maenas, the European shore crab, (Figure 6E) was placed in a separate category due to its known potential as a major bivalve predator (Gee et al., 1985; McGrorty et al., 1990). *C. maenas* comprised 8.17% of the total macrobenthos and there was no evidence of *C. maenas* predation in any of the cages.

5.9.5 DISCUSSION

Enclosure effects

Increasing density has a hierarchical effect on soft sediment bivalves; the first response is density-dependent emigration, then a decrease in growth rate and reproductive effort, and finally an increase in mortality (Peterson, 1982b). In this study density dependent emigration was effectively eliminated by the enclosures. In the absence of fences, experimental animals display enhanced immigration into enclosure-free plots, a process which over time would have

eroded the density treatments if manipulations had been attempted without enclosures (Peterson & Black, 1993). Despite the necessary use of enclosures, the effect of enclosure artefacts on the results cannot be overlooked. Previous studies comparing enclosed and enclosure free plots to examine the effect of enclosing on individual growth, demonstrated a 50% reduction in growth of *Katelysia* spp. in roofed cages (Peterson & Black, 1993), although cages had no effect on any other variable measured. In this study the presence of enclosure artefacts would not prevent a test of density on growth because identical enclosures were used for all density treatments. In fact the lack of, or minor biological impact, of density effects, even with the enclosures, strengthens the conclusion that density within the range of $20.58m^2$-$679.14m^2$ has little effect on this species.

Survival

Several authors have reported that increased density, even under conditions of extreme crowding, does not easily produce mortality or competitive exclusion (Creese & Underwood, 1982; Peterson, 1982b; Peterson & Black, 1993), although few studies have investigated the combined effects of increased density and tidal position. Our results from Moulting Lagoon indicated that both density and tidal position have a significant effect on survival of *K. scalarina*, primarily at the high tide level at elevated densities. The trend of decreased survival at higher tidal levels may be due to greater exposure causing physiological stress (Newell, 1979; Vincent *et al.*, 1994; Roegner & Mann, 1995). Similarly, Peterson & Black (1988) found that mid shore survival rates of *Circe lenticularis* were 50% lower than for subtidal treatments. In contrast**,** Peterson & Black (1987) found that survival of clams higher in the intertidal zone was greater than treatments lower on the shore, largely due to predation of clams in low shore treatments. All experimental treatments at Moulting Lagoon were enclosed in cages and this, coupled with the fact that dead shells displayed no obvious signs of predation**,** makes predation an unlikely explanation for low survival levels. A more likely explanation is mortality due to increased exposure or resource depletion.

Growth

K. scalarina in experimental treatments displayed decreased growth rates with increased shore height. Clams in low shore treatments displayed growth increases double that of high shore treatments. Previous authors have reported that growth of suspension feeders decreases with tidal height (Peterson & Black, 1987, 1988, 1991; Jensen, 1992; Beukema, 1993; Jensen, 1993; Vincent et al., 1994; Roegner & Mann, 1995). The inverse relationship of growth and tidal height can be explained by the duration of tidal submersion (Jensen, 1992; Vincent et al., 1994) which determines the feeding period of suspension feeding and thus their food supply (Jensen, 1992). However, the prevailing tidal regime at Moulting Lagoon and flat broad nature of the tidal flat indicate that duration of tidal submersion would not vary greatly between tidal positions (pers. obs) making this explanation any unlikely one.

Other authors have suggested that submersion time alone does not explain growth reductions and a number of factors may be responsible (Peterson & Black, 1991; Jensen, 1993; Vincent *et al.*, 1994). Firstly, the physiological effects of exposure during low tide may reduce growth of suspension feeders due to high temperatures and fluctuating salinities (Beukema, 1985; Hummel, 1985). Physiological stress in terms of desiccation varies between shore positions at Moulting Lagoon, despite the fact that tidal duration is similar across tidal heights. Sediments higher on the shore dry out significantly more than those lower on the shore (pers. obs.). Another explanation for decreased growth rates is the depletion of sestonic food concentrations, as the water moves up an intertidal gradient, due to suspension feeding organisms lower on the tidal

flat (Hummel, 1985; Peterson & Black, 1991). Previous hydrodynamic studies have illustrated the ability of suspension feeders to deplete local supplies of benthic phytoplankton (Frechette & Bourget, 1985a,b; Frechette et al. ab, 1989; Monismith et al., 1990). The high abundance of bivalves low on the shore at Moulting Lagoon make sestonic depletion of food resources a possible explanation for the observed patterns. Clearly, any combination of the above factors could be involved.

Density of *K. scalarina* at Moulting Lagoon also displayed a statistically significant effect on growth, however, as in Manuscript 8, the impact of density on growth was of relatively little biological importance. Numerous studies have reported reduced growth of suspension feeding bivalves (Peterson & Andre, 1980; Peterson & Black, 1988; Olafsson, 1986; Vincent et al., 1994) and it is generally associated with competition for limited resources (Peterson, 1992; Peterson & Black, 1993). However, there was no significant interaction between beach position and density for growth rate or condition index, indicating that depletion of food within tidal flows at elevated beach positions was unlikely to be the cause of depressed growth at such positions.

Tidal position and density displayed a significant effect on the meat ratio of *K. scalarina* at Moulting Lagoon. It has been suggested that rates of shell and soft tissue growth do not occur simultaneously, and that shell growth may precede growth of soft tissues especially at high tidal levels (Hilbish, 1986, Harvey & Vincent, 1990). In contrast Beukema (1993) suggests that growth of soft tissues is dependent on tidal level. However, shell growth *K. scalarina* at higher shore positions is more suppressed than meat growth and hence the meat to shell ratio increases. However, while statistically significant, the effect was relatively small in absolute terms. Maguire and Kent (1994) also found that tidal position had much greater biological impact than density and the trends were similar to this study with repressed shell growth and higher condition index for Pacific oysters *Crassostrea gigas* at an elevated growing height.

Recruitment

Previous studies have detected negative influences of adult density on larval settlement or juvenile recruitment (Peterson & Andre, 1980; Peterson, 1982b; Andre & Rosenberg, 1991; Jensen, 1992; see Manuscript 8). In contrast, Peterson & Black (1993) suggested that *K. scalarina* demonstrate higher rates of recruitment where adult populations are more dense. In this study the numbers of recruits were highest in the low and mid density treatments rather than the high density treatments. Recruitment also increased with tidal height, rising from a total of 5 recruits.cage^{-1} at the low shore treatments to 8 cage^{-1} at high shore treatments. Jensen (1992) suggests that the abundance of *Cerastoderma edule* spat along a tidal gradient was positively correlated with submersion time. However, recruits at Moulting Lagoon were more abundant at high tidal positions, however as the total number of recruits were low it is difficult to infer trends. It has been suggested that this pattern may be due to passive settlement of larvae due to depth and velocity of the overlying water mass (Jensen 1992). In natural populations at Moulting Lagoon juveniles and small size classes are generally distributed higher on the shore than larger size classes (Bellchambers, 1993) although opposite trends have been observed for another Tasmanian waterway (Woodward, 1985).

Macrobenthos

A growing body of evidence suggests that increased densities of benthic suspension feeders in soft sediments do not cause significant reductions in recruitment of benthos (Peterson, 1982b; Hunt et al., 1987; Peterson & Black, 1988; Manuscript 8). In these last two studies total densities

of smaller invertebrates failed to respond to changes in density of clams (*Katelysia* spp.). This was also the case at Moulting Lagoon as the abundance of potential competitors in terms of other macro-invertebrates did not increase at low clam densities. There also appeared to be no real trend in the distribution of macro-invertebrates along the tidal gradient, with a total of 159 present. However, when the macrobenthos is categorised clearer trends emerge.

The crabs *Mictyris platycheles* and *Paragrapsus gaimardii* composed 39.62% of the collected macrobenthic assemblage. Both these species are common estuarine crabs and are abundant in Moulting Lagoon, do not pose any predation threat to *K. scalarina*. Crabs display a clear preference for mid-tidal treatments, with 76% of their total abundance distributed in these treatments.

Bivalves composed 32.07% of the cage macrobenthos, as total numbers were low it is unlikely that they were competing significantly, with *K. scalarina*, for food. Unlike the crabs, bivalves present in the macrobenthic samples were more abundant in the low shore treatments and this is not surprising given this is where bivalves reach there greatest abundance at Moulting Lagoon (pers. obs.).

Experimental manipulations failed to exclude all macrobenthos including potential predators such as naticid gastropods and *Carcinus maenas*. These types of predation leave distinctive signs: naticid gastropods leave characteristically drilled shells behind, whereas crab predation can be similarly inferred from crushed or chipped shells. Despite the reports of other authors that *Carcinus* is a voracious predator of bivalves (Gee *et al*., 1985; Hunt *et al*., 1987; McGrorty *et al.,* 1990) no signs of predation were evident on any dead shells. The only evidence of naticid predation was in one treatment cage. Therefore, predation was not a significant cause of mortality, which supports the suggestion that high shore treatments experienced higher levels of mortality due to starvation through depletion of local food resources or environmental stress.

5.9.6 CONCLUSION

Artificially increasing the density of natural populations of *K. scalarina* has been suggested as a means of both sustaining and increasing the productivity of natural populations. However, the reduction in survival and growth of *K. scalarina* exposed to high tidal positions particularly at high densities, coupled with the absence of predation pressure, indicates that resource limitation of some type is occurring. Alternatively, increased mortality and depressed growth rates of high shore treatments may be due to increased exposure and physiological stress caused by variation in environmental conditions. The mechanisms limiting the growth and survival of *K. scalarina* at high shore positions are still not clear. Further research is required to determine the factors determining the distribution and abundance of *K. scalarina* as these factors are important not only in terms of improving yields for aquaculture but to provide an explanation for patterns observed in natural populations and to ensure sustainable practices are implemented. However, choice of beach position is likely to have a major effect on the success of farming with this species.

5.9.7 ACKNOWLEDGMENTS

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5.9.8 REFERENCES

Andre, C. and Rosenberg, R. 1991**.** Adult-larval interactions in the suspension-feeding bivalves *Cerastoderma edule* and *Mya arenaria*. *Mar. Ecol.- Prog. Ser.* **71**, 227 - 234.

Bellchambers, L. M. (1993). Aspects of the ecology of *Katelysia scalarina* (Bivalvia: Veneridae), a commercially exploited cockle. Unpublished Honours Thesis, University of Tasmania, Hobart.

Bertness, M. D. and Grosholz, E. 1985. Population dynamics of the ribbed mussel, *Geukensia demissa*: the costs and benefits of an aggregated distribution. *Oecologia* **67**, 192-204.

Beukema, J. J. 1993. Successive changes in distribution patterns as an adaptive strategy in the bivalve *Macoma balthica* (L.) in the Wadden Sea. *Helgol. Wiss. Meeresunters.* **47**, 287-304.

Creese, R. G. and Underwood, A. J. 1982**.** Analysis of inter- and intra- specific competition amongst intertidal limpets with different methods of feeding. *Oecologia* **53**, 337 - 346.

Frechette, M. and Bourget, E. 1985a**.** Energy flow between the pelagic and benthic zones; factors controlling particulate organic matter available to an intertidal mussel bed. *Can. J. Fish. Aq. Sci.* **42**, 1158 - 1165.

Frechette, M. and Bourget, E. 1985b**.** Food-limited growth of *Mytilus edulis* in relation to the benthic boundary layer. *Can. J. Fish. Aquat. Sci.* **42**, 1166 - 1170.

Frechette, M., Bulman, C. A. and Geyer, W. R. 1989**.** The importance of boundary-layer flow processes in supplying phytoplankton to the benthic suspension-feeder *Mytilus edulis*. *Limnol. Oceanogr* **34**, 19 - 36.

Gee, J. M., Warwick, R. M., Davey, J. T. and George, C. L. 1985**.** Field experiments on the role of epibenthic predators in determining prey densities in an estuarine mudflat. *Est. Coast. Shelf Sc.* **21**, 429-448.

Gray, J. S. 1981. *The Ecology Of Marine Sediments*. Cambridge University Press, Cambridge.

Harvey, M. and Vincent, B. 1990. Density, size distribution, energy allocation and seasonal variations in shell and soft tissue growth at two tidal levels of a *Macoma balthica* (L.) population. *J. Exp. Mar. Biol. Ecol.* **142**, 151-168.

Hilbish, T. J. 1986. Growth trajectory of shell and soft tissue in bivalves: seasonal variations in *Mytilus edulis* L. *J. Exp. Mar. Biol. Ecol.* **96**, 103-113.

Hummel, H. 1985**.** Food intake of *Macoma balthica* (Mollusca) in relation to seasonal changes in its potential food on a tidal flat in the Dutch Wadden Sea. *Neth. J . Sea. Res.* **19**, 52-76.

Hunt, J. H., Ambrose, W. G. and Peterson, C. H. 1987**.** Effects of the gastropod, *Ilyanassa obsoleta* (Say), and the bivalve, *Mercenaria mercenaria* (L.), on larval settlement and juvenile recruitment of infauna. *J. Exper. Mar. Biol. Ecol.* **108**, 229-240.

Jensen, K. T. 1992**.** Dynamics and growth of the cockle, *Cerastoderma edule*, on a intertidal mud flat in the Danish Wadden Sea: effects of submersion time and density. *Neth. J. Sea Res.* **28**, 335-345.

Jensen, K. T. 1993**.** Density-dependent growth in cockles (*Cerastoderma edule*): evidence from interannual comparisons. *J. Mar. Biol. Ass. U.K.* **73**, 333 - 342.

Kent, G. N., Maguire, G. B., John, M., Cropp, M. and Frankish, K. 1998. Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina* (Lamark 1818). Journal of Shellfish Research, 17(4): 1065-1070.

McGrorty, S., Clarke, R. T., Reading, C. J. and Goss-Custard, J. D. 1990**.** Population dynamics of the mussel *Mytilus edulis*: density changes and regulation of the population in the Exe estuary, Devon. *Mar. Ecol. Prog. Ser.* **67**, 157-169.

Maguire, G. B., and Kent, G., 1994. Performance of triploid and diploid Pacific oysters at four combinations of growing heights and stocking densities. In: J.A. Nell and G.B. Maguire (Editors), Evaluation of Triploid Sydney Rock Oysters (*Saccostrea commercialis*) and Pacific Oysters (*Crassostrea gigas*) on Commercial Leases in New South Wales and Tasmania. FRDC Grant Report.

Menge, B. A. and Sutherland, J. P. 1987**.** Community regulation: variation in disturbance, competition , and predation in relation to environmental stress and recruitment. *Am. Nat.* **130**, 730-757.

Monismith, S. G., Koseff, J. R., Thompson, J. K., O'Riordon, C. A. and Nepf, H. M. 1990**.** A study of model bivalve siphonal currents. *Limnol. Oceanogr* **5**, 680 - 696.

Newell, R. C. 1979 Biology of intertidal animals. Elsevier, New York.

Olafsson, E. B. 1986**.** Density dependence in suspension-feeding and deposit-feeding populations of the bivalve *Macoma balthica*: a field experiment. *J. Animal Ecol.* **5** , 517 - 526.

Olafsson, E. B., Peterson, C. H. and Ambrose, W. G. 1994. Does recruitment limitation structure populations and communities of macro-invertebrates in marine soft sediments: the relative significance of pre- and post-settlement processes. *Oceanogr. Mar. Biol. Annu. Rev.* **32**, 65-109.

Peterson, C. H. 1982a**.** The importance of predation and intra- and interspecific competition in the population biology of two infaunal suspension feeding bivalves, *Protothaca staminea* and *Chione undatella*. *Eco. Mono.* **52**, 437 - 475.

Peterson, C. H. 1982b. Clam predation by whelks (*Busycon* spp.): Experimental tests of the importance of prey size, pry density, and seagrass cover. *Mar. Biol.* **66**, 159-170.

Peterson, C. H. 1992**.** Competition for food and its community-level implications. *Benthos Research* **42**, 1-11.

Peterson, C. H. and Andre, S. V. 1980**.** An experimental analysis of interspecific competition among marine filter feeders in a soft-sediment environment. *Ecol.* **61**, 129-139.

Peterson, C. H. and Black, R. 1987**.** Resource depletion by active suspension feeders on tidal flats: Influence of local density and tidal elevation. *Limnol. Oceanogr.* **32**, 143-166.

Peterson, C. H. and Black, R. 1988**.** Density- dependent mortality caused by physical stress interacting with biotic history. *Am. Nat.* **31**, 257 - 270.

Peterson, C. H. and Black, R. 1991**.** Preliminary evidence for progressive sestonic food depletion in incoming tide over a broad tidal sand flat. *Estuar. Coast. Shelf Sc.* **32**, 405-413.

Peterson, C. H. and Black, R. 1993**.** Experimental tests of the advantages and disadvantages of high density for two coexisting cockles in a southern ocean lagoon. *J. Anim. Ecol.* **62**, 614 - 633.

Roegner, G. C. and Mann, R. 1995**.** Early recruitment and growth of the American oyster *Crassostrea virginica* (Bivalvia: Ostreidae) with respect to tidal zonation and season. *Mar. Ecol. Prog. Ser.* **117**, 91-101.

Toba, D. R., Thompson, D. S., Chew, K. K., Anderson, G. J. and Miller, M. B. 1992. *Guide to Manila Clam Culture in Washington*.. University of Washington, Washington.

Vincent, B., Joly, D. and Harvey, M. 1994**.** Spatial variation in growth of the bivalve *Macoma balthica* (L.) on a tidal flat : effects of environmental factors and intraspecific competition. *J. Exp. Mar. Biol. Ecol.* **181**, 223-238.

Woodward, I. O. 1985. The structural dynamics of a tidal flat mollusc community. Unpublished PhD Thesis. University of Tasmania, Hobart.

5.9.9 FIGURES

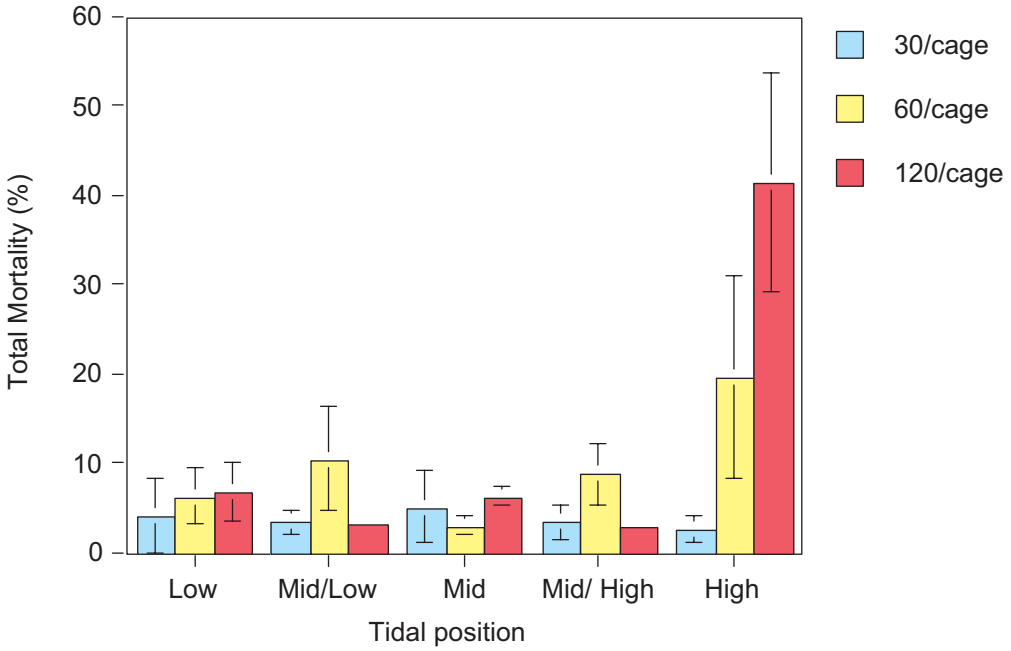

Figure 1. Mortality of *K. scalarina* in relation to stocking density and tidal position (mean±S.E., n=4 replicate baskets).

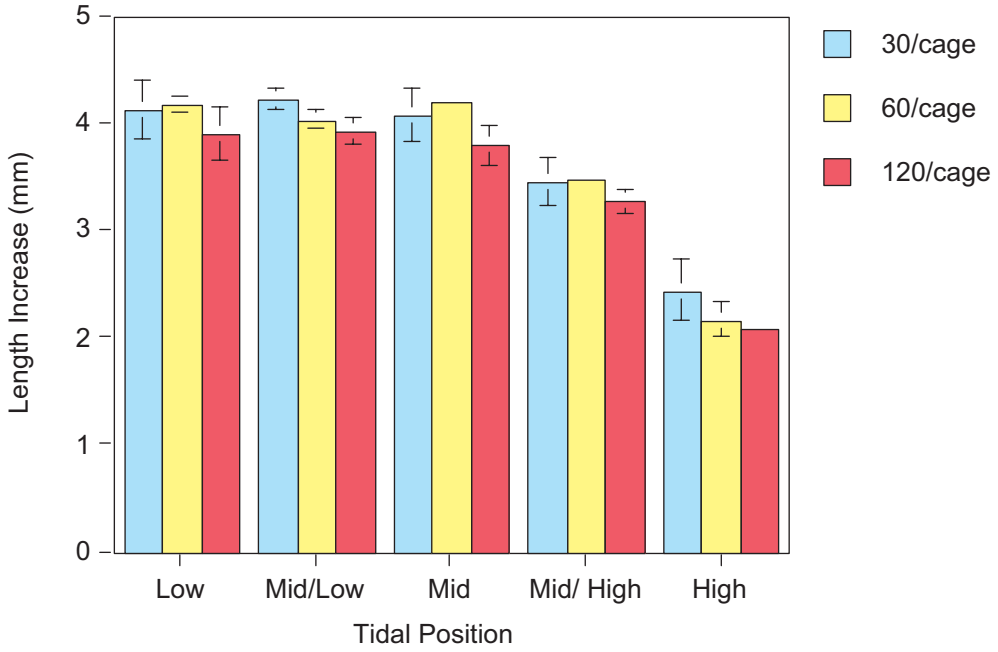

Figure 2. Growth of *K. scalarina* in experimental treatments measured as increased in length.(mm) over one year. (mean±S.E., n=4 replicate baskets).

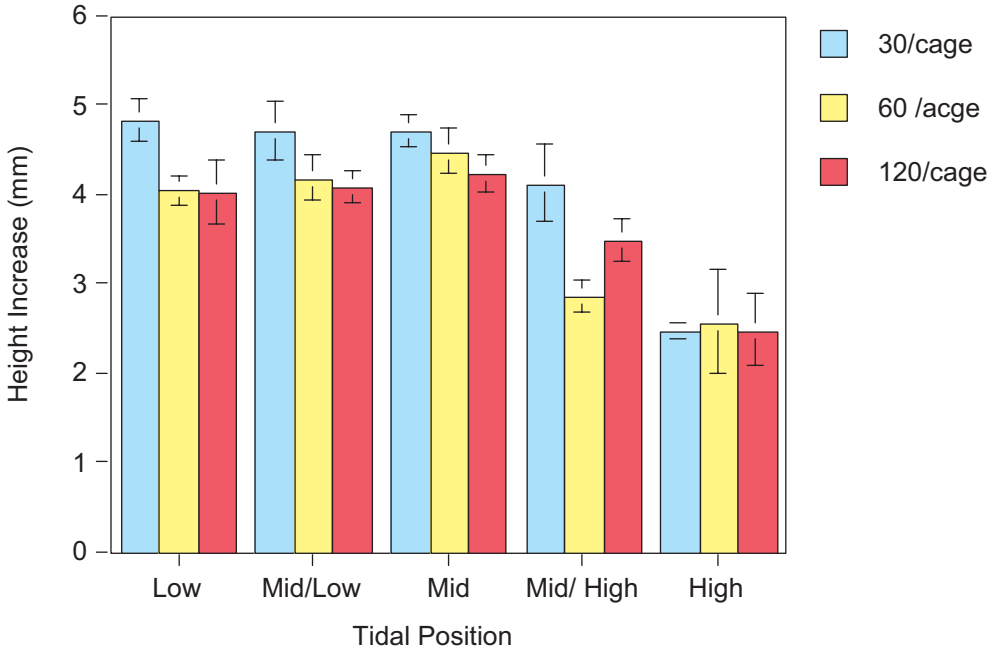

Figure 3. Growth of *K. scalarina* in experimental treatments measured as increases in height (mm) over one year (mean±S.E., n=4 replicate baskets).

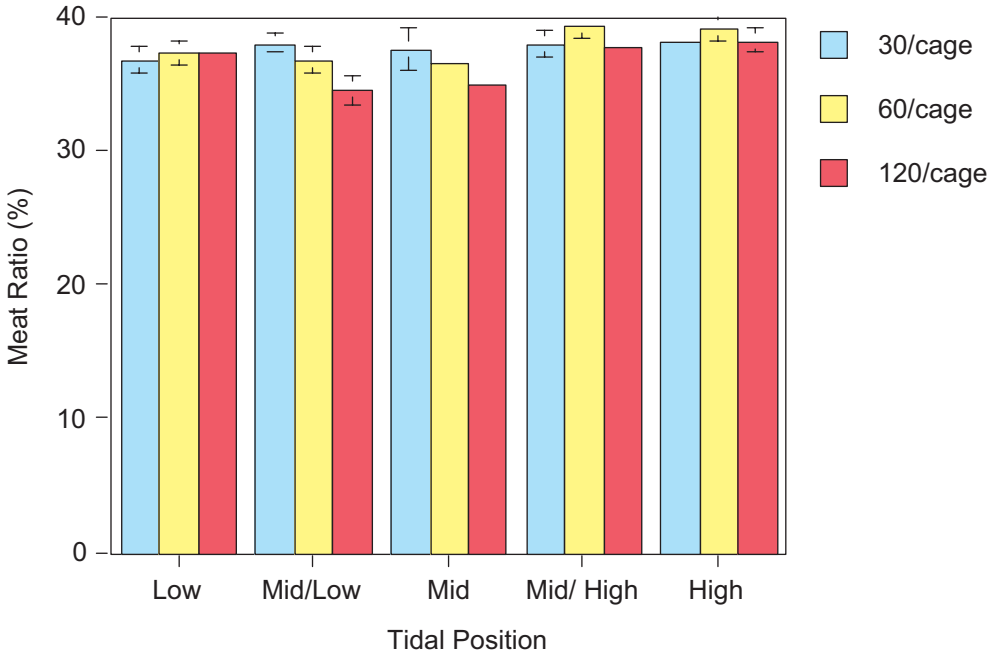

Figure 4. Percentage meat weight of total weight of *K. scalarina* in experimental treatments. (mean±S.E., n=30).

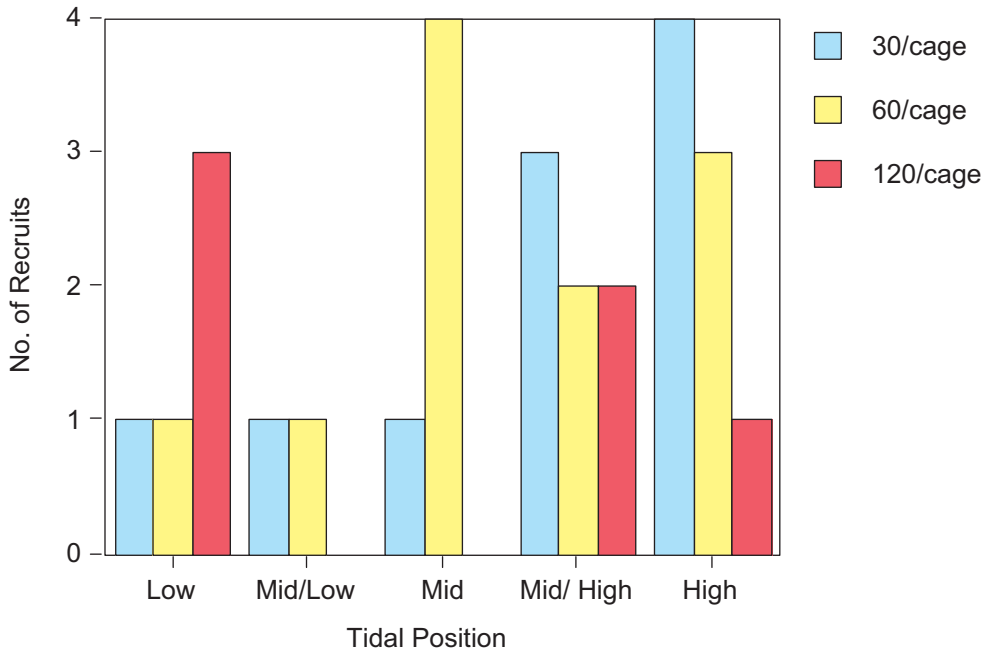

Figure 5. Recruitment of juvenile *K. scalarina* to experimental treatments (n=27). Each column represents the total number of recruits at the corresponding density and tidal position.

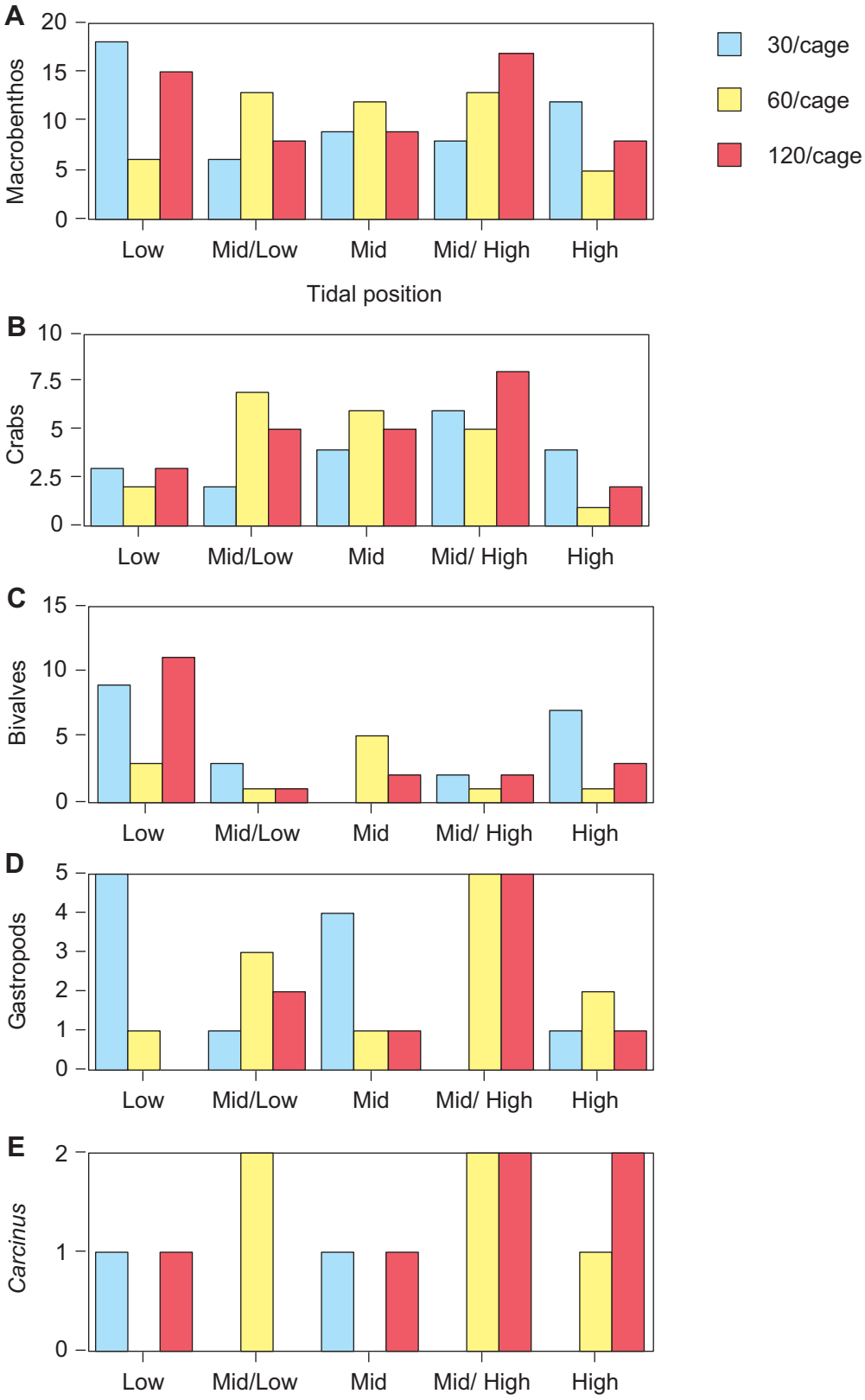

Figure 6. Numbers of macrobenthos present in experimental treatments. A) Total numbers of macrobenthos (n=159) B) Number of crabs (n=63) C) Number of bivalves (n=51) D) Number of gastropods (n=32) E) Number of *Carcinus maenas* (n=13). Each column represents the total number for the corresponding density treatment at the given tidal height.

5.10 MANUSCRIPT 10

The effect of growout site on the mortality of *Katelysia scalarina* (Lamarck, 1818) (Bivalvia: Veneridae).

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5.10.1 ABSTRACT

Five growout trials were conducted over periods of about one month with juveniles collected from natural populations being grown in mesh enclosures near intertidal oyster leases in Tasmania. Survival rates (10-26%) were low in two trials at Moulting Lagoon and Pittwater, probably because of inappropriate cage design and installation leading to sediment loss from the cages. In two subsequent trials at Pipeclay Lagoon and Cockle Creek survival was again low (about 15%) because sites were chosen to avoid anoxic sediment near the oyster racks and hence the clams were located high in the intertidal zone and therefore suffered from increased aerial exposure although the cage design was more appropriate. The final trial at Duck Bay, near Smithton was conducted under newly established oyster racks with little buildup of organic matter. Survival rates were high (65-95%) and led to subsequent long term ($\geq$ 1 year) growout trials in Duck Bay under and away from oyster racks (Manuscript 12) and at Moulting Lagoon, distant from oyster racks, which yielded negligible mortality (Manuscripts 8-9) unless cages were located high in the intertidal zone. In general, the use of intertidal oyster leases for polyculture systems involving *K. scalarina* does not appear promising.

5.10.2 INTRODUCTION

Site selection is a key determinant of profitability of aquaculture ventures (Treadwell *et al.*, 1992) and is particularly critical for forms of bivalve culture which do not involve supplemental feeding (Treadwell *et al.*, 1991). The suitability of sites for clam culture is influenced by a wide variety of factors and small scale trials are often needed to evaluate each potential site (see Castagna & Kraeuter, 1981; Manzi & Castagna, 1989; Toba *et al.*, 1992). In Tasmania *Katelysia scalarina* is distributed from Stanley in the north of the state to Cockle Creek in the south, with the majority of populations found in the sheltered bays and estuaries of the East coast (see Manuscript 13). Despite the wide distribution of *K. scalarina* around the state, the selection of study sites was dependent on several important factors. Firstly, study sites had to be located on an existing oyster lease, to assess the feasibility of growing clams under oyster racks on marine farms. The use of oyster farms for clam culture has been identified as offering advantages in terms of dual use of existing infrastructure and available microbial monitoring data for waterways from quality assurance programs (Maguire, 1991). In addition, *K. scalarina* was known to occur on several of the existing Tasmanian Pacific oyster, *Crassostrea gigas*, leases in Moulting Lagoon near Coles Bay and Duck Bay near Smithton.

Several criteria were used for the selection of oyster farms, the first criteria for selection was substrate characteristics. *K. scalarina* is located primarily in the intertidal zone of sheltered bay and estuaries in fine to medium grain sediment, approximately 2-4 cm below the substrate surface (Bellchambers & Richardson, 1995). As *K. scalarina* is sensitive to sediment stability and oxygen levels, areas that experience large scale sediment movement and anoxia cause mortality of the species. The presence of seagrass beds appears to limit the ability of *K. scalarina* to

bury into the substrate and natural populations are rarely found inhabiting these areas in high densities. Secondly, oyster leases had to have stable salinity profiles as long term salinity fluxes cause large scale mortality (Manuscript 5). In this study, a number of sites were trialed utilising two cage types to select a suitable site for the location of growth and gut contents trials. At one site, cages were positioned under and away from the oyster racks to see if *K. scalarina* derived benefit from the fallout of faeces and pseudoforces produced by the oysters.

5.10.3 METHODS

Initial sites

Initially two sites, at Moulting Lagoon and Pittwater, were used on existing oyster leases (Trials 1-2). Adult clams (35.0 - 40.0 mm shell length) were only collected from Moulting Lagoon as they were either absent or in insufficient numbers at other experimental sites for the initial and subsequent trials (Trials 1-5). A sample of the clams were sent to Mount Pleasant Laboratories for disease testing before being translocated. The genetic similarity of Tasmanian populations (Manuscript 15) has indicated that genetic issues associated with such translocation were not significant.

Experiments were conducted inside cages to prevent migration, over the experimental period. Cages were also designed to prevent the access of large mobile consumers such as crabs and wading birds (see Toba *et al*., 1992). All density treatments were enclosed identically; any additional effects of enclosures were held constant across treatments. Cages were constructed of 15 mm Nylex^{R} mesh (151.0 cm long x 101.0 cm wide x 14.0 cm deep) with treated wooden frames and fastened with plastic cable ties to wooden stakes, driven into the substrate. Eight cages were established at both sites with 180 clams per cage. Four cages were placed under oyster racks and four cages away from racks as a series of paired samples in four patches.

Trial 1 was established on 29/11/94 at Freycinet Marine Farm, Moulting Lagoon, Coles Bay. *K. scalarina* is widely distributed and abundant throughout Moulting Lagoon and is present on several of the existing oyster leases. Experiments were located under and away from intertidal oyster racks approximately 150 m from the high tide mark and experienced the full range of tidal movement. Sediment was composed of sand with a high proportion of organic matter especially under the existing oyster racks. Aquatic vegetation, in the form of seagrass beds, was present although not abundant. Experiments were re-sampled on 25/02/95 and mortalities recorded.

Trial 2 was established on the 13/12/94 at Coal River Oysters, Pittwater. Experiments were located as above in a region with frequent tidal flushing. The sediment at Pittwater was composed of fine sand and mud with a high component of organic matter, aquatic vegetation was absent. Experimental treatments were re-sampled on 27/02/95 and mortalities recorded.

Final site trials

After the failure of experiments at the first two sites a number of other potential sites were investigated. Alternative sites were located at Pipeclay Lagoon (Trial 3), Cockle Creek (Trial 4) and Duck Bay (Smithton) (Trial 5). The cages used in the initial trials had timber frames and these may have contributed to loss of sediment from or partial displacement of the cages via resistance to water flow. Due to these problems a new cage design was employed. Cages were constructed from 9 mm Nylex mesh (33 cm wide x 53 cm long x 14 cm) fastened with plastic cable ties to wooden stakes and driven into the substrate. Cages were partially refilled with sediment at allow clams to rebury.

Trial 5 at Duck Bay, established on 6/06/95 for a month long pilot study, was particularly noteworthy. Oyster racks at this site were newly established and experienced the full range of tidal movement. Sediment was composed of fine to medium grain sand with little organic matter even under existing oyster racks. Six cages containing 20 clams were located under existing oyster racks for a period of one month. No "away from racks" treatment was included. Experiments were re-sampled on the 4/07/95.

Maps of these growout areas are provided in Manuscripts 12-13.

Temperature and salinity data for Tasmanian oyster leases are available in Maguire et al. (1994) but the theft of data logging equipment from an experimental clam growout site restricted opportunities for detailed data collection at the sites discussed in this study.

5.10.4 RESULTS

Survival of *K. scalarina* at Moulting Lagoon (Trial 1) never exceeded 15% (Figure 1). There was no significant effect of location (under or away from oyster racks) on the survival of *K. scalarina* and survival was consistent between patches, ranging from 8.3 % to 14.6%. Results are consistent between paired samples within patches (see Manuscript 12 for design strategy) with the largest difference between paired cages in "under" and "away" treatments recorded for cage position 1 (3.5%) (Figure 1).

Percentage survival at Pittwater (Trial 2) ranged between 26.2 - 18.7% (Figure 2). No significant difference the survival of under and away treatments or between patches was evident. Survival at Pittwater was approximately 10% higher than survival at Moulting Lagoon.

Trials 3-4 from both Cockle Creek and Pipeclay Lagoon proved unsuccessful with approximately 85% mortality of experimental clams at both sites (Table 1).

Table 1. Survial of *K. scalarina* over one month at Cockle Creek (Trial 3) and Pipeclay Lagoon (Trial 4) (mean $\pm$ S.E., n=6).

Site	Survival
Cockle Creek	15.83% $\pm$ 1.30
Pipeclay Lagoon	14.00% $\pm$ 1.26

Survival at Smithton (Trial 5) ranged between 65-95% (Figure 3). Although the trials at Smithton were only conducted under oyster racks, percentage survival is significantly higher than at both Moulting Lagoon and Pittwater, with differences in mortality between patches evident.

5.10.5 DISCUSSION

Site location had a significant effect on the survival of *K. scalarina* in experimental trials. The high mortality of *K. scalarina* in initial trials (1-2) established at Moulting Lagoon and Pittwater may in part be attributed to cage design. Cages had large wooden frames which hindered effective burial of the cages in the substrate and aided flotation thereby restricting effective burrowing into the substrate to avoid variations in environmental conditions.

Although natural populations of *K. scalarina* are abundant and widely distributed throughout Moulting Lagoon**,** clams at this site in Trial 1 displayed high mortality, coinciding with a freshwater influx from a period of high rainfall in conjunction with high temperatures, causing up to 80% mortality in some treatments. Large scale mortality of oysters on the Freycinet

Marine Farm also occurred in the same period (pers. comm. Andrea Cole). Increased freshwater input also caused an algal bloom (*Etocarpus spp.*) which coated many of the cages in a layer of seagrass thus limiting water flow.

Natural populations of *K. scalarina* are not present in Pittwater although another intertidal suspension feeder *Anapella cycladea* is abundant and widely distributed. The sediment at Pittwater was a fine grained sand which was highly mobile due to strong tidal currents. Cages used at this site (Trial 2) experienced at high degree of sediment movement and scouring around the edges. With reduced sediment levels in the cages *K. scalarina* ability to bury was limited thus exposing them to fluctuating environmental conditions. Another possible factor responsible for the high mortality of *K. scalarina* at Pittwater was the high percentage of organic matter and anoxic nature of the sediment. Mortality of *K. scalarina* displayed no significant difference between under and away treatments, this may be due to the fact that cages away from racks experienced a higher level of sediment loss while clams under the rack were exposed to anoxic sediments.

The significant increase in *K. scalarina* survival at Smithton (Trial 5) compared to the previous two sites is due to a number of factors. Cages trialed at Smithton were more effective in maintaining sediment levels so clams could rebury to avoid fluctuations in environmental conditions. Secondly, unlike the Moulting Lagoon site, Smithton was also characterised by a stable salinity profile. Finally, oyster racks used were recently established and therefore had little organic build up and oxic sediments. This simulated a possible management strategy of adding clams as racks are constructed, to help avoid organic accumulation in the sediment caused by biodeposition of faeces and pseudofaeces from the oysters. Unfortunately, subsequent research (Manuscript 12) indicated that *K. scalarina*, despite anecdotal assessments by other researchers, did not prove to be a deposit feeder.

Site selection at Pipeclay Lagoon (Trial 4) was limited by the often shallow layer of fine grained sand as the shell grit layer of the sediment profile is very close to the surface in this area. The extensive tidal range at Pipeclay Lagoon often meant that areas with suitable sediment characteristics were exposed for a large proportion of the tidal cycle, thus exposing the clams to increased desiccation and temperature stress. The poor survival at Cockle Creek (Trial 3) was due again to the fact that areas suitable for the location of trials i.e. absence of anoxic sediment, meant that clams were often located high in the intertidal zone and therefore suffered from increased exposure.

Subsequent to these five trials, a 14 month growout trial was established at Duck Bay and cumulative mortality over that period was only about 35% with little consistent difference in growth or survival rates between under and away locations (Bellchambers, 1998; Manuscript 12).

To minimise the mortality of *K. scalarina* a number of criteria are important in the selection of oyster leases for marine farming of the species. The type of enclosure used must cause minimal scouring and sediment movement. This maximises the sediment inside enclosures thereby allowing clams to bury into the substrate to avoid extremes in environmental conditions. To ensure survival rates are acceptable, sites should be located in areas free of long term sporadic salinity fluctuations which may cause significant mortality of the species and induce unfavourable conditions, such as undesirable algal blooms. *K. scalarina* is sensitive to sediment characteristics so future sites should be located in areas with stable, well oxygenated sediments. Sites with these sediment characteristics may be difficult to locate within existing oyster leases, hence the most feasible solution may be to establish clam beds higher in the intertidal zone on existing

leases in areas not currently occupied by oyster cultivation. Use of intertidal oyster baskets, supported off-bottom by post and rail structures, has not been successful for *K. scalarina* (C. Dyke, pers. comm.). Alternatively, clam farming industries based on this species may have to be developed independent of the physical resources of oyster industries other than for the use of multi-species hatcheries.

In contrast, *Ruditapes largillierti* are harvested from the main fishery at St. Helens from highly anoxic shell deposits (see Manuscript 11). Further research should be undertaken on its dietary preferences as it could be suited to growout below subtidal commercial oyster cages, in higher current flow areas, both to generate additional income and to possibly ameliorate any organic buildup caused biodeposition onto the benthic area around the cages. Subtidal oyster and mussel farming is undertaken at St. Helens.

5.10.6 ACKNOWLEDGMENTS

The assistance of cooperating oyster farmers was greatly appreciated.

5.10.7 REFERENCES

Bellchambers, L. M. 1998. Ecology and ecophysiology of *Katelysia scalarina*, (Bivalvia: Veneridae), a commercially important clam. Unpublished PhD Thesis, University of Tasmania, Launceston.

Bellchambers, L. M. & Richardson, A. M. M., 1995. The effect of substrate disturbance and burial depth on the venerid clam, *Katelysia scalarina* (Lamarck, 1818). J. Shellfish Res., 14(1): 41-44.

Castagna, M. & Kraeuter, J. N., 1981. Manual for growing the hard clam *Mercenaria*. Special report in Applied Marine Science and Ocean Engineering N. 249, Virginia Institute of Marine Science, Gloucester Point, VA.

Maguire, G. B., 1991. Small clams and cockles - are they valuable for fisheries and aquaculture? Austasia Aquaculture Magazine, 5(12): 48-49.

Manzi, J. J. & Castagna, M., 1989. Clam Mariculture in North America. Developments in Aquaculture and Fisheries Science, vol 19. Elsevier, Amsterdam.

Toba, D. R., Thompson, D. S., Chew, K. K., Anderson, G. J . & Miller, M. B., 1992. Guide to Manila Clam Culture in Washington. Washington Sea Grant Program, University of Washington.

Treadwell, R., McKelvie, L. & Maguire, G. B. 1991. *Profitability of Selected Aquacultural Species*. Australian Bureau of Agricultural and Resource Economics Discussion Paper 91.11, ABARE, Canberra, 85 pp.

Treadwell, R., McKelvie, L. & Maguire, G. B. 1992. *Potential for Aquaculture in Australia.* Australian Bureau of Agricultural and Resource Economics Research Report 92.2, ABARE, Canberra, 81 pp.

5.10.8 FIGURES

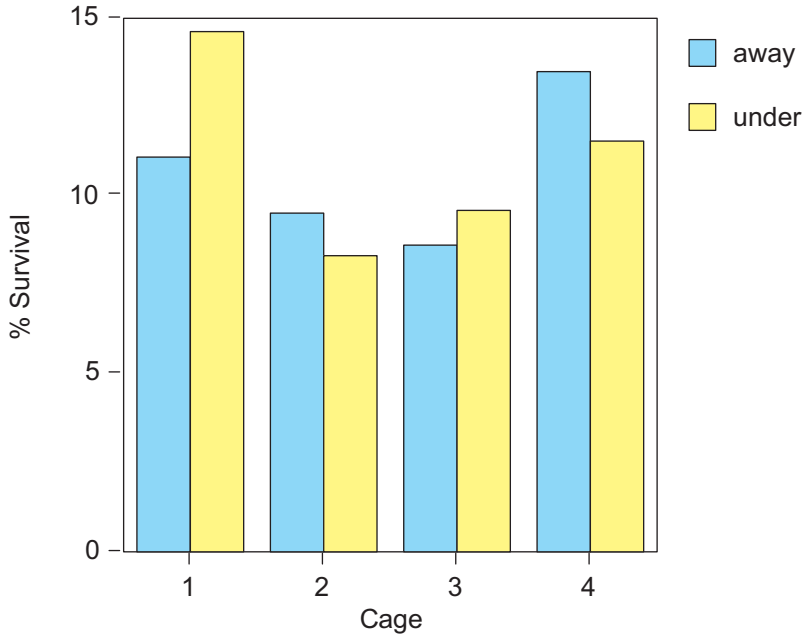

Figure 1. Percentage survival of *K. scalarina* under and away from oyster racks at Moulting Lagoon (n=180/cage) (Trial 1).

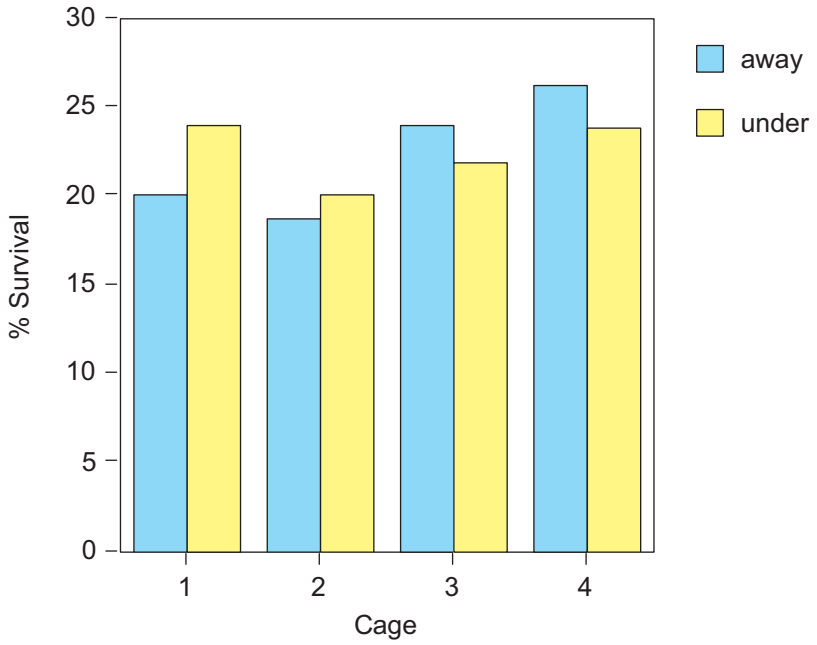

Figure 2. Percentage survival of *K. scalarina* under and away from oyster racks at Pittwater (n=180/cage) (Trial 2).

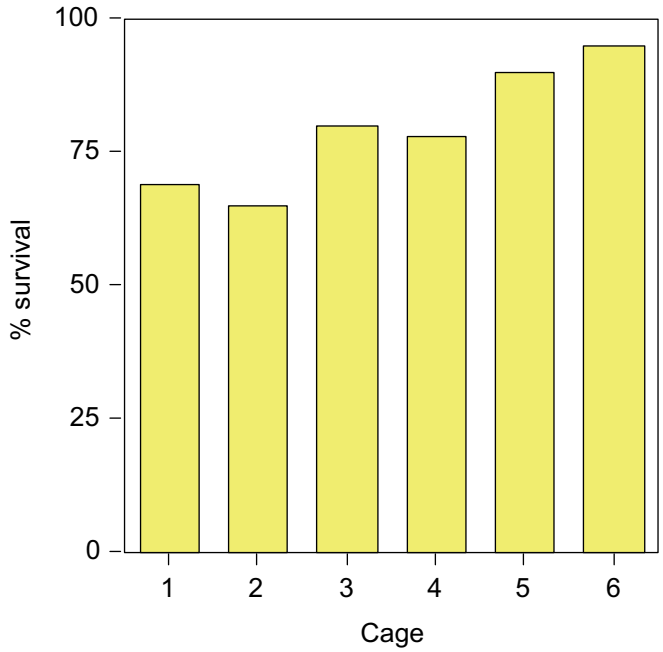

Figure 3. Percentage survival of *K. scalarina* under oyster racks at Duck Bay, Smithton (n=20 clams/cage) (Trial 5).

5.11 MANUSCRIPT 11

Performance of clams, *Ruditapes largillierti* (Phillipi, 1849), stocked at different densities and sizes in experimental cages in Georges Bay, Tasmania

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5.11.1 ABSTRACT

Two size groups of clams *Ruditapes (=Venerupis) largillierti* (average initial size of 27.4 mm shell length, 5.7 g whole weight or 43.5 mm shell length, 20.1 g whole weight, respectively) were grown for 8 months (April to December 1993) in partially-buried plastic mesh cages on a shallow subtidal flat at densities of 200 or 400 clams/m^2. Initial planting size significantly affected final size ($P<0.001$), however, neither initial density nor the size group x density interaction significantly influenced growth as measured by whole weight or length ($P>0.05$). Dry soft tissue (meat) weight of small clams was not affected by treatments but was significantly lower ($P<0.05$) when large clams were grown at the higher stocking density. Large clams exhibited slower absolute growth rates but higher condition index (CI) values than small clams ($P<0.05$) at harvest and CI was also density independent ($P>0.05$). Minimum CI values were observed in October 1993, coinciding with maximum moisture content values and the occurrence of numerous spawned individuals. A high proportion of the clams probably spawned between August and October. Gonadal stage was not influenced by density or sex but males were more common in the smaller size class. In the initial sample (April) far more of the small clams were in an indeterminate stage in comparison to large clams. By the end of the study, fewer of the small size class were in a post-spawned or regressive stage than for the large size class.

This study indicates that the species has considerable potential for aquaculture particularly because overall survival was high (84%) and independent of the treatments. However, around minimum commercial size (40-45 mm total length) growth was reduced thereby favouring marketing of small size grades. Although the management of shallow subtidal seabed plots is less convenient than intertidal plots, the major limitation is the identification of sites which do not suffer from periodic high current flow and hence sediment loss from the cages.

Keywords: Clam; venerid; *Ruditapes largillierti*; *Venerupis*, density; growth; spawning; condition; glycogen.

5.11.2 INTRODUCTION

To grow clams successfully in natural waterways, it is essential that seed clams be protected from predators (Toba et al., 1992). Recoveries of planted Manila clams *R. philippinarum* (Anderson and Chew, 1980; Spencer et al., 1992), the quahog *Mercenaria mercenaria* (Eldridge et al., 1979; Walker and Humphrey, 1984), and yearling surf clams *Spisula solidissima* (Goldberg and Walker, 1990) improve significantly when protection from predation is provided. The use of cages offers the advantages of maintaining clams in a substrate, excluding most macro-predators and facilitating harvests.

Stocking density is one of the major variables that influence profitability in aquacultural systems (Treadwell et al., 1991). Effects of changing stocking density are not always consistent between different published studies on clams including those involving *Mercenaria mercenaria*, however, reported effects include changes in growth or survival rates (Eldridge et al., 1979; Manzi et al., 1980; Hadley and Manzi 1984; Walker 1984), soft tissue weight and gonad size (Vincent et al., 1989). Size at stocking can also influence growth and survival size (Kraeuter and Castagna, 1985; Beal and Kraus, 1991) and recommended stocking densities have differed depending on initial clam size (Menzel 1971; Eldridge et al., 1979). Density effects in natural clam populations are reviewed in Manuscript 8 and Appendix 6.

The genus *Ruditapes* is one of the most commercially significant venerid groups largely because of importance of the carpet shell clam *Ruditapes decussatus* in Atlantic and Mediterranean fisheries (Shafee and Daoudi, 1991) and the Manila clam *R. phillipinarum* which is farmed widely (Toba et al., 1992). *R. largillierti* is endemic to New Zealand but its range has extended to Tasmania, by 1963 and probably much earlier, where it remains indistinguishable from New Zealand populations, on the basis of allozyme analysis (manuscript 16). *R. largillierti* grows to a length of 70 mm and a height of 50 mm and is found subtidally in both muddy and sandy substrates in shallow estuarine waters (Gabriel and Macpherson 1962). The only commercial fishery for this species in Tasmania is at St Helens and it is being evaluated for aquaculture (Kent et al., 1999).

This present study documents combined effects of stocking density and initial size in growth, gonad maturation and condition index for *R. largillierti* at St Helens and complements a longer study of seasonal patterns in gonadal development for this species (manuscript 2).

5.11.3 MATERIALS AND METHODS

Experimental animals

The subtidal clams used in this experiment were collected from Humbug Point, Georges Bay, St. Helens on the east coast of Tasmania, Australia (41°18' S, 148°17' E), 0.5 to 1.5 m below mean low tide. The bottom sediment was transferred with a garden fork to a floating sieve to

reveal the clams which were manually graded into small and large size groups (17.80 to 35.20 mm and 36.60 to 50.70 mm shell length, respectively).

Culture techniques

Clams were contained in 10 mm plastic (polyethylene) square mesh cages with dimensions of 50 x 30 x 15 cm (L x W x H). The 32 cages were partially buried so that they contained 10 cm of sediment. Random groups of four cages were attached to a pole via 1 m lengths of polyethylene rope to prevent baskets being displaced from the growout site. This was near the above collection site towards the mouth of Georges Bay, an estuarine embayment.

Experimental designs

Four combinations of two densities and two size groups with four sampling times (excluding the initial samples) were used to form a three-factor design. The four different treatments with two replicates in four sampling times required 32 experimental cages as two cages were removed permanently for each combination at each sampling time. The densities used were 30 and 60 clams/cage^{-1} (equivalent to 200 clams/m^2 and 400 clams/m^2 seabed area, respectively). The average initial shell length and whole weight of the small size group were 27.4 $\pm$ 4.4 mm (n=90; mean $\pm$ s.d) and 5.7 $\pm$ 2.5 g respectively and for the large size group were 43.5 $\pm$ 3.4 mm (n=90; mean $\pm$ s.d) and 20.1 $\pm$ 4.0 g respectively.

Sampling and handling

Initial samples were taken in April 1993 when the clams were positioned within baskets in the seabed. Samples were collected bimonthly beginning in June and continuing through August, October, and December, 1993 (Table 1). Water temperature was recorded near the cages on each occasion (Fig. 1). All clams sampled were covered with wet towel and packed in an insulated foam box which were then transported from the field to the laboratory by road for 3 h. Prior to processing, all samples were held for a minimum 15 h in recirculating system containing ambient sea water at the University of Tasmania, Launceston. Growth, meat condition and gonadal development were estimated using the surviving clams at each sampling time.

Growth indices

Clams were blotted dry with paper towel before weighing. The weights of individual clams were measured with a two decimal place balance. Vernier callipers were then used to determine length, height and depth to the nearest 0.1 mm. The length was defined as the longest possible measurement from anterior to posterior, while the height was measured from dorsal to ventral and depth as the greatest distance between the outer side of right and left valves (Quayle and Newkirk, 1989). The wet soft tissue was then removed and blotted dry before weighing. The valves were left to air dry for 24 h before being weighed.

In the initial sample, 70 clams from each size group were used for condition index and moisture content calculation. In the subsequent samples (June to December), from both clam size groups, all 10 or 20 clams taken from each replicate of low and high density were used for condition index, moisture content and glycogen content. The remaining clams were used for assessing gonad histology. The clams used for condition index and moisture content determination were processed as described in Manuscript 2.

Glycogen analyses

Dried soft tissue from condition index determinations were used for glycogen analysis. All clams from each a replicate cage were finely ground with a Sieb-technik™ geological rock crusher for 15-20 seconds and pooled prior to analysis.

The glycogen analysis method used in this study was modified from Keppler and Decker (1974) (B. Day and G.B. Maguire, unpublished data, 1999). The accurately weighed dry ground powder (approximately 3.0 g) was hydrated by adding four times the dry weight as distilled water. The rehydrated clam tissue was then mixed with a drop of Triton-X (wetting agent). The mixed solution was homogenised in a domestic blender with approximately 5 parts of 0.6M perchloric acid ($HClO_4$) for 3 minutes. Approximately 40 ml sample was taken from this homogenate and stored on ice for up to 1 h.

When all samples had been homogenised, 0.2 ml homogenate was taken and added with 0.1 ml $KHCO_3$ and 2 ml Amyloglucosidase reagent and incubated at 38°C in a water bath for 2 h with regular agitation. Duplicate subsamples of the test homogenate were taken from each sample. The reaction was stopped by adding 1 ml of 0.6M $HClO_4$. These test samples were then centrifuged for 15 min at 2800 rpm. Blank samples were prepared by mixing 2.0 ml of sample homogenate with 1.0 ml $KHCO_3$. These were centrifuged as for test samples. Blanks were also processed as duplicates.

Glucose content was then analysed on an Abbot 100 Biochromatic Analyser under the following conditions:

340/380 nm filter

1:51 dilution (ie. 5 ml of sample is added to 250 ml of reagent

[Hexokinase + G6PDH + ATP + NAD])

37°C incubation temperature

5 minute analysis time

reaction end point

A water blank and four standards (3 x 150 mg/100 ml Beckman, and 1 x 100 mg glucose/ 100 ml Agent) were included in each sequence of samples. Standards were used to calibrate the analyser at 150 mg glucose/100 ml, results were then read as mg glucose per 100 ml. Samples that resulted in errors greater than 18% between subsamples were repeated. The readings were then corrected for the various dilution and results expressed as g glycogen/100 g dry soft tissue weight after correction for the non-glycogen glucose content.

Gonad analyses

In the initial sample 20 clams from each size group were prepared for histological analysis. The clams in the subsequent samples (June to December) were randomly taken and accounted for about 30% of the clams in each cage. Therefore, an average of 10 and 20 was respectively collected from low and high density group. Prior to processing for histology the wet soft tissue was preserved in 10% phosphate buffered formalin. Processing, staining and gonad staging criteria were as described by Maguire and Kent (Manuscript 2).

Statistical analysis

A three factor Analysis of Variance (ANOVA) was conducted to assess differences for a range of performance indices in relation to time, density, and size group as fixed factors. Some variables were also analysed using two factor ANOVA (Table 1). When ANOVA showed a significant difference, Fisher's LSD (least significant difference) was used where necessary to identify the means that were significantly different from each other. Normality of the data was tested using

Shapiro-Wilk test (Zar, 1984) and homogeneity of variance was confirmed with the Cochran's test (Sokal and Rohlf, 1981). To satisfy the assumption of normality and homogeneity of variance, survival data were arcsine square root transformed prior to ANOVA. Size variation was quantified as Coefficient of Variation (100 x s.d./mean; Sokal and Rohlf, 1981). Chi-square contingency tests were used to assess the differences in gonad development stages in relation to treatments (Dowdy and Wearden, 1991).

5.11.4 RESULTS AND DISCUSSION

Survival was not significantly affected by any of the treatments ($P>0.05$) and overall survival by December was 84% (Figs. 2-3). Clearly the use of a subtidal site and mesh cages reduced the potential for losses due to environmental fluctuations and predators. As expected, the average whole clam weight, and morphometric measurements (length, height and depth) were significantly affected by initial planting size ($P<0.01$) and sampling time ($P<0.001$; Figs. 4 and 7, Table 1). However, no significant difference ($P>0.05$) in whole weight was found between clams held at different densities (Fig. 4) and there were no significant interaction terms ($P>0.05$). Fastest growth occurred within the first two months (April to June) for both size groups (Fig. 4) and subsequently in the warmer months (Table 1) particularly for small clams (October to December). Similar patterns of better growth during warmer months have been reported for *R. philippinarum, Meretrix lusoria* and *Mya arenaria* (Bourne 1982; Chen, 1990; Newell 1991).

The larger clams exhibited slower absolute growth rates on a whole weight ($P<0.001$) and shell length gain basis ($P<0.001$) (Figs. 5 and 8). Thus the average growth (shell length) rate of *R. largillierti* was 1.5 and 0.5 mm/month for small and large clams respectively (Fig. 7). Size group effects were similar for shell height (1.2 and 0.3 mm/month) and shell depth (0.8 and 0.3 mm/month). This study confirms reports of previous investigators that growth rate declines with increasing size. Eldridge et al. (1979) showed that aged clams *M. mercenaria* (mean size, 13 mm shell length) grew 1.7 mm/month to reach an average size of 44 mm, while another six months were required to attain a mean shell length of 51 mm (1.2 mm/month). Manila clams grew from 10 mm to 30; 30 to 42 and 42 to 51 mm with the rates of 1.7; 1.3 and 0.7 mm/month, respectively (Spencer et al., 1991). Growth data presented in this grant report for *K. scalarina* grown in baskets, indicate that *R. largillierti* is a faster growing species and this has been confirmed by sampling of tagged clams within the major fisheries for these species at Ansons Bay (Manuscript 13) and St Helens respectively (S. Riley, pers. comm., 1999).

Size variation is another factor of commercial significance. For this variable there was a significant size x density interaction ($P<0.05$) and hence size groups were considered separately. The large size class had much lower initial variation on a whole weight basis, measured as coefficient of variation, and this changed little throughout the study (Fig. 6), however, variation decreased through time for the smaller size group ($P<0.05$) and higher stocking density increased size variation ($P<0.01$). Similarly, Holliday et al. (1993) found that higher stocking densities increased size variation among Sydney rock oysters, *Saccostrea glomerata*, in mesh trays.

Meat size and Condition Index can be even more commercially significant. For dry soft tissue weight (meat weight), a significant size x density interaction necessitated separate analyses for the two size classes (Table 1). Predictably, larger clams contained more soft tissue and meat weight increased during warmer months (Fig. 9). Larger clams, but not the smaller clams, lost meat weight during the cooler months and for this group meat size was consistently better at the lower stocking density. Causal factors probably include spawning activity and competition for food at the elevated biomass levels in cages stocked with large clams at a high density.

The condition of both size groups, measured as Condition Index, maintained at either low or high density showed a similar trend ($P>0.05$) throughout the study (Fig. 10). Condition index, however, was significantly different between size groups ($P=0.01$) and through time ($P<0.001$). The clams were in best condition in June. The condition dropped dramatically by August and reached the lowest levels in October. A recovery then occurred between October and December. Large clams showed better condition throughout the sampling period. The maximum and minimum Condition Index values, based on sample means for the large size class, were both lower than equivalent values recorded by Maguire and Kent (Manuscript 2) for samples taken directly from the fishery. This could reflect the influence of the mesh enclosures or spatial patterns as the cages were in shallower water than commercially fished stocks. Moisture content data (not presented) exhibited an inverse pattern to the Condition Index data but variation between samples was not commercially significant (minimum was 78% for large clams at the higher density in June and the maximum was 81% for small clams at the lower density in October). This is a similar range to that reported for a large size class by Maguire and Kent (Manuscript 2).

Initial size and density of the clams had no significant effect on their glycogen content ($P>0.05$) but differences in glycogen content through time were highly significant ($P<0.001$) and mirrored changes in Condition Index (Fig. 11). Glycogen content increased markedly from April and reached a peak in June. A sharp decline in glycogen occurred after June but the rate of decrease was slower between August and October. From October onward, there was a considerable increase in glycogen content until the end of the study.

Chi-square contingency tests were used to assess the effects of density and size group on gonad development (Fig 13). No significant effect of density on gonad stages was found either in females or males in both size groups throughout the sampling times ($P>0.05$). The size groups, however, differed in gonadal development and sex ratio (Fig. 14) and there were distinct changes through time for the former but not the latter.

Among the clams that could be sexed in April samples, the majority were in developing stages (38% D1 and 26% D2; Fig. 13). No mature gonads (Fig. 6.03), however, were detected at that time. Gamete development progressed extensively during the Autumn period (5% D1, 58% D2, and 32 % advanced stages in June). Maturation largely occurred between June to August when the dominant reproductive stage changed from developing (63 %) in June to advanced (82%) in August. Only 26% remained in an advanced stage of maturation by October and regressive stages was common (33%). It is likely that most of the clams spawned between August and October. This is evident is the proportion of clams (all treatments pooled) in spawning condition (Fig. 12). However, spawning was not necessarily synchronous as advanced stages were observed from June to December (Fig. 13) and spawned individuals were evident in October and December samples. Developing stages (42%) was also detected in October (Fig. 6.09). This indicates that renewed gametogenesis may occur soon after spawning. The differences in gonad stages due to size group were only observed in December for females while no difference was observed for males. All stages were detected in large females at both densities and the majority (50%) was post-spawned and regressive stages (Fig. 6.04 and 6.05). In contrast, few post-spawned and regressive stages (<5%) were detected in small clams and most stages (90 %) were developing (D1 and D2). It is also noteworthy that in the initial sample (April) >60% of the small clams were indeterminate while the equivalent value for large clams was 10%. As discussed by Maguire and Kent (Manuscript 2), the much higher proportion of females in the large size class is consistent with a pattern of size related sex change although sex ratio did not change as the clams grew during this study (Fig. 14).

The maturation of gametes from June onward (during winter) was consistent with the low values of glycogen in this period, as gonad development occurs at expense of glycogen. In addition, temperature in winter, 11.8 to 12.7°C (Table 3.6) may not affect gonad maturation as much as somatic tissue growth (Section 3). Gauthier and Soniat (1989) showed that gametogenic development of oysters, *Crassostrea virginica* cultured in Gulf Coast of Mexico was not interrupted by winter dormancy as long as temperatures were not unusually low. The subsequent spawning period of August-October is consistent with condition index and moisture content data (Fig. 10). The poorest condition and the maximum water content in meat occurred in October probably as a result of spawning.

5.11.5 CONCLUSIONS

Manipulation of stocking density is an important management tool in aquaculture. If satisfactory survival and growth are maintained, higher stocking densities may result in lower production costs. In the present study, the stocking densities used did not cause significant differences in terms of growth rates in whole weight, shell weight and shell length, gonad development, condition index, glycogen and moisture content. Effects on dry soft tissue were relatively minor as well as differences in variability of size (coefficient of variation). Therefore, trends of decreasing growth with increase in density were not discernible in this trial. As previously indicated, this conflicts with the results on the effects of density on performance of other species of clams in some published studies. However, density did not have a major influence on the performance of *K. scalarina* in cages (Manuscript 8).

This study suggests that culture of the clams *R. largillierti* is cages at densities of 400 clams. m^{-2} or more is appropriate. However, at this higher density, adverse effects of meat weight were just evident for large clams and this density may be close to optimum for that size class. Further studies may be required to ascertain the optimum stocking density for smaller clams. Algal biofouling was becoming significant by the end of the study and could be addressed by in situ cleaning of the mesh or by moving stock to clean baskets at a time when density reductions were appropriate as the clam to a larger size. Such density manipulations are, however, labour intensive.

The cages used proved to be efficient in excluding macro predators as evident by low mortality rates (about 2%/month). The major problem was sediment loss from some cages during periods of high current flow. Enclosure of planted, subtidal seabed with a light, overlying mesh rather than use of baskets may negate the impact of sediment movements (Heasman et al., 1998).

Differences in growth due to initial sizes, however, were shown in this experiment. The difference is a characteristic of the lessening growth rate with size and age commonly observed in other bivalves (Beninger and Lucas, 1984; Ruwa, 1990). In the present study, it is clear that for both density groups, faster growth rates were observed in small clams. The growth rate of *R. largillierti* , therefore, is comparable to that of other commercial clam species including *M. mercenaria* (Craig et al., 1988), and *T. phillipinarum* (Spencer et al., 1991). Growth rates of seed *M. mercenaria* was found to range from 0.9 to 1.5 mm/month, however where water circulation was greatest, a growth rate of up to 2.8 mm/month was recorded for *M. mercenaria* (Craig et al., 1988). Walker and Humphrey (1984) reported that *M. mercenaria* seed imported from Massachusetts and planted in the coastal waters of Georgia grew from 12.8 to 23.9 mm in their first year (0.93 mm/month). Quahog clams (mean length 19.5 mm) planted at various sites, achieved growth rates of 1.0 to 2.2 mm/month depending upon location (Goldberg and Walker, 1990). Spencer et al. (1990) showed that growth rates of Manila clams (10 mm mean length) were between 0.75 to 1.70 mm/month depending on initial planting sizes. All of these studies had been carried out over $\geq$ 1 year.

Growth data obtained in the present study were mostly based on growth during cooler months. Rates of growth observed in the warmer months were of 2.7 and 1.1 mm (shell length)/month respectively for small and large clams respectively (April - June), and 1.8 and 0.4 mm/month for small and large clams respectively from October to December. Therefore, the annual growth rates of the clams *V. largillierti* may well have been higher than the data indicate. The fast growth and high survival rate attained by the clams *V. largillierti* may make this bivalve species a valuable candidate for cage culture at high stocking density in Tasmanian waters. However, many questions of a biological nature remain to be answered before a complete analysis of economic feasibility can be attempted. Growth rate for large clams, for instance, was relatively low suggesting that more time may be required for growth before large clams will reach a market size of about 45 mm in shell length (A. Flintoff, pers. comm.). A successful marketing, strategy may require that other markets for smaller clams to be investigated. This should be possible, as another Tasmanian clams *Katelysia scalarina* is generally marketed at a smaller size than *R. largillierti*.

An increase in condition index often associated with the onset of gametogenesis is commonly observed in many bivalve species e.g. clams *Tapes decussatus* and *T. philippina*rum (Beninger and Lucas, 1984), and oysters *Crassostrea gigas* (Maguire et al., 1994). In the present study, however, condition index was observed to decline from June when gonadal maturation process was most intensive. A progressive decrease in condition index into August was observed as gonadal maturation peaked (Chapter 6). This phenomenon of decreasing condition index with an increase in the rate of gonadal maturation may be related partly to the poor condition for growth during the cold months. As a consequence, glycogen reserves may be rapidly utilised to facilitate gametogenesis while energy intake could not compensate for energy expenditure. This limited energy intake was also evidenced by the low growth rates (whole, shell, dry soft tissue weights and shell length) between June and October. Condition and growth improved after October. *R. largillierti* showed typical seasonal variations in glycogen content in relation to gametogenesis and spawning and perhaps due to water temperature during winter. From June onwards there was a rapid decrease in glycogen production as gametogenesis progressed through to spawning by in October.

The histological analyses performed revealed no evidence to suggest simultaneous occurrence of male and female gametes present in individual samples prepared. This indicates that *R. largillierti* is not hermaphroditic, that is, a mature adult is not able to produce both male and female gametes. In addition there was also very similar rate of gonad development stages between females and males (Paturusi, 1984). The possibility of several stages of gonad maturation being present within a given adult clam implies that multiple spawning events (partial spawning) may be possible year round. In terms of both the production of juveniles and the utilisation of facilities, the capital outlay for the construction of which may have been substantial, the benefits derived may be significant. The year round production of harvestable clams may mean that the supply of clams to the market would not be restricted had the broodstock only spawned during a certain time in the year, such as during the spring months. To date, mature *R. largillierti* adults have been successfully spawned to produce viable gametes during both autumn and summer months under artificial conditions (Maguire and Kent, unpublished data, 1994). Furthermore, the year round use of costly and extensive hatchery facilities would better ensure a capital return sufficient to justify the initial expense of such facilities. However, the winter maturation pattern observed in the present study coincides with a relatively inactive period for commercial bivalve hatcheries in Tasmania and this represents a commercial opportunity if there is a sufficient demand for *R. largillierti* spat.

5.11.6 ACKNOWLEDGMENTS

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5.11.7 REFERENCES

Anderson, G.J. and Chew, K.K., 1980. Intertidal culture of the Manila clam *Tapes japonica* using hatchery-reared seed clams and protective net enclosures. ICES, Copenhagen, 16 pp.

Beal, B.F. and Kraus, M.G., 1990. The importance of initial size and density on the survival and growth of hatchery-reared individuals of *Mya arenaria*. (Abstract). J. Shellfish Res., 8: 462-463.

Beninger, P.G. and Lucas, A., 1984. Seasonal variations in condition, reproductive activity, and gross biochemical composition of two species of adult clam reared in a common habitat: *Tapes decussatus* L. (Jeffreys) and *Tapes philippinarum* (Adams and Reeves). J. Exp. Mar. Biol. Ecol., 79:19-37.

Bourne, N., 1982. Distribution, reproduction, and growth of Manila clam, *Tapes philippinarum* (Adams and Reeves), in British Columbia. J. Shellfish Res., 2: 47-54.

Chen, T.P., 1990. Aquaculture in Taiwan. Fishing News Books, Oxford.

Craig, M.A., Bright, T.J. and Gittings, S.R., 1988. Growth of *Mercenaria mercenaria* and *Mercenaria mercenaria texana* seed clams planted in two Texas Bays. Aquaculture, 183-207.

Dowdy, S. and Wearden, S., 1991. Statistics for Research. Wiley series in probability and mathematical statistics. John Wiley & Sons, New York, 629 pp.

Eldridge, P.J., Eversole, A.B. and Whetstone, J.M., 1979. Comparative survival and growth rates of hard clams, *Mercenaria mercenaria*, planted in trays subtidally and intertidally at varying densities in a South Carolina estuary. Proc. Natl. Shellfish. Assoc. 69: 30-39.

Gabriel, C.J. and Macpherson, J.H., 1962. Marine Molluscs of Victoria, Melbourne University Press, 475 pp.

Gauthier, J.D. and Soniat, T.M., 1989. Changes in the gonadal state of Louisiana oysters during their autumn spawning season. J. Shellfish Res., 8: 83-86.

Goldberg, R. and Walker, R.L., 1990. Cage culture of yearling surf clams, *Spisula solidissima* (Dillwyn, 1817), in coastal Georgia. J. Shellfish Res. Vol. 9. No. 1. 187-193.

Hadley, N.H. and Manzi, J.J., 1984. Growth of seed clams, *Mercenaria mercenaria*, at various densities in a commercial scale nursery system. Aquaculture, 36:369-378.

Heasman, M.P., O'Connor, W.A., O'Connor, S.J. and Walker, W.W., 1998. Enhancement and Farming of Scallops in NSW using Hatchery Produced Seedstock. FRDC Project No. 94/084. NSW Fisheries Final Report Series, No 8, 146 pp.

Holliday, J.E., Allan, G.L. and Nell, A., 1993. Effects of stocking density on juvenile Sydney rock oysters, *Saccostrea commercialis* (Iredale & Roughley), in cylinders. Aquaculture, 109: 13-26.

Kent, G. N., Maguire, G. B., and Duthie, I., 1998. Spawning, settlement and growth of the New Zealand venerid *Ruditapes largillierti* (Philippi 1849). NZ J. Marine and Freshwater Research, 33: 55-62.

Keppler D. and Decker, K., 1974. Glycogen Determination with Amyloglucosidase. In: H.U. Bergmeyer (Editor), Methods of Enzymatic Analysis. Verlag Chemie Weinheim, Academic Press, Inc., New York. pp. 1127-1131.

Kraeuter, J.N. and Castagna, M., 1985. The effects of seed size, shell bags, crab traps, and netting on the survival of the northern hard clam *Mercenaria mercenaria* (Linne). J. Shellfish Res., 5: 69-72.

Maguire, G.B., Gardner, N.C., Nell, J.A., Kent. G. and Kent, A., 1994. Studies on triploid oysters in Australia. II. Growth, condition index, glycogen content and gonad area of triploid and diploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania. In: J.A. Nell and G.B. Maguire (Editors), Evaluation of Triploid Sydney Rock Oysters (*Saccostrea commercialis*) and Pacific Oysters (*Crassostrea gigas*) on Commercial Leases in New South Wales and Tasmania. FRDC Grant Report., pp. 37-71.

Menzel, R. W., 1971. Quahog clams and their possible mariculture. Proc. Ann. Workshop World Mariculture Soc., 23-36.

Newell, C.R., 1991. The soft clam *Mya arenaria* (Linnaeus) in North America. In: W. Menzel (Ed.), Estuarine and Marine Bivalve Mollusk Culture. CRC Press, Inc., Boca Raton., pp. 1-9.

Paturusi, A., 1994. Effects of stocking density and initial size on the performance of the clam *Venerupis largillierti* (Phillipi, 1849) in experimental cages in Georges Bay, Tasmania. Unpublished thesis, University of Tasmania.

Quayle, D.B. and Newkirk, G.F., 1989. Farming Bivalve Molluscs: Method for Study and Development. The World Aquaculture Society, Baton Rouge, L.A., 294 pp.

Ruwa, R.K., 1990. Growth of *Crassostrea calcullata* Born (Bivalvia) at different levels in the intertidal zone. Aquaculture, 88:303-312.

Shafee, M.S. and Daoudi, M., 1991. Gametogenesis and spawning in the carpet-shell clam, *Ruditapes decussatus* (L.) (Mollusca: Bivalvia), from the Atlantic coast of Morocco. Aquaculture and Fisheries Management, 22: 203-216.

Sokal, R.R. and Rohlf, F.J., 1981. Biometry. W.H. Freeman and Company, New York, NY, 895 pp.

Spencer, B.E., Edwards, D.B. and Millican, P.F., 1991. Cultivation of Manila clams. Laboratory Leaflet No. 65. Ministry of Agriculture, Fisheries and Food Directorate of Fisheries Research. Lowestoft. 27 pp.

Spencer, B.E., Edwards, D.B. and Millican, P.F., 1992. Protecting Manila clams (*Tapes phillipinarum*) beds with plastic netting. Aquaculture, 105: 251-268.

Toba, D.R., Thompson, D.S., Chew, K.K., Anderson, G.J. and Miller, M.B., 1992. Guide to Manila Clam Culture in Washington. Washington Sea Grant Program, University of Washington. 80 pp.

Treadwell, R., McKelvie, L. and Maguire, G.B., 1991. Profitability of Selected Aquacultural Species. Australian Bureau of Agricultural And Resource Economics Discussion Paper 91.11, ABARE, Canberra, 85 pp.

Vincent, B., Joly, D. and Brassard, C., 1989. Effects of density on the growth of the bivalve *Macoma balthica* (L.) in the intertidal zone. J. Exp. Mar. Biol. Ecol., 126: 145-162.

Walker, R.L., 1984. Effects of density and sampling time on the growth of the hard clam *Mercenaria mercenaria* planted in predator free-cages in coastal Georgia. Nautilus, 98: 114-119.

Zar, J.H., 1984. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J., 718 p.

5.11.8 TABLES AND FIGURES

Table 1. Summary of ANOVA results for a range of variables measured for of two size groups of *Ruditapes largillierti* in subtidal cages at different densities. (*** = P<0.001; ** = P<0.01; * = P<0.05; NS = P>0.05; NA indicates a two factor ANOVA in which this variable was not included).

Variable	Size	Density	Time	Significant interactions
Survival	NS	NS	NS	
Whole weight	***	NS	***	
Whole weight gain	**	*	NA	
Coefficient of variation C.V. (whole weight)	***	*	*	
C.V. (whole weight) (small clams)	NA	**	*	
C.V. (whole weight) (large clams)	NA	NS	NS	
Shell length	***	NS	***	Size x Time **
Dry meat weight	***	=0.05	**	Month x Size *
Dry meat weight (small clams)	NA	NS	NS	
Dry meat weight (large clams)	NA	*	**	
Condition index	=0.01	NS	***	
Moisture content of soft tissue	**	NS	***	
Glycogen content	NS	NS	***	

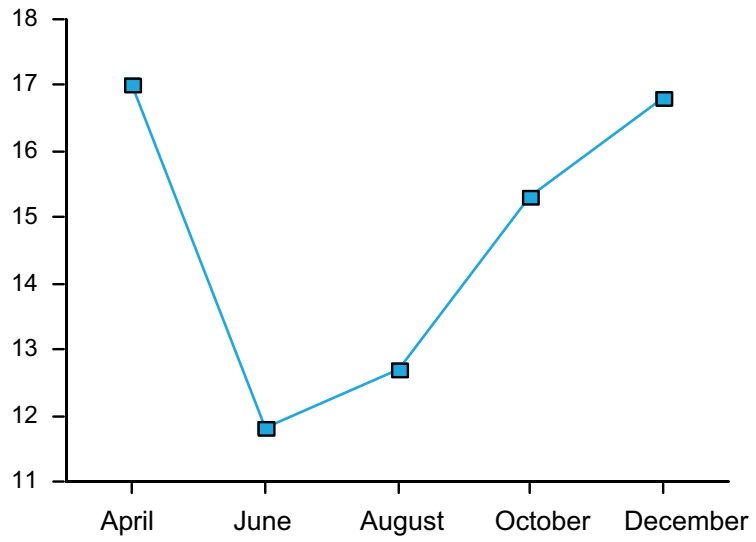

Figure 1. Water temperature at the subtidal growout site at St. Helens, Tasmania in 1993.

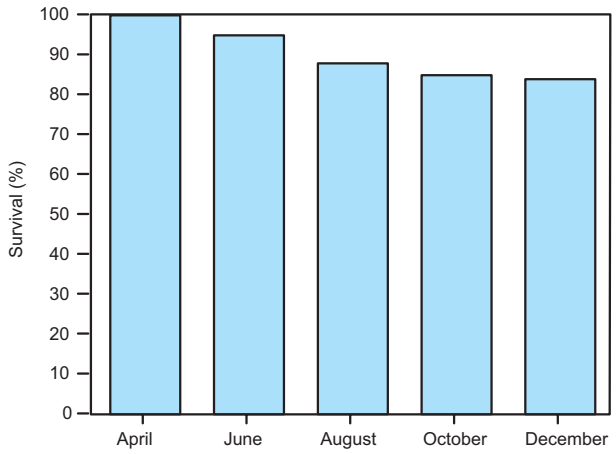

Figure 2. Overall survival of *Ruditapes largillierti* in cages (data for size group and density pooled).

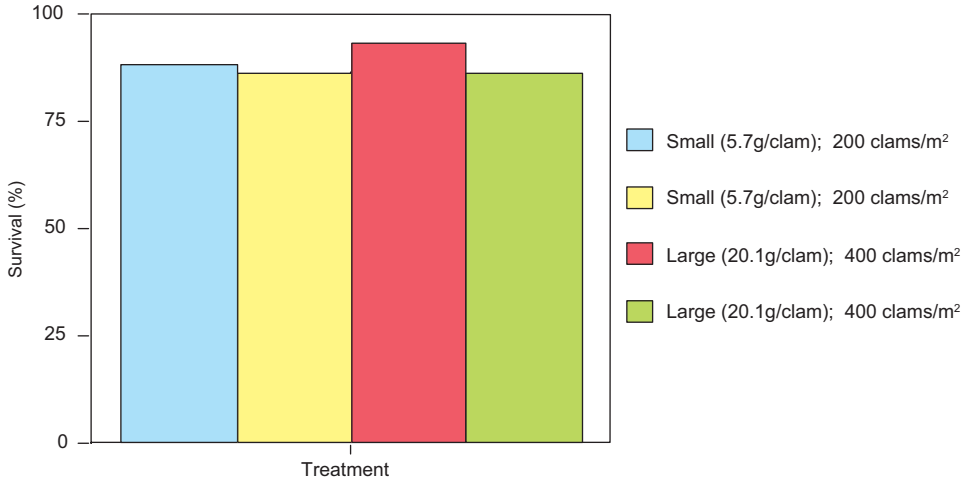

Figure 3. Overall survival of two size groups of *Ruditapes largillierti* in subtidal cages at different densities.

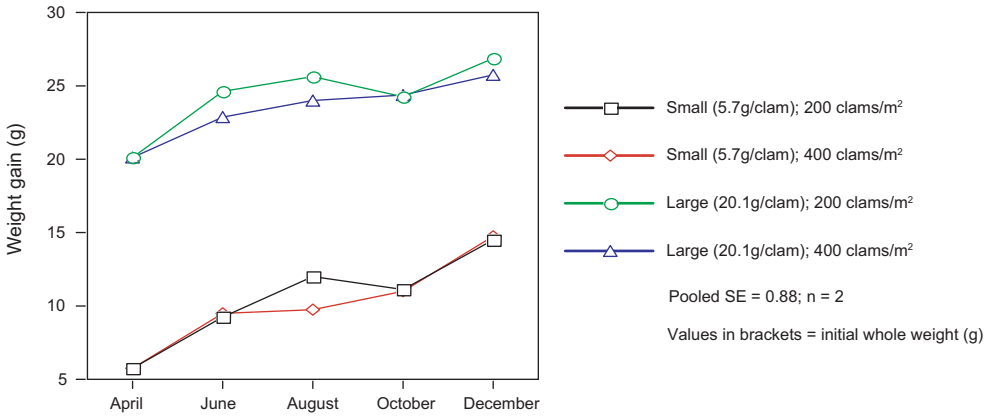

Figure 4. Growth (whole weight) of two size groups of *Ruditapes largillierti* in subtidal cages at different densities.

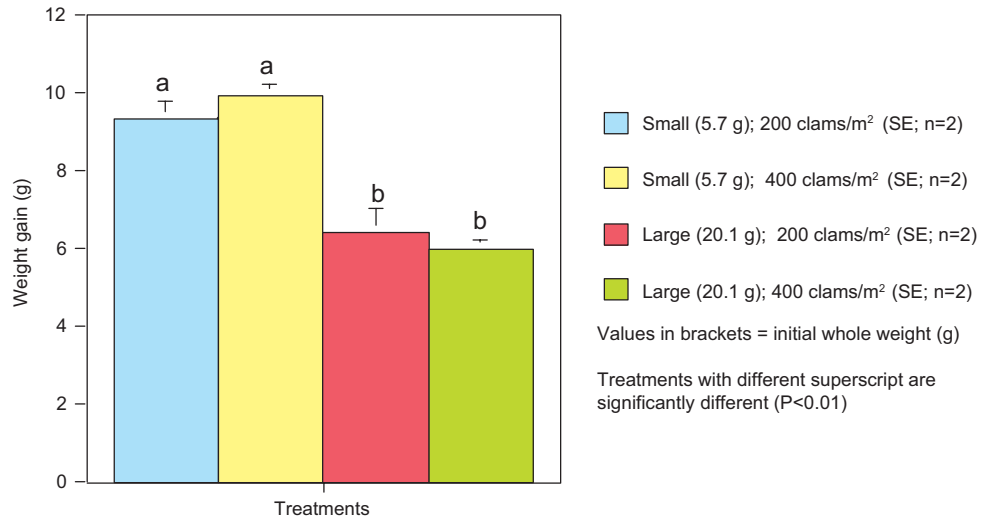

Figure 5. Whole weight gain of two size groups of *Ruditapes largillierti* in subtidal cages at different densities.

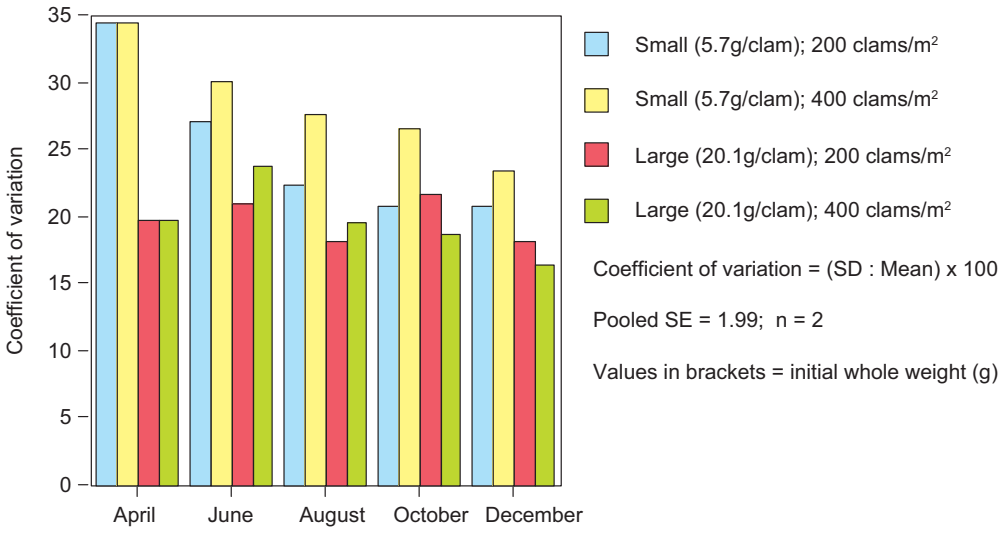

Figure 6. Coefficient of variation (CV) for whole weight (g/clam) for two size groups of *Ruditapes largillierti* in subtidal cages at different densities.

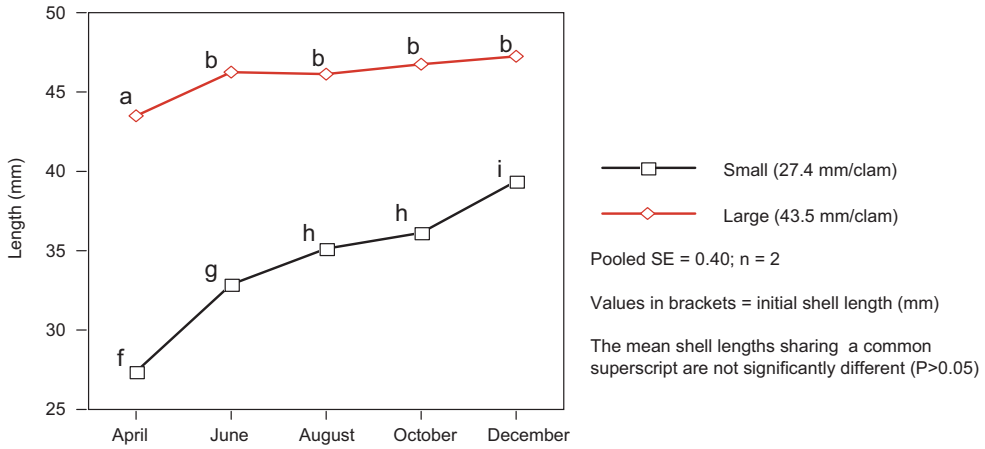

Figure 7. Growth (shell length) of two size groups of *Ruditapes largillierti* in subtidal cages (data for two stocking densities pooled).

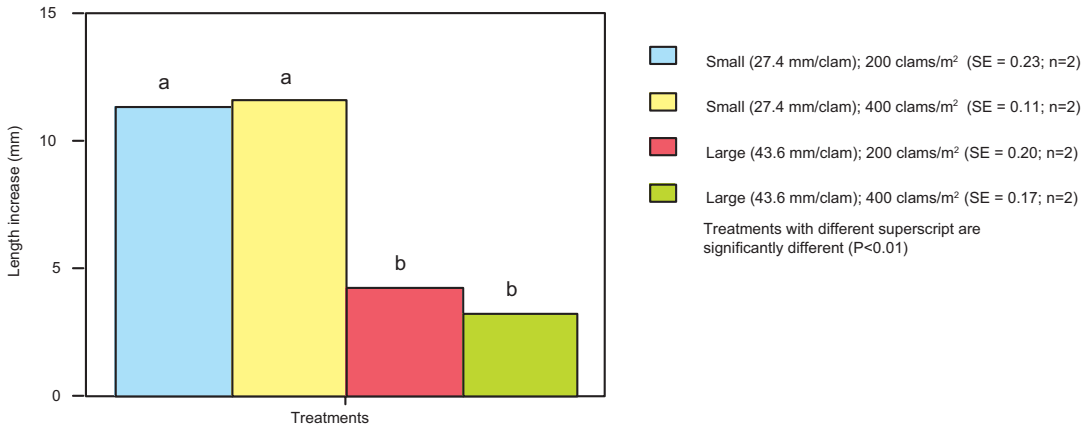

Figure 8. Shell length gain of two size groups of *Ruditapes largillierti* in subtidal cages at different densities.

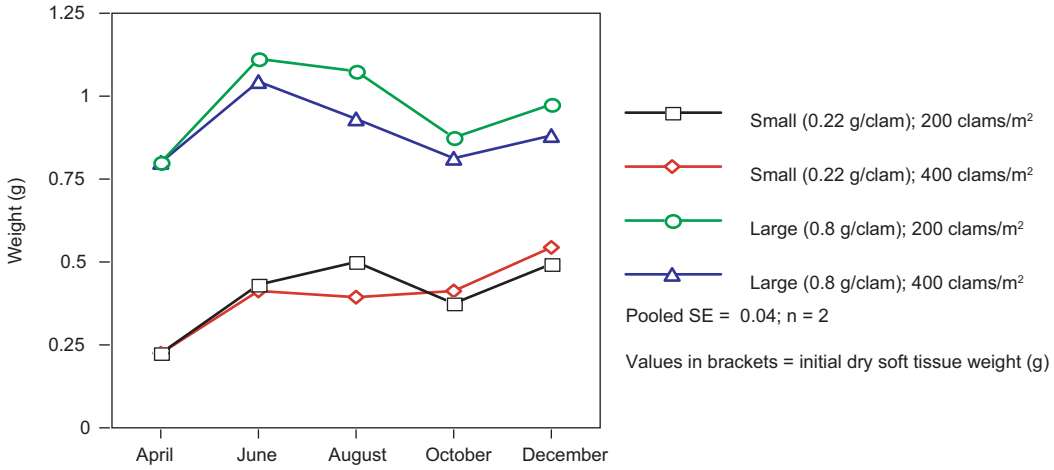

Figure 9. Growth (dry soft tissue weight) of two size groups of *Ruditapes largillierti* in subtidal cages at different densities.

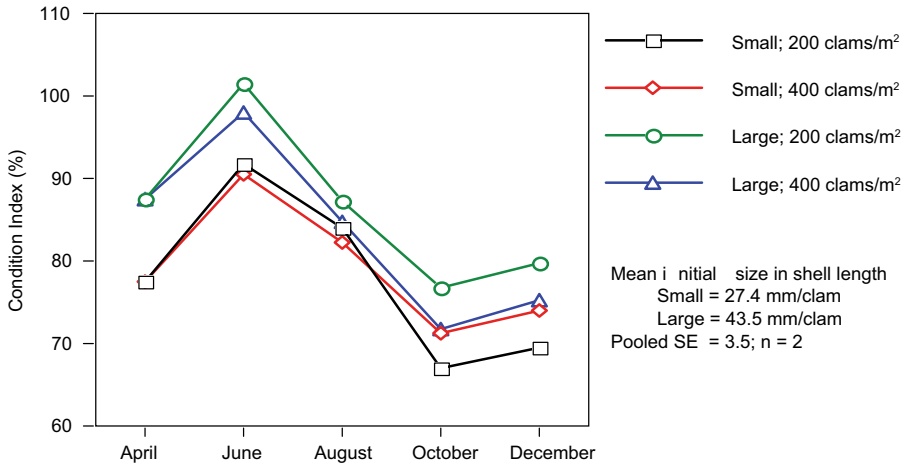

Figure 10. Condition index of two size groups of *Ruditapes largillierti* in subtidal cages at different densities.

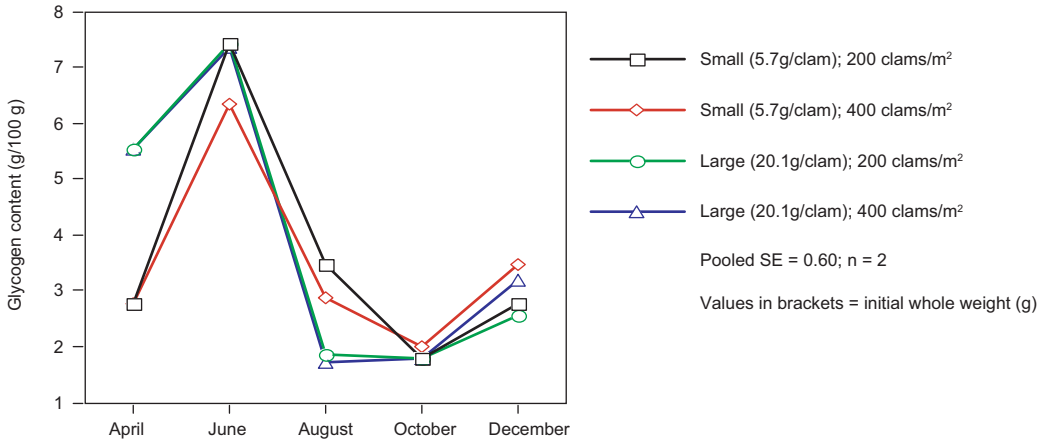

Figure 11. Glycogen content of two size groups of *Ruditapes largillierti* in subtidal cages at different densities.

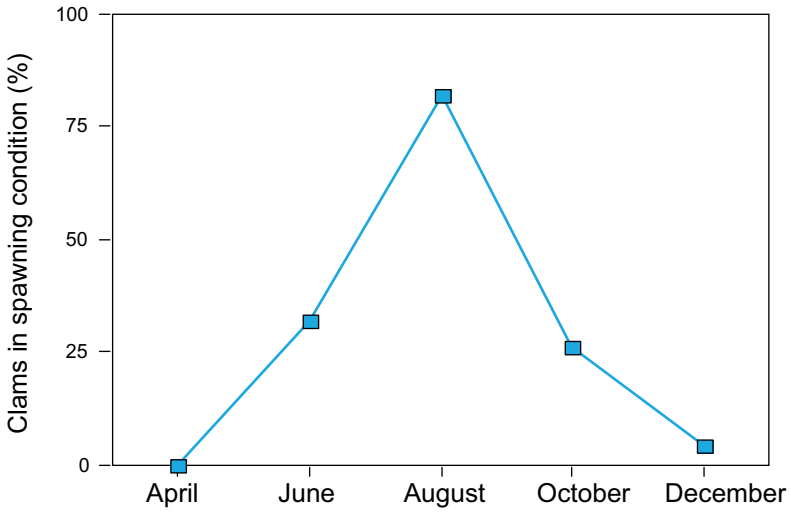

Figure 12. Spawning condition of *Ruditapes largillierti* in subtidal cages (data two size groups at different densities pooled).

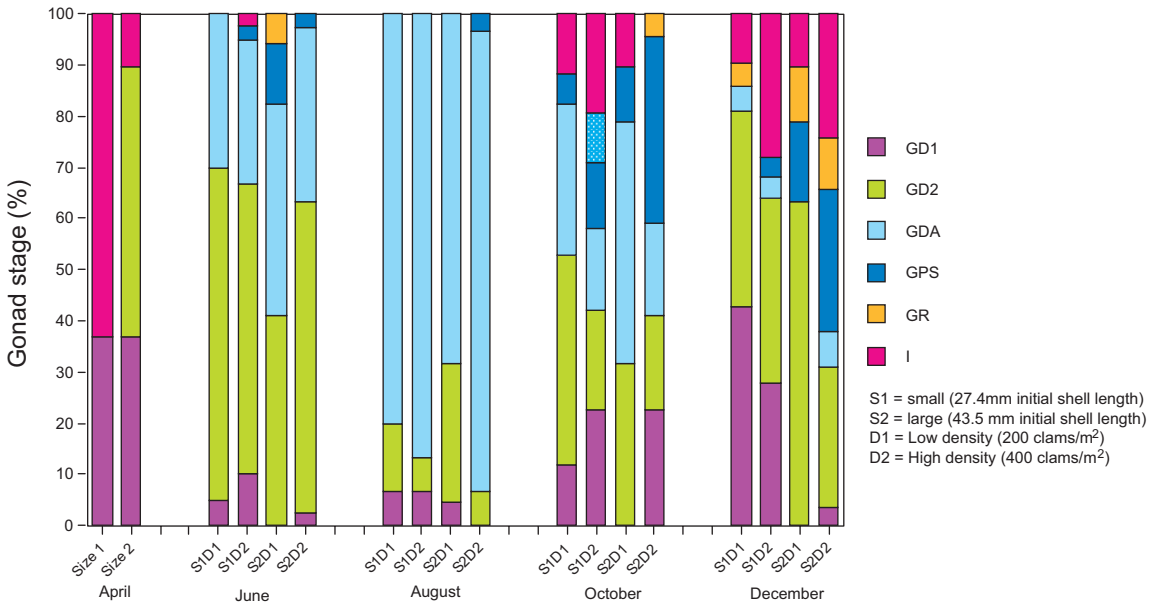

Figure 13. Gonad development stage of two size groups *Ruditapes largillierti* grown at different densities (sexes pooled). GD1-2 are developing gonad stages with GDA indicating advanced gonad development. GPS and GR represent post-spawning and regressive stages with I indicating an indeterminant stage with contracted follicles and germ cells that are not sexually differentiated.

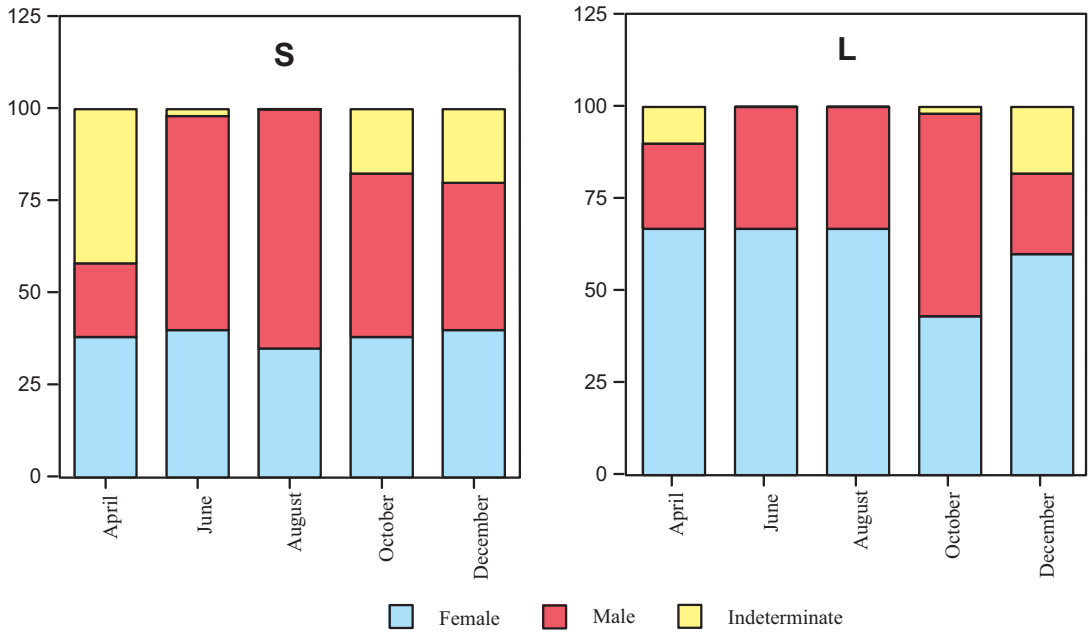

Figure 14. Sex ratio (%) of small (S) and large (L) *Ruditapes largillierti* from subtidal cages (data pooled for density).

5.12 MANUSCRIPT 12

Diet of *Katelysia scalarina* (Lamarck 1818) (Bivalvia: Veneridae) on a Pacific oyster (*Crassostrea gigas*) farm

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5.12.1 ABSTRACT

K. scalarina were placed in cages under and away from intertidal oyster racks at Duck Bay Smithton, Tasmania to determine the major food source of the species and to investigate the potential for polyculture with Pacific oysters *Crassostrea gigas.* The gut contents of *K. scalarina* on the two treatments were similar in composition and were comprised primarily from benthic diatoms from the genera *Navicula, Nitzschia* and *Cocconeis*. The abundance of benthic diatoms in the stomach contents and water samples, coupled with the absence of detritus, suggests that *K. scalarina* is predominantly a suspension feeder rather than a deposit feeder. As such *K. scalarina* may derive little trophic benefit from being located below an intertidal, off-bottom oyster rack and any benefits are far outweighed by the accumulation of the anoxic sediments. Since these benthic diatoms are a common component of the diet of Pacific oysters, the potential for competition for food resources exists if *K. scalarina* is grown in oyster growing areas in southern Australia.

Key words: algae, clams, food sources, *Katelysia*, oysters.

5.12.2 INTRODUCTION

Food can be a limiting factor for bivalve production (Beukema & Cadee, 1986) particularly as dilution of phytoplankton concentrations by suspension feeding organisms may lower food supply for other suspension feeders. It is generally assumed that filter feeders rely on phytoplankton suspended in the water column as their main food source (Shumway *et al.*, 1987; Newell & Shumway, 1993). However, in unconsolidated sediments, seston and benthic phytoplankton are important food sources for benthic organisms (Langdon & Newell, 1990).

Katelysia scalarina is a small (40 mm, shell length), intertidal, clam found in sheltered bays and estuaries along the southern coast of Australia (Wells & Roberts, 1980; Coleman, 1982; Roberts, 1983). Tasmanian populations are genetically similar, but are distinguishable from southern mainland populations (Soh *et al*., 1998). *K. scalarina* inhabits fine to medium grain sand approximately 2-4 cm below the surface and comprises the dominant macrofaunal component of many bays and estuaries on Tasmania's east coast (Bellchambers and Richardson, 1995). Since 1987 a small commercial fishery for *K. scalarina* has operated in several of the larger bays and estuaries of Tasmania's east coast and now has an annual value of approximately $Aus 200, 000 depending on market value. However, fishery managers are concerned that the current harvesting rates are unsustainable and are examining other methods of ensuring the sustainability of clam populations such as aquaculture (Kent *et al*., 1998).

The Pacific oyster, *Crassostrea gigas*, was first introduced into Tasmanian waters in the late 1940's and early 1950's. By January 1993 there were 91 oyster leases occupying 1353 ha, which were estimated in 1995 to be worth approximately $15 million annually to the Tasmanian

economy (Wilson *et al.,* 1996). Traditionally oyster farming has consisted of placing oysters in mesh baskets strung between wooden racks in the intertidal zone, so that the oysters are suspended in the water column and faeces and pseudofaeces sink to the sediment below. Thus the use of oyster farms for clam culture has been identified as offering advantages in terms of use of existing infrastructure and microbial monitoring data for waterways, arising from quality assurance programs (Maguire, 1991). In addition, *K. scalarina* occurs on several of the existing Pacific oyster leases in Moulting Lagoon and Duck Bay.

No published work has been conducted on the feeding of *K. scalarina* and there is some debate as to the primary feeding strategy and major dietary components of this species. For the species to be successfully farmed, especially in a polyculture system, this information is essential to ensure competition for food resources is minimised. Therefore a series of trials were conducted at Duck Bay, Smithton to determine the feasibility of growing *K. scalarina* under oyster racks on existing marine farms. Experiments were situated under, and away from, existing oyster racks for two reasons. Firstly, the aim was to investigate whether *K. scalarina* and *C. gigas* compete for food resources. Such competition is likely to render the potentially attractive idea of growing clams on otherwise unused space on oyster leases unacceptable to farmers concerned about carrying capacity of waterways used for farming oysters. Secondly, to determine whether *K. scalarina* obtained any positive benefits from being located beneath oyster racks in terms of additional food resources from oyster faecal and pseudofaecal biodeposition. The stocking of clams able to utilise benthic organic deposits on existing oyster leases has the potential to reduce the localised environmental impact of oyster farms (Maguire, 1992).

5.12.3 METHODS

Site Description

Experiments were established at Duck Bay Shellfish's Pacific oyster lease on the 30/08/95 at Duck Bay, Smithton. Duck Bay is a large continuously open bay on the North West coast of Tasmania, Australia (54°81'S,145°38'E) (Figure 1). Buffered from the strong northerly seas by Perkins Island and the westerly projection of the Tasmanian mainland, Duck Bay experiences less wave action and a faster tidal current than many of the North West coast bays (Last, 1983). The bay is predominantly marine but receives freshwater in the southern end from the Duck River and is dominated by extensive subtidal and tidal flats dissected by braided channels. The tidal cycle is semi-diurnal with a period of approximately 3 hours between high and low tide. The coast is of low relief mainly fringed with salt marshes and mixtures of mud, sand and shingle. There are no significant seagrass beds or mangrove areas in the vicinity.

Duck Bay is approximately 12 km^2 in area, however marine farming is only permitted in West Duck Bay, from Pelican Point to East Perkins Island, an area of approximately 7.5 km^2. There are three oyster leases in Duck Bay with a total area of 45 ha and approximately 32-35 ha are currently under cultivation.

Experimental Design

Adult *K. scalarina* (30.0 - 40.0 mm shell length) were collected from Moulting Lagoon, Coles Bay, Tasmania, Australia (42°05'S, 148°10'E) as insufficient numbers were available in Duck Bay. Clams were placed inside cages to prevent migration over the experimental period and to prevent access of large mobile consumers, such as crabs and wading birds, to the clams. All treatments were enclosed identically, therefore any additional effects of enclosures were held constant across the treatments. All cages were constructed of 9 mm Nylex® mesh (33 cm wide

x 53 cm long x 14 cm) and fastened with plastic cable ties to wooden stakes driven into the substrate. A total of 256 cages were placed in a total of eight blocks within four patches, so that each patch had an "under oyster rack" area and an "away from oyster rack" area (Figure 2).

Oysters were located in baskets at commercial densities (Treadwell *et al*., 1991) directly above the "under rack" treatments and remained in this position for the duration of the experimental period. Each block had eight cages each containing 25 clams. Clams were sampled for gut content determination every two months with one cage removed from each block at every sampling period, making a total of eight cages sampled per period (four from each treatment in a single patch)**.**

Two 2 L bottles of seawater were collected from the surface layers of the water column between the oyster rack and the away treatment at each sampling period for identification and estimation of the relative abundance of plankton assemblages. These were preserved using Lugols fixative or gluteraldehyde and stored in darkened 2 L bottles for later identification and quantification.

Samples were generally collected on the outgoing tide, 1-2 h before low tide. After collection, clams were scrubbed to remove any epiphytes, gently prised apart with a scalpel and injected with 10% formalin from a hypodermic syringe to prevent digestion of the gut contents before examination. Clams were then placed in labelled plastic bottles containing 10% formalin and stored for later dissection. As the experimental site had to be accessed by boat, approximately 1-2 h lapsed between the collection of water and clam samples and their preservation.

Laboratory Techniques

To identify and count algal species present in the water column, samples preserved in Lugols were settled in 1 L measuring cylinders for at least a week (Richardson, 1991). The supernatant was siphoned off and the remaining 100 mL centrifuged at 1000 rpm for 10 minutes. The supernatant was again siphoned off and the remaining pellet re-suspended. A concentrated sub sample was then placed in a Sedgwick Rafter cell and examined on a Carl Zeiss Axiovert 25 inverted microscope. Fifty divisions of the counting cell were counted (approximately 300 algal cells) and the algae were identified to genus and, where possible, to species level.

A sub sample of ten clam guts from each treatment were used for enumeration and identification of gut contents. Guts (mid gut and stomach) were carefully dissected from the preserved clams, any excess tissue removed and rinsed in distilled water. Gut contents (< 1 mm) were collected by slicing through the stomach with a scalpel blade and removing the contents with the edge of the scalpel blade. The gut contents were wet mounted on a microscope slide with distilled water and the phytoplankton present were counted and identified using a Carl Zeiss Axioskop phase contrast microscope at x400 magnification.

Scanning Electron Microscopy SEM

SEM was used to obtain photos of the dominate algal types present in water and gut samples and to allow easier identification (see following plates). Algae were identified by comparing preserved and photographed specimens with the descriptions and illustrations of Round *et al*. (1990). Algal identifications were confirmed by consultation with algal taxonomists.

Algal species identified from the water samples and gut contents of *K. scalarina* were tabulated (see Bellchambers, 1998). Data presented in this paper are expressed as weighted abundance percentages which were calculated by dividing the total species abundance for each genera by the total phytoplankton abundance for that sample period and multiplying this number by 100.

The use of weighted abundance percentages reduced the influence of any identification errors which may have occurred at the species level.

Algal Disappearance

Adult *K. scalarina* (30.0 - 40.0 mm shell length) were collected from Moulting Lagoon, Coles Bay. Clams were maintained in the laboratory at 15°C in aerated seawater ($35^0/_{00}$) prior to trials. Clams were starved for one week prior to the trials to ensure that the guts of the clams in the experimental treatments had no residual algal cells present. Four different algal species were utilised *Pavlova lutheri* (pymnesiophtye), *Tetraselmis suecica* (green flagellate) *Nitzschia longipes* (diatom) and *Navicula jeffreyi* (diatom).

Experimental treatments were established by placing four clams in plastic containers (500 ml) with seawater ($35^0/_{00}$), to which one of the four experimental algae was added. Each treatment was replicated (n=2) and a control containing clams without algae was used. Clams were allowed to feed for 2 h and then removed from the water for a further 2 h. A two hour digestion period was used to mimic field preservation techniques. The clams were then injected with 10% formalin to prevent digestion. Clam guts were examined in the same manner as used for field samples to determine whether the experimental algal species could still be observed in the gut contents.

5.12.4 RESULTS

Water Samples

Excluding dinoflagellates, 66 phytoplankton species from 29 genera were identified from the water column at Duck Bay, Smithton. The majority of the phytoplankton species identified were benthic diatoms, with 22 of the 29 genera identified being pennate diatoms, indicating that benthic pennate diatoms are a major component of the phytoplankton assemblage at Duck Bay. Several species of dinoflagellate were also present but could not be identified. The majority of phytoplankton species in the water column at Duck Bay were epiphytic (living and growing on other aquatic plants) or epipelic (growing and living on inorganic objects) in origin. Some diatoms were present in water samples over the entire study period. These included diatoms from the genera *Amphora*, *Coscinodiscus*, *Cocconeis*, *Navicula*, *Nitzschia*, *Pleurosigma,* and *Synedrav* and the species *Grammatophora oceanica*. Despite the consistent appearance of these diatoms in every sampling period and the wide array of phytoplankton genera identified, the phytoplankton assemblage was primarily composed of four genera; *Cocconeis* (35.64%), *Navicula* (25.03%), *Nitzschia* (9.68%) and *Amphora* (6.45%).

Survival

K. scalarina in under and away treatments did not display major differences in mortality over the experimental period (Figure 3) with the exception of the November sample when mortality was much higher in the away treatment. However, cumulative mortality increased greatly over time with the January sample displaying approximately 37% and 35% in under and away treatments respectively.

Gut Contents

The abundance of phytoplankton species in the gut contents of *K. scalarina* was generally consistent between "under" and "away" treatments within sampling periods (Figure 4). However, for samples taken on the 13/11/96, there was a substantial difference between the gut contents of *K. scalarina* in under and away treatments on an abundance, but not taxonomic

composition, basis. Phytoplankton abundance was highest in the first sampling period with a total of 463 algal cells identified from ten clams in "away" treatments and 397 cells from ten clams in "under" treatments. A sudden decrease in contents is evident in the subsequent sample (away 71, under 100) and, while there was an increase in subsequent sample periods, algal abundance did not reach the level of the initial summer sample.

Excluding dinoflagellates, 53 species of phytoplankton from 28 genera were identified in the gut contents of *K. scalarina* from Duck Bay, Smithton. Of the 28 genera 22 were pennate diatoms of benthic origin. The majority of the gut contents of *K. scalarina* were represented by three genera: *Cocconeis* (30.79%), *Nitzschia* (26.03%) and *Navicula* (14.07%). These three genera were also the major component of the water column phytoplankton assemblage in Duck Bay thus phytoplankton present in the gut of *K. scalarina* generally reflected the composition of the surrounding water column (Figure 5). However, although some genera appear to be more abundant in the gut contents of *K. scalarina* than their abundance in the water column would suggest, for example, *Nitzschia* and *Achnanthes*. Other species are less abundant in the gut than the water assemblage, for example, *Navicula* and *Entomeneis*. Some species are present in the water but not in the gut contents, for example, dinoflagellates, indicating that *K. scalarina* may be displaying food selection, with *Achnanthes* and *Nitzschia* being preferred.

While the abundance of phytoplankton in the gut contents between sample periods varied, the species composition was consistent between "under" and "away" treatments across all five sample periods (Figure 6A-E). In general, *Cocconeis* was the dominant genus present in the gut samples, followed by *Nitzschia* and *Navicula*, with the exception of 1/04/96 where *Nitzschia* was the dominant species followed by *Cocconeis*. The order of dominance generally reflected the proportion of the three major diatom genera present in the water samples. The presence or abundance of the other minor dietary phytoplankton varied between treatments but again remained consistent between "under" and "away" treatments, and in general reflected the presence or abundance of phytoplankton in the water.

Algal Disappearance

All of the algal species used in the algal disappearance trials were present in the gut contents of *K. scalarina* despite the 2 h period between removal from water and subsequent preservation. In fact, the algal species used in the trials were the only recognisable items in the guts of the clams examined. Clams in control trials had empty guts with no recognisable items present indicating that the algal species observed in the guts of experimental clams were consumed during the trial and had been adequately preserved.

5.12.5 DISCUSSION

The water column algal assemblage at Duck Bay, Smithton was dominated by benthic pennate diatoms, with over 90% of the species identified belonging to this group. Previous authors have report that the phytoplankton assemblage of several Tasmanian estuaries are also dominated by diatoms and nanoplankton (Hallegraeff *et al*. 1986; Van den Enden 1994). Similarly, water samples from Duck Bay were dominated by pennate benthic diatoms from the genera *Cocconeis*, *Navicula*, and *Nitzschia*. In contrast, Hummel (1985) working in the Wadden Sea reported that the water column phytoplankton assemblage was composed primarily of centric diatoms such as *Melosira*, *Thalassiosira*, *Rhaponesis* and *Biddulphia* while the sediment assemblages was composed of pennate diatoms. The Tasmanian estuaries studied by Hallegraeff *et al*. (1986), van den Enden (1994) and this study are shallow areas influenced by wave, tidal and current action which cause the resuspension of benthic diatoms into the water column.

The stomach contents of *K. scalarina* displayed a strong resemblance to the algal composition of water samples, and were dominated by benthic diatoms. Fifty three species from 28 genera were identified from the gut contents of *K. scalarina*. Similarly, Hummel (1985) identified 24 species of phytoplankton in the stomach of *Macoma balthica* from the Wadden Sea, of which 8 species were dominant (appearing in 50% of samples with abundances of up to 89%). *Cocconeis*, *Navicula* and *Nitzschia* were the dominant algal species in the stomach of adult *K. scalarina* in > 85% of samples. Newell *et al*. (1989) identified over 38 items from the stomach contents of *Mytilus edulis* and reports that >65% of the algal species identified were of benthic origin. The prevalence of benthic diatoms in the gut contents of *K. scalarina* may be explained by surface deposits and epiphytes on shells, sediment, macrophytes and cultivation trays; being stirred into suspension by wave, current or tidal action and thus made available to suspension-feeders in the form of seston (Shumway *et al*., 1987). The predominance of benthic algae in the diet of *K. scalarina* is indicative of resuspension of the benthic layer, common in wave and current dominated areas, rather than an indication that *K. scalarina* is capable of switching its feeding mode as in other bivalve species. This is supported by the dominance of benthic diatoms in the phytoplankton assemblage of the water column. It is possible that *K. scalarina* relies to some extent on the sediment surface as a food source, but given the predominance of benthic phytoplankton in the water column it is difficult to draw any conclusions concerning the importance of the benthic environment in the diet of *K. scalarina.*

Some algal species, especially small forms (<10 µm), may be quickly digested and therefore may not be evident in the stomach contents (Shumway *et al*. 1987). Furthermore, much of the food may be too delicate to remain intact after digestion, such as naked and minute nanoplankton. In this study, the presence of both *P. lutheri* and *T. suecica* in the gut contents of *K. scalarina* in algal disappearance trials suggests that the absence of flagellates in the guts of field samples is not due to rapid digestion or inadequate preservation techniques. A more plausible explanation for the absence of flagellates from the gut contents of *K. scalarina* is that they comprised a relatively minor part of the phytoplankton assemblage of this area. This explanation is in part supported by the absence of flagellates in water samples.

Previous authors agree that algae, micro organisms and detritus are all potential food for bivalves although algae are regarded as a particularly good food source (Fenchel, 1972; Fenchel & Jørgensen, 1977) while detritus is thought to be of low nutritional value (Hummel, 1985). Despite the suggestions of previous authors that detritus is an important food source for bivalves (Tsikhon-Lukanina, 1982), the gut contents of *K. scalarina* were composed primarily of benthic diatoms with little detritus present. Detritus is likely to be digested slowly and if present would be evident. Therefore, the majority of *K. scalarina* diet appears to reflect the availability of phytoplankton species in the clams' immediate habitat.

Despite expectations that *K. scalarina* may derive some benefit in terms of additional food resources by being placed underneath existing oyster racks, due to biodeposition and pseudofaeces production, the gut contents of *K. scalarina* were consistent between under and away treatments. The limited amount of detritus in the gut of *K. scalarina* may indicate that detritus is not an important food source to this species.

It has been suggested that tidal flat suspension feeders can cause a reduction of phytoplankton (Peterson & Black, 1991). Similarly dense populations of clams may deplete the natural food resources of the commercially more valuable oysters. Throughout the duration of this study Pacific oysters were collected from the overlying oyster racks, for stomach content analysis. The stomach contents of these oysters revealed a large amount of partially digested matter, that

precluded identification of algal species without the use of further cleaning techniques. As the techniques required to adequately identify algal species in oyster stomachs were not amenable to those used for the identification of clam gut contents, the results of previous studies are used for the purpose of comparison between the two species. However, it must be noted that the techniques used for the identification of algal species in previous studies are not identical to those used here and a degree of caution must be used when making comparisons.

A number of previous studies have investigated the gut contents of natural populations of oysters at various locations around the world (see Table 1). Many of the phytoplankton species found in the gut contents of oyster species are common components of the diet of other bivalves (Thangavelu, 1988; van den Eden, 1994). Van den Eden (1994) working on *Crassostrea gigas* at Little Swanport, Tasmania, reports that over 60% of the stomach contents were of benthic origin indicating that the benthic phytoplankton community may be a very important food resource to oysters, with the most preferred species being from the genera *Bacillaria*, *Cocconeis*, *Grammatophora*, *Licmorphora*, *Navicula*, *Pleurosigma*, *Striatella* and *Synedra* (see Table 1). The majority of the species identified were also a common component of the gut contents of *K. scalarina* especially *Cocconeis* and *Navicula*. However, previous authors have reported that oysters consume phytoplankton up to 180 µm in length (Richardson, 1991; van den Enden, 1994) whereas the phytoplankton present in the gut contents of *K. scalarina* were approximately 10 µm in length. Therefore potential food competition between the two species, in terms of dominant algal species, may be avoided to a degree by size selection of food particles.

Besides the potential for dietary overlap, farming *K. scalarina* on existing oyster leases may not be a valid option due to increased mortality of clams. Clams held in cages on existing oyster leases displayed mortality up to four times higher than clams in previous trials, regardless of their position in relation to oyster racks (Bellchambers, 1998). The increased mortality of clams on oyster farms was attributed to sediment characteristics. The sediment on oyster leases is frequently fine grained anoxic sand which is subject to scouring by tidal currents, whereas natural populations of *K. scalarina* are predominant in sheltered areas with well oxygenated fine to medium grain sand.

5.12.6 CONCLUSION

Benthic diatoms dominate the gut contents of both Pacific oysters (van den Enden, 1994), Sydney rock oysters *Saccostrea glomerata* (Richardson, 1991) and *K. scalarina*. Therefore, the potential for competition for food resources exists if *K. scalarina* is grown in oyster growing areas in southern Australia. The abundance of benthic diatoms in the stomach contents and water samples suggests that *K. scalarina* is predominantly a suspension feeder rather than a deposit feeder. As such, *K. scalarina* may derive little trophic benefit from being located below an oyster rack. As dense beds of suspension feeding bivalves are capable of substantially decreasing the phytoplankton biomass of an area, outplanting dense beds of *K. scalarina* on existing oyster farms is unlikely to be a viable option for oyster farmers already concerned with the carrying capacity of waterways.

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5.12.8 REFERENCES

Bellchambers, L. M. 1998. Ecology and ecophysiology of *Katelysia scalarina*, (Bivalvia: Veneridae), a commercially important clam. Unpublished PhD Thesis. University of Tasmania, Launceston.

Bellchambers, L. M. and Richardson, A. M. M. 1995. The effect of substrate disturbance and burial depth on the venerid clam, *Katelysia scalarina* (Lamarck, 1818). *J. Shellf. Res.* **14**, 41-44.

Beukema, J. J. and Cadee, G. C. 1986. Zoobenthos responses to eutrophication of the Dutch Wadden Sea. *Ophelia* **26**, 55-64.

Coleman, N. 1982. Population density and biomass of the bivalves *Tellina mariae* and *Katelysia rhytiphora* from a seagrass bed in Western Port, Victoria. *J. Malacol. Soc. Aust.* **5**, 141-149.

Fenchel, T. 1972. Aspects of decomposer food chains in marine benthos. *Verh. dt. zool. Ges.* **65**, 14-23.

Fenchel, T. M. and Jørgensen, B. B. 1977. Detritus food chains or aquatic ecosystems: the role of bacteria. *Adv. Microb. Ecol.* **1**, 1-58.

Hallegraeff, G. M., Tyler, H. L. and Sumner, C. E. 1986. Phytoplankton abundance and growth of the Pacific oyster (*Crassostrea gigas*) in Pittwater (Tasmania). *Unpublished Report*.

Hummel, H. 1985. Food intake of *Macoma balthica* (Mollusca) in relation to seasonal changes in its potential food on a tidal flat in the Dutch Wadden Sea. *Neth. J . Sea. Res.* **19**, 52-76.

Kent, G. N., Maguire, G. M., Cropp, M., Frankish, K. and John, M. 1998. Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina*. *J. Shellf. Res*. **17(4),** 1065-1070.

Langdon, C. J. and Newell, R. I. E. 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension feeders, the oyster Crassostrea virginica and the mussel *Geukensia demissa*. *Mar. Ecol. Prog. Ser.* **58**, 299-310.

Last, P. 1983. Ecology and zoogeography of Tasmania shore fishes. unpublished PhD. Thesis. University of Tasmania, Hobart, Australia.

Maguire, G. B. 1991. Small clams and cockles - are they valuable for fisheries and aquaculture? *Austasia Aquaculture*, **5:** 48 - 49.

Maguire, G. B. 1992. What does the public think of oyster farms? *Austasia Aquaculture*, **6:** 45-46.

Martin, G. W. 1923. Food of the oyster. *Bot. Gazette* **75**, 143-169.

Newell, C. R. and Shumway, S. E. 1993. Grazing of natural particulates by bivalve molluscs: a spatial and temporal perspective. In: R. F. Dame (Ed.) Bivalve Filter Feeders in Estuarine and Coastal Ecosystem Processes. Spriger-Verlag, Berlin, pp. 85-148.

Newell, C. R., Shumway, S. E., Cucci, T. L. and Selvin, R. 1989. The effects of natural seston particle size and type on the feeding rates, feeding selectivity and food resource availability for the mussel *Mytilus edulis* Linnaeus, 1758 at bottom culture sites in Maine. *J. Shellf. Res.* **8**, 187-196.

Peterson, C. H. and Black, R. 1991. Preliminary evidence for progressive sestonic food depletion in incoming tide over a broad tidal sand flat. *Estuarine Coastal Shelf Sci.* **32**, 405-413.

Richardson, D. L. 1991. Some aspects of food and feeding in the Sydney rock oyster *Saccostrea commercialis* (Iredale & Roughley) in Port Stephens, NSW. Unpublished Honours Thesis. University of New England, Armidale, NSW.

Roberts, D. 1983. The genus *Katelysia* (Bivalvia: Veneridae) in southern Australia. *J. Malacol. Soc. Aust.* **6**, 191-204.

Roughly, T. C. 1926. An investigation of the cause of an oyster mortality on the Georges River, N.S.W. *Proc. Linnean Soc. NSW.* **51**, 473-481.

Round, F. E., Crawford, R. M. and Mann, D. E. 1990. *The Diatoms. Biology and Morphology of the Genera*. Cambridge University Press. Cambridge.

Savage, R. C. 1925. Food of the oyster. *Fishery Investigation, Series II* **8**, 1-50.

Shumway, S. E., Selvin, R. and Schick, D. F. 1987. Food resources related to habitat in the scallop *Placopecten magellanicus* (Gmelin 1791): a quantitative study. *J. Shellf. Res.* **6**, 89-95.

Soh, S. W. L., Maguire, G. B. and Ward, R. D. 1998. Genetic studies of the venerid clam genus *Katelysia*. *J. Shellf. Res*. **17(4)**, 1057-1064.

Thangavelu, R. 1988. Natural food of the edible oyster *Crassostrea madrasensis* (Preston) of Pulicat lake, south India. *Proc. Indian Nat. Sci. Acad. (Anim. Sci.)* **97**, 463-470.

Treadwell, R., McKelvie, L. and Maguire, G. B. 1991. *Profitability of Selected Aquacultural Species*. Australian Bureau of Agricultural and Resource Economics Discussion Paper 91.11, ABARE, Canberra, 85 pp.

Tsikhon-Lukanina, Y. A. 1982. Food spectra of bottom molluscs. *Oceanology* **22**, 751-754.

van den Enden, R. 1994. The role of phytoplankton in the diet of juvenile and adult Pacific oysters (*Crassostrea gigas*) cultured at Little Swanport Lagoon. unpublished BSc Hons. Thesis. University of Tasmania, Hobart, Australia.

Wells, F. E. and Roberts, D. 1980. Molluscan assemblages on an intertidal sandflat in Princess Royal Harbour, Western Australia. *Aust. J. Mar. Freshwater Res.* **31**, 499-507.

Wilson, J., Crawford, C. and Mitchell, I. 1996. Predictive modelling of carrying capacities of the oyster (*Crassostrea gigas*) farming areas in Tasmania. Final FRDC Report, DPIF, Hobart, Tasmania.

5.12.9 TABLES AND FIGURES

Table 1. Dominate algal assemblages in stomachs of oyster worldwide.

Species	Location	Algal Species	Reference
Ostrea edulis	Wales	*Nitzschia, Rhizosolenia, Skeletonema, Navicula, Melosira, Coscinodiscus, Pleurosigma spp.*	Savage, 1925
O. edulis	France	*Cocconeis, Coscinodiscus, Melosira, Navicula, Diploneis, Grammataphora spp.*	LeRoux, (in Hendey 1964)
Saccostrea glomerata	Sydney	*Pleurosigma, Navicula, Coscinodiscus, Bacillaria, Amphora spp.*	Roughly, 1926
S. glomerata	Port Stephens	*Melosira, Thalassiosira, Nitzschia, Navicula, Amphora, Pleurosigma spp.*	Richardson, 1991
Crassostrea madrasensis	India	*Navicula, Coscinodiscus, Nitzschia, Pleurosigma, Rhizosolenia, Amphora spp.*	Thangavelu, 1988
C. gigas	Little Swanport, Tasmania	*Amphora, Bacillaria, Cocconeis, Diploneis, Dinophysis, Grammatophora, Gyrosigma, Navicula, Nitzschia, Pleurosigma, Prorocentrum, Striatella, Synedra spp.*	van den Eden 1994
C. virginica	USA	*Coscinodiscus, Melosira, Pleurosigma, Navicula, Amphora, Nitzschia spp.*	Martin, 1923

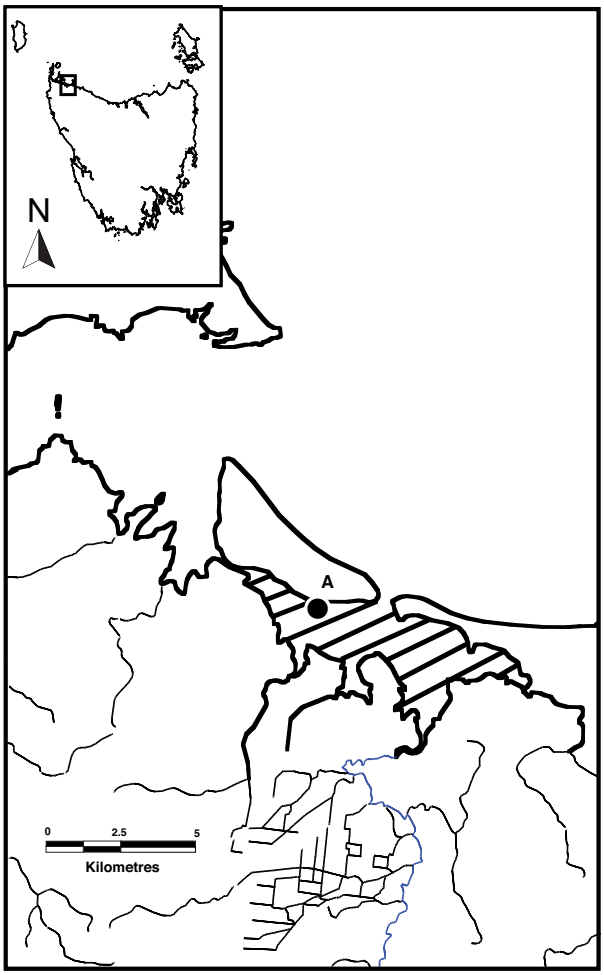

Figure 1. Map of Duck Bay, Smithton, Tasmania, Australia. Location of experimental treatments indicated by A.

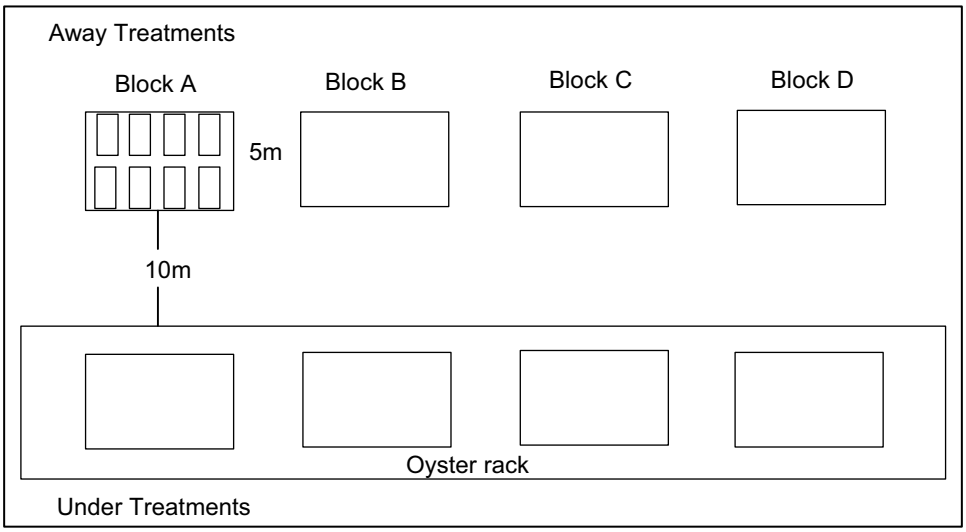

Figure 2. Representation of experimental design at Duck Bay, Smithton.

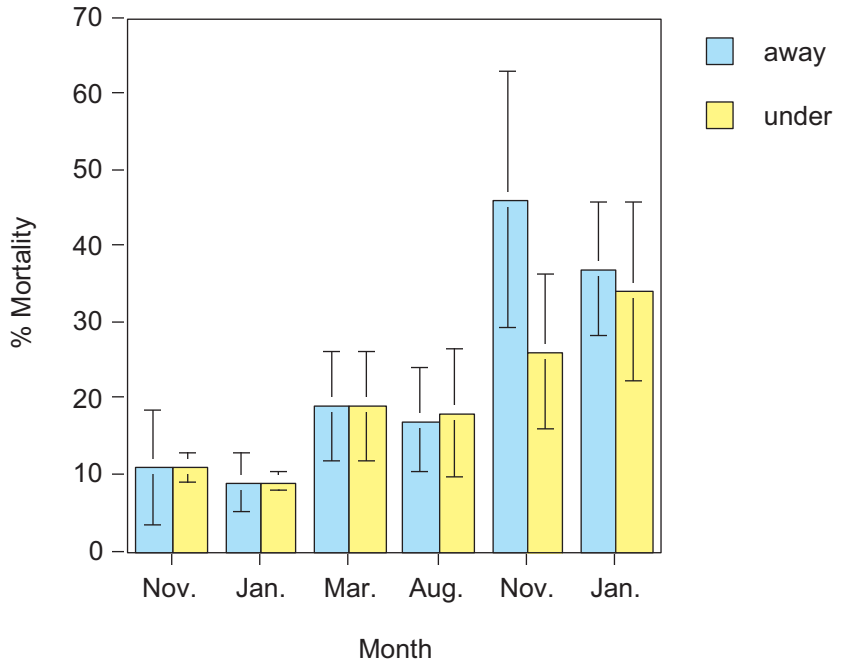

Figure 3. Cumulative mortality of *K. scalarina* under and away from oyster racks at Duck Bay, Smithton, over the experimental period (mean±S.E. n=4 cages).

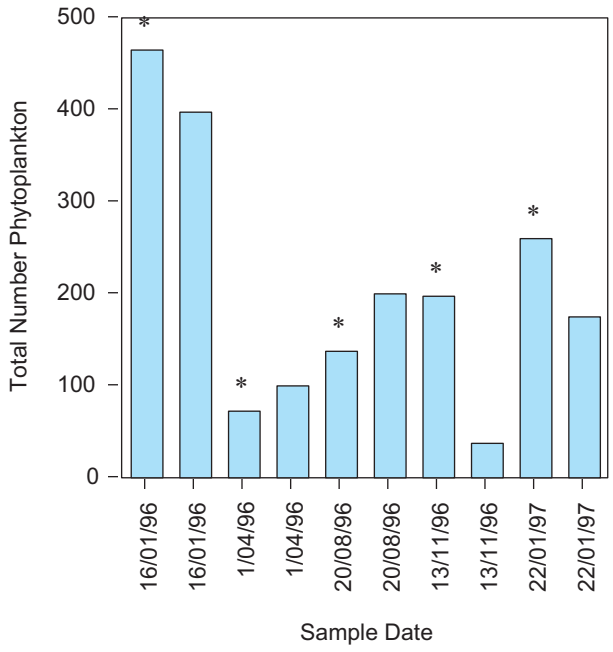

Figure 4. Phytoplankton abundance in the gut contents of *K. scalarina* at each sampling period. * denotes treatments away from oyster racks (n=40 clams/sample period).

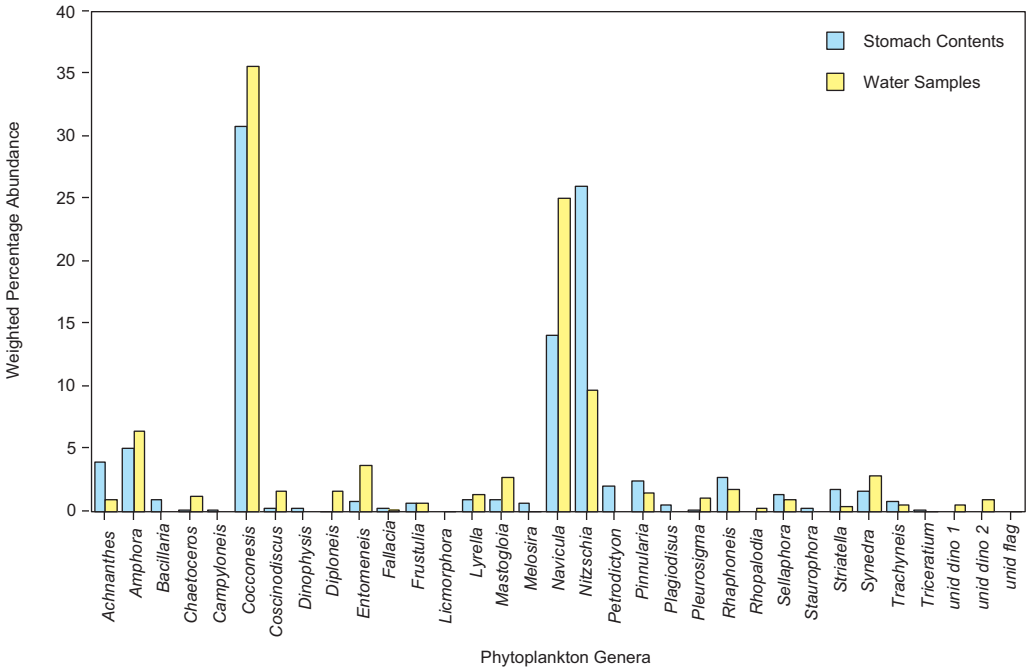

Figure 5. Comparison of algal composition of gut contents of *K. scalarina* and water algal assemblage.

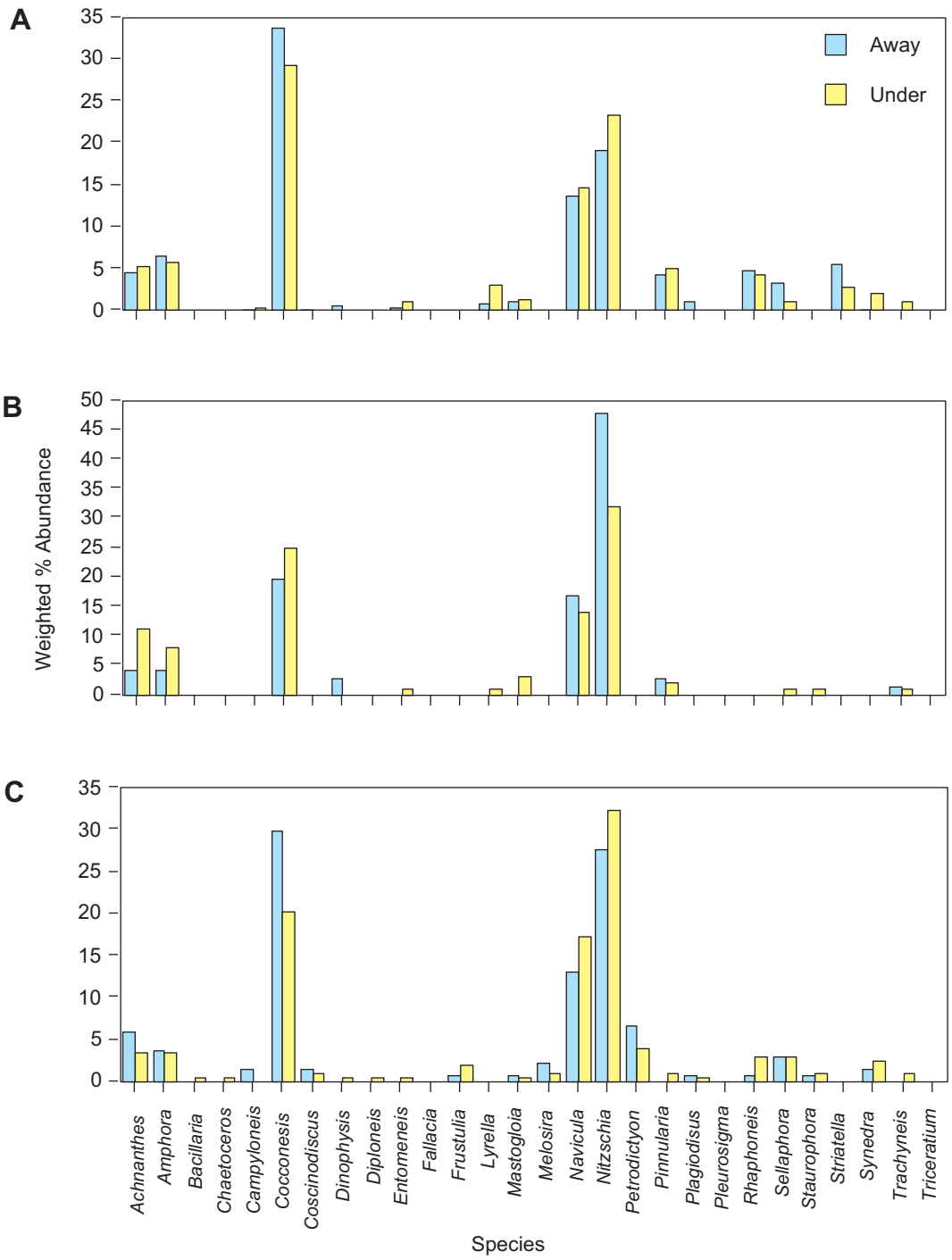

Figure 6. Phytoplankton identified in the gut contents of *K. scalarina*. A)16/01/96 B)1/04/96 C)20/08/96 D)13/11/96 E)20/01/97

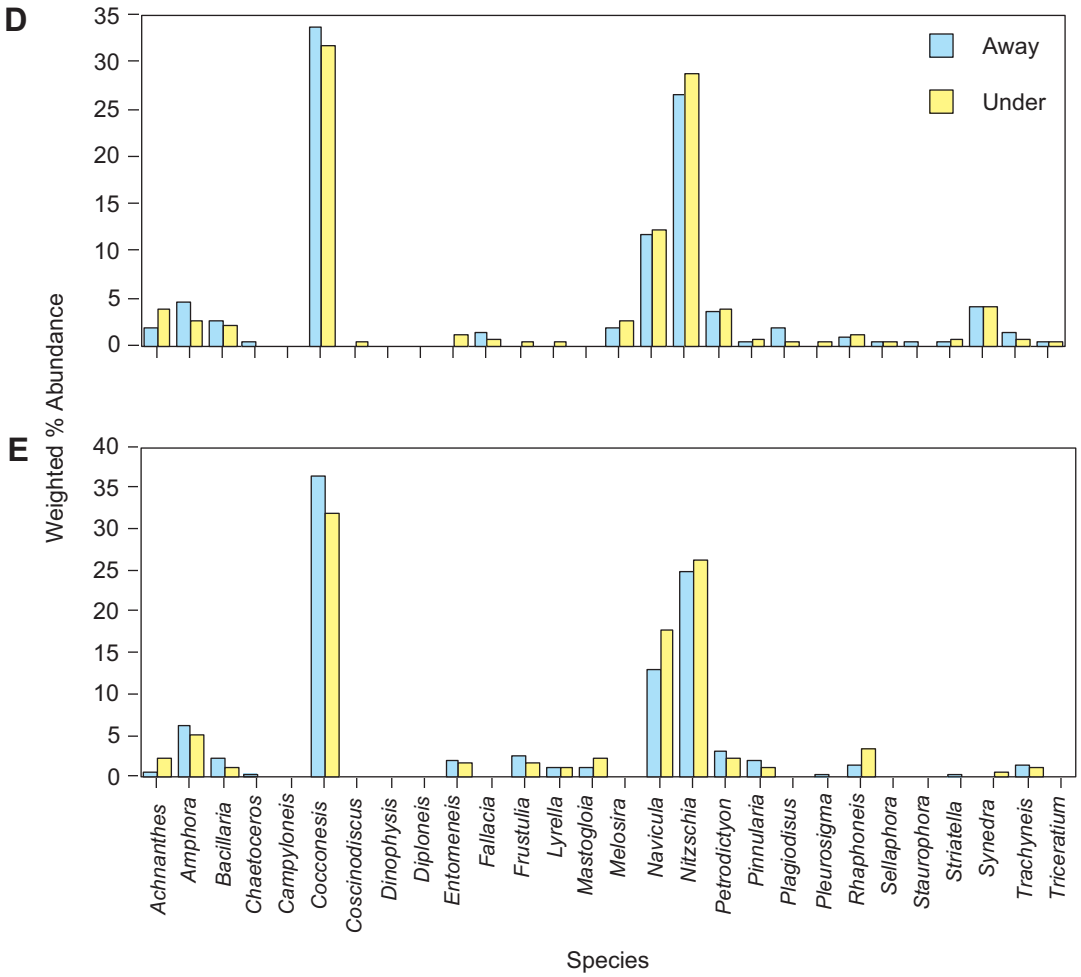

Figure 6 cont.

5.13 MANUSCRIPT 13

Growth models and age determination for the intertidal venerid clam *Katelysia scalarina* (Lamarck 1818) from three sites in Tasmania, Australia.

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5.13.1 ABSTRACT

Age and growth information was obtained for the intertidal clam *Katelysia scalarina* at three coastal sites in Tasmania. Age determinations from acetate peel replicas of transverse shell sections and growth increment data from tagging were used to determine the von Bertalanffy growth parameters and construct growth curves for *K. scalarina* at three demographically isolated locations in Tasmania.

The three sites, in order from highest to lowest growth rate, are Ansons Bay, Little Musselroe Bay and Cockle Creek. Preliminary results from age determinations of *K. scalarina* indicate that it takes approximately 4 years for clams to grow to commercial size (≥ 32 mm) at Ansons Bay, 5 years at Little Musselroe Bay and 6 years at Cockle Creek. Similarly, estimates of age

from tagging trials for each sample location suggest that *K. scalarina* takes approximately 4-5 years to reach commercial size at Ansons Bay, 5-6 years at Little Musselroe Bay and 6 years at Cockle Creek. Growth rates for clams varied both within and between sites.

Estimates of natural mortality (*M*) obtained in this study remained relatively constant through time at Ansons Bay (0.24-0.25 year^{-1}) and varied slightly at Little Musselroe (0.33-0.29 year^{-1}). Estimate of *M* obtained for Cockle Creek (0.11-0.35 year^{-1}) varied markedly between fishdowns. The lower estimate of *M* for this site (0.11 year^{-1}) was obtained after a release period of 2.8 years. These values are comparable to estimates of *M* for the New Zealand clam species clam species *Mactra murchisoni* (0.40-0.46), *Mactra discors* (0.28-0.34) and *Dosinia anus* (0.17-0.23).

There are significant implications for the commercial development and aquaculture of the inshore clam fishery from these findings on growth and mortality of *K. scalarina*. The prospect of enhancing existing clam stocks with cultured clam spat must address and consider issues relating to optimisation of growth and survivorship of farmed clams through appropriate site selection and the need for maintenance of inshore coastal ecosystems in the sustainable management of clam resources.

5.13.2 INTRODUCTION

Age and growth information on fishes and marine invertebrates is commonly used in age-structured and surplus yield models, and for estimates of mortality and recruitment for fisheries assessment purposes (Hancock, 1992). However, the usefulness and relevance of growth and ageing information in these models is dependent on the validity and accuracy of growth and age determinations.

The primary objective of the present study was to optimise and verify age determination techniques for clams, to provide information for fisheries management and to assess the potential for aquaculture.

Management arrangements for the inshore clam fishery first came into effect in 1993 to prevent unsustainable expansion in fishing capacity and degradation of fishing areas by mechanical fishing methods. At that stage, the inshore clam fishery had been in existence for six years and there was little information on the dynamics or biology of clam resources for formulating management strategies. The Tasmanian Department of Primary Industry and Fisheries embarked on a co-operative research project with the University of Tasmania in an attempt to collect valuable biological and fishery information for the sustainable management of clam resources.

The Tasmanian inshore clam fishery exploits two bivalve species, *Katelysia scalarina* and *Venerupis* (= Ruditapes) *largillierti. K. scalarina* is an intertidal species found in sheltered, sandy bays and occasionally in estuarine conditions. It is the major commercial species and accounts for approximately 75% of the total inshore catch. *V. largillierti* is a subtidal clam species that occurs in gravelly sand or shell grit substrates in sheltered bays. This species is only harvested in commercial quantities at one location (Georges Bay, St Helens) by one of the four commercial clam harvesters.

Marine farming for clams is already established on the eastern and western seaboard of the United States as well as in Asia and Europe. In these regions, culture and farming techniques have been developed for the hard clam (*Mercenaria mercenaria*) and the Manila clam (*Ruditapes philippinarum*) to supplement established wild native clam fisheries (Manzi and Castagna, 1989; Britton, 1991). It was anticipated that enhancement of wild stocks could also be useful for increasing production in Tasmania. Growth information was considered important for

assessing the benefit of enhancing Tasmania's clam resources because of the need for predicting potential production levels for clam farming. An assessment of growth and age was undertaken in this study using two complementary methods. Estimations of age from ageing and tagging studies were used to determine the von Bertalanffy growth parameters for *K. scalarina* at three commercial clam harvesting sites in Tasmania.

5.13.3 MATERIALS AND METHODS

(i) Age Estimation of K. scalarina

Age estimation of bivalves has traditionally been undertaken by physical examination of the external surface of valves to identify particular growth check patterns that result from biological disturbances (Richardson and Walker 1991). More recently, ageing studies have used shell microgrowth patterns in cross sections or acetate peel replicas of transverse shell sections. Age determinations have been undertaken in this manner for several bivalve species, including *Arctica islandica* (Thomson, Jones and Dreibelbis, 1980); *Mercenaria mercenaria* (Richardson and Walker 1991) and *Spisula solidissima similis* (Walker and Heffernan, 1994). The present study used the method of preparation and examination of transverse sections, described by Sheppard (1984), to age specimens of *K. scalarina*.

Three hundred (300) randomly selected clams were collected from each of the commercial clam harvesting sites located on the east coast of Tasmania (Figure 1). Clams were numbered and measured for shell length (mm), shucked and the shells cleaned in a weak solution of hydrogen peroxide. Further preparation of clam shells included embedding the left valve of each clam in clear polyester casting resin to prevent shell fracture during sectioning.

Resin blocks containing the valves were sectioned with a smooth edged diamond saw blade along the axis of maximum growth, from the umbo to the outer shell margin. The transverse shell sections were initially polished by hand using progressively finer grit (400, 600, 800, 1200) of wet and dry carborundum paper and finally polished on a lapidary wheel with 3 µm diamond paste. The polished sections were etched in 1M HCl for 10-15 seconds, rinsed in running water and dried prior to the preparation of acetate peel replicas.

Acetate peel replicas were formed by flooding acetone over the surface of each transverse section and applying 100 µm clear acetate film over each section for 20-30 minutes, or until dry. The acetate film was then peeled from the transverse shell section and mounted between two glass slides for microscopic examination.

Examination of the acetate peel replicas (1 per clam) included classification of peels as either readable or unreadable. Annual growth cycles were determined by pairing of alternate light and dark layers in the inner, middle and outer layers of the shell and where the darker opaque zone forms at the ventral margin. It is worth noting that only 50% of acetate peel replicas could be read successfully using the method of Sheppard (1984).

This technique assumes that growth checks are laid down in annual lines formed each winter when growth rates are significantly reduced. Although annual internal growth bands have been used reliably in the age determination of subtidal clam species, there remains some doubt in the validity of the method to intertidal clam species, where environmental fluctuations are more severe and occur more frequently. To ensure consistency, readings of acetate peel replicas in this study were undertaken by one reader only. However, periodic validations by a second reader were also undertaken.

The von Bertalanffy growth equation was used to construct growth curves for *K. scalarina* at each sample location from age at length estimations. The variables length, L, to age, T, were used to estimate the von Bertalanffy growth parameters L_∞, the asymptotic mean length, k, the growth coefficient of the rate at which maximum size is reached and t_0, the theoretical age at zero from the equation:

$$L = L_\infty (1 - e^{-k(T-t_0)})$$

(ii) Tag and Recapture Trials

Tag and recapture trials were conducted in the present study to complement age estimation from acetate peel replicas of transverse shell sections. It is important to emphasise that tag and recapture information cannot be directly compared to age at length estimation, because tagging describes growth as a function of length and the latter as a function of age. The present study applies Fabens' (1965) model for estimating the von Bertalanffy growth curve from tag and recapture data for *K. scalarina*.

Length increment data from three sample locations (Figure 1) was obtained for *K. scalarina* and used to estimate growth. One thousand (1000) clams were randomly selected, measured for shell length (mm), tagged and released within a 10 m x 2 m area at each of the locations. Red polyethylene "Hallprint" tags measuring 8 mm x 3 mm were attached to clams using "super glue". The release areas were located approximately mid tide and marked at the corners to assist locating recaptures at later dates. These areas did not prevent movement of clams into or out of the area.

Intermittent sampling was undertaken at each of the three sites on an irregular basis (Table 2). Samples large enough to provide an adequate indication of the size structure of tagged clams at each location were collected, measured and released within the marked area.

Total harvests (fishdowns) were conducted approximately annually at each site. Fishdowns included the removal of all tagged clams within the vicinity of the release area. Tagged specimens were then measured and released within the marked area.

Growth increment data were analysed using Fabens' (1965) parameterization of the von Bertalanffy growth equation. Estimates of the von Bertalanffy growth parameters L_∞ and k were obtained from the equation:

$$\Delta L = L_\infty - L_i)(1 - e^{-k\Delta t})$$

where ΔL is the growth increment (mm), L_i is the length at initial release (mm) and Δt is the time at liberty (years). Growth curves of best fit were constructed from tagging data for total fishdowns to estimate age at length for *K. scalarina* at each sample location.

5.13.4 RESULTS

Age determinations from examinations of acetate peel replicas of transverse shell sections for *K. scalarina* at Ansons Bay, Little Musselroe Bay and Cockle Creek (Figure 2) include the best fit von Bertalanffy growth curves from age at length data. The von Bertalanffy growth parameter estimates for the asymptotic mean length (L_∞), growth coefficient of the rate at which maximum size is reached (k), theoretical age at zero length (t_0) and the asymptotic standard errors (parentheses) for each sample location were estimated (Table 1).

Absolute age determined for *K. scalarina* specimens collected from each sample and the best

fit von Bertalanffy growth curve derived from age at length data suggest that there is a general trend for the growth rate of *K. scalarina* to decrease with age (Figure 2). However, there is some indication that the rate of growth varies between sites. Values of k vary from 0.74 at Ansons Bay, 0.40 at Little Musselroe Bay and 0.08 at Cockle Creek. The lower value of k for Cockle Creek may be explained by the comparatively high value of L_∞(47.3) obtained in this study. As no small clams were available at Cockle Creek (all ≥ 26.3 mm shell length), the estimates for this site should be interpreted with caution.

Age determination for *K. scalarina* at all three sample locations suggest that the size structure of clam populations at each site is composed of a broad range of year classes. For instance, age determinations for *K. scalarina* ranged from 2-20 years at Ansons Bay, 1-22 years at Little Musselroe Bay and 4-29 years at Cockle Creek. Clearly, *K. scalarina* is a relatively long lived bivalve species.

Estimates of the age of clams at the commercial size (32 mm) were obtained using the von Bertalanffy growth curves derived from age determinations. These suggest that *K. scalarina* reaches commercial size at 4 years of age at Ansons Bay, 5 years of age at Little Musselroe Bay and 6 years of age at Cockle Creek. It is important to note that the commercial size (32 mm) value was determined from measuring commercial catches of *K. scalarina*. This minimum size is driven by the commercial demand for clams of this species 32 mm shell length. Preliminary microscopic and macroscopic examinations on gonad development suggest that *K. scalarina* reaches sexual maturity before reaching the minimum commercial size with advanced gonad development evident down to 19 mm.

Growth curves for *K. scalarina,* using Fabens' (1965) parameterization of the von Bertalanffy growth equation, use the length increment of recaptured clams over time at liberty to describe the expected growth (Fig. 3). Total harvest of experimental plots (fishdowns) occurred twice at Ansons Bay and Little Musselroe Bay but only once at Cockle Creek. Values for the von Bertalanffy parameters and the asymptotic standard error (parentheses) (Table 3) show the same general pattern as predicted by age determinations for all sample locations. Estimates of k from the tagging trials are largest for the Ansons Bay site and lowest at Cockle Creek.

Although growth curves derived for Ansons Bay and Little Musselroe Bay are similar for both fishdowns, an overall decrease in the value of k is observed for the latest recapture dates. This may reflect an overall increase in the growth of the sample over time at liberty. The growth coefficient k is a measure of the rate at which the length L_∞ is reached, and as the sample population increases in shell length over time, a gradual decrease in the value of k should be observed. The progression in size of the tagged populations over time at liberty is given in Figure 4. The percentage frequencies at each sample location represents both total fishdowns of the sample sites and the intermittent sampling of the tagged populations. Increases in mean shell length of recaptures also provide evidence of the overall increase in shell length of the tagged population samples over time at liberty.

Interpretations on growth of *K. scalarina* should be restricted to the size range of recaptures collected in this study. The growth curves derived from the tagging data (Figure 3) do not give any indication of clam growth below 25.3 mm shell length. Age of clams at the commercial size (32 mm) using the tag and recapture method have a similar pattern to the age obtained from acetate peel replicas for each of the sample locations. The tag and recapture method suggests that *K. scalarina* reaches commercial size at approximately 4-5 years of age at Ansons Bay, 5-6 years of age at Little Musselroe Bay and 6 years of age at Cockle Creek.

Estimates of natural mortality (*M*) for tagged clams at each sample location are given in Table 3. Estimations of *M* assume that tag loss is 1% (L. Bellchambers pers. comm., 1997), fishing mortality is nil, and that there was no movement out of the sampling site. Therefore, estimates of *M* are likely to overestimate the natural mortality if additional tag loss, fishing mortality or emigration from sample plots occurred between fishdowns.

The range of natural mortality estimates obtained in this study was greatest for Cockle Creek (0.11 $year^{-1}$-0.34 $year^{-1}$). Estimates of natural mortality were more consistent between fishdowns at both Ansons Bay and Little Musselroe Bay, where *M* varied by 0.06 (0.18 $year^{-1}$ - 0.24 $year^{-1}$) between fishdowns at Ansons Bay and 0.04 (0.28 $year^{-1}$ - 0.32 $year^{-1}$) at Little Musselroe Bay.

5.13.5 DISCUSSION

Calculation of growth parameters for *K. scalarina* from tagging and age determinations demonstrate the usefulness of the von Bertalanffy growth equation in estimating growth as a function of age and as a function of length. However, it is important to recognise inherent differences between estimates of growth using both methods, as any comparison of growth information from age at length and tagging data is equivalent to comparing mean growth rates at age and mean growth rates at length, and should be discouraged on the basis that both methods do not simultaneously incorporate both the age and length aspects of growth.

Originally, it was proposed that this study optimise and verify ageing techniques for clams. However, the study evolved to incorporate tagging studies to investigate growth patterns of *K. scalarina* over time at liberty as a means of providing limited insight into the potential for clam farming in Tasmania. The two separate techniques provide complementary estimates of growth parameters for *K. scalarina* at three demographically isolated locations in Tasmania.

A major premise of age determinations for clams in this study is that growth bands on acetate peels were formed as annual increments. These findings have been demonstrated for subtidal clam species using the method described by Sheppard (1984), but are not proven for intertidal species. It is possible that growth bands on acetate peel replicas for *K. scalarina* represent variations in physical and nutritional factors. Key environmental factors affecting clam growth include water temperature, salinity, tide and current flows, concentration of suspended sediments, freshwater flooding and desiccation. The nutritional factors affecting growth include both the quantity and quality of detritus and other organic particulate matter within the system (Rice and Pechenik, 1992). On many occasions during this study, extreme environmental factors have been observed, such as prolonged exposure by extended periods of low tides during hot weather. Further research should incorporate the use of oxytetracycline as a chemical tag to validate age determinations of *K. scalarina* using transverse shell sections.

Analysis of age determination results (Figure 2) and their subsequent application to the construction a growth curve for *K. scalarina* should take into account technical errors arising from the ageing process.

Values for t_0 obtained from the von Bertalanffy growth equation (Table 1) are not significant in terms of clam growth in this study, but may infer differences in the rate of growth between small and large clams. Since the range of sizes of clams used in age determinations in this study includes a relatively small size range (15.8-47.3 mm), the von Bertalanffy curve provides for statistical fit over the range of interest. However, the use of an alternative model to describe growth may well have been considered in this study if the range of interest had been significantly larger (< 15 mm). Nash (1995) suggests that the Gompertz curve provides a better fit for some

species where the size or age range of a population is extensive and where the growth curve is sigmoid in shape due to the influence of smaller individuals in the sample.

Limitations associated with these tagging trials include the relevance of Fabens' parameterization of the von Bertalanffy growth equation in describing individual variability of growth for *K. scalarina*. Sainsbury (1980) points out that although all fish grow according to a von Bertalanffy curve, each individual fish has its own growth parameters and k. Sainsbury (1980) and Francis (1988) model variability in growth by using the maximum likelihood estimation of growth from tagging data. Sainsbury (1980) also suggests that the conventional approach of fitting tagging data to a von Bertalanffy model tends to underestimate values of k.

Cryer (1997) argued that the von Bertalanffy model underestimates growth and documented the independent determinations of k from ageing (k=0.44) and simultaneous length frequency analysis (k=1.10) for the cockle *Austrovenus stutchburyi* in New Zealand. The latter value has been used in subsequent yield per recruit analyses in the management of this cockle fishery on the basis that it provides a more realistic evaluation of growth parameters.

Additional limitations of estimating age as a function of length by tagging includes transcription errors and the effect of tagging on growth. The present study did not investigate the effect of tagging on growth and mortality.

The tagging trials conducted in the present study show a similar overall trend to the age determinations using acetate peel replicas for growth of *K. scalarina* at each sample location. Preliminary results from age determinations of *K. scalarina* indicate that it takes between 4-6 years for clams to grow to commercial size. Growth rates for clams are vary both within and between sites. Similarly, estimates of age from tagging trials for each sample location suggest that *K. scalarina* takes approximately 4-5 years to reach commercial size at Ansons Bay, 5-6 years at Little Musselroe Bay and 6 years at Cockle Creek.

Both methods indicate that the order from highest to lowest growth rate follows the order of Ansons Bay, Little Musselroe Bay and Cockle Creek. It is possible that these observed differences in growth rate are due to differences in the physical and nutritional factors between sample locations. These differences highlight the importance of appropriate site selection in the development of successful clam farming practices. Further development of this study will incorporate the use of length frequency analysis of *K. scalarina* to compare estimates of growth parameters at each sample location.

The commercial implications of these findings should be considered in the context of development potential for clam production in Tasmania. For example, similar exploitation rates for the wild fishery at each sample location would have a different effect on the standing crop or biomass of clams. Similarly, yield to prospective clam farmers would be increased by re-seeding areas that maximised growth rates. Furthermore, it can be argued that the management strategies used to regulate the harvesting of wild clam resources should address these inherent differences in growth between commercial harvesting sites in any determinations of sustainable yield.

Nell and Patterson (1997) have documented growth during pilot farming trials for the estuarine clams *Tapes dorsatus* and *Katelysia rhytiphora* in New South Wales. *T. dorsatus* recorded the fastest growth from spat to commercial size (approx. 38 mm shell length) in 48 weeks at Brisbane Water (NSW) and attributed this high growth rate to warm water temperature, a low growing height and an extended growth season in spring and summer. *K. rhytiphora*, which does not grow as large as *T. dorsatus*, grew slower, but exhibited superior tolerance to lower temperatures.

The development of a clam farming industry may be more competitive in New South Wales than in Tasmania, on the basis that growth rates obtained for *T. dorsatus* in warmer climates would provide clam farmers with a more efficient return on investment. Nell and Patterson (1997) also note that appropriate site selection for clam farming is important for maximising clam growth in any location.

Estimates of natural mortality (*M*) obtained in this study varied slightly through time at both Ansons Bay (0.18-0.24) and Little Musselroe (0.32-0.28). However, estimates of *M* for Cockle Creek decreased markedly between the first and second fishdowns and can be attributed to inefficient searching for tagged clams during the first fishdown at this site. The second estimate of *M* (*M*=0.11) obtained for *K. scalarina* at Cockle Creek after a release period of 2.8 years is relatively low when compared with other bivalve species and suggests that predation is not an important factor affecting the natural mortality of clams (29.4 mm) at this site. Cranfield *et al.* (1993) estimated natural mortality rates for the New Zealand clam species *Mactra murchisoni* (0.40-0.46), *Mactra discors* (0.28-0.34) and *Dosinia anus* (0.17-0.23), but noted that the natural mortality rate of different stocks of the same species depends on the environment. It is difficult to explain differences in estimates of *M* for *K. scalarina* between sample locations without an adequate understanding of the physical and nutritional factors acting at each site. Nonetheless, these values obtained provide important information for yield per recruit analyses.

The development of enhancement techniques to supplement existing wild stocks should also consider the effect of clam density on mortality. Peterson and Black (1988) have documented the effect of density and mortality of *K. scalarina* and *K. rhytiphora* by manipulating clam density and measuring mortality after controlled physical disturbances. They observed a relationship between increased density and mortality following severe physical stress and concluded that density dependent mortality is caused by the interaction with the history of increased density or crowding. Therefore, excessive crowding of clams by enhancement of cultured clam spat might, in the event of sudden physical stress, increase natural mortality to levels above those obtained in this study. Excessive mortality of clams can also occur as a result of predation. For instance, Beattie (1992) documents the survivorship of <1% of 18 million broadcast seeded geoduck clams (*Panopea abrupta*) after 2-3 years release time and identified predation from crabs, starfish and flatfish as the major cause of mortality. The subsequent use of predator exclusion devices increased the survivorship of the geoducks by more than twenty fold.

Prospective clam farmers should also assess the likelihood of catastrophic or severe physical events acting on clam populations and causing massive synchronous mortalities. There is also evidence that environmental deterioration and eutrophication acts as a major mechanism for mortality of *K. scalarina and K. rhytiphora*. Peterson *et al.* (1994) provide a detailed account of the effect of eutrophication on a coastal ecosystem in Western Australia and the associated increase in mortality and decline in biomass of *K. scalarina* and *K. rhytiphora*. Furthermore, they note significant recruitment failure arising from a decline in environmental conditions.

The Tasmanian Shellfish Quality Assurance Program (TASQAP) is responsible for maintaining the public health integrity of the inshore clam fishery and classifies and manages clam fishing areas under a national shellfish sanitation control program. Commercial clam harvesting areas are regularly monitored to evaluate any potential health risks and pollution sources. This involves bacteriological analysis of water samples collected from appropriately selected sites over a range of environmental conditions. Specific clam harvesting areas are closed to fishing when water quality parameters do not meet the minimum requirements for harvesting shellfish for human consumption.

There is some concern that an increases in the population of residents at Ansons Bay may impact on the classification status of this commercial harvesting site by the TASQAP. There is already some evidence of elevated levels of faecal coliforms in Ansons Bay in close proximity to existing clam beds during periods of low salinity. It is therefore crucial for the development of the inshore clam fishery that ecological, environmental and human interactions are rigorously monitored to prevent irreparable damage to the faunal and floral components that sustain existing clam resources.

Estimates of growth and mortality obtained in this study for wild populations of *K. scalarina* provide a useful foundation for assessing the potential for farming this species in areas where they occur naturally. There is little doubt that the development of clam farming in Tasmania depends on enhancement of existing clam resources with hatchery reared clam spat. The future of the current wild fishery is uncertain, as it appears that populations of *K. scalarina* are susceptible to over exploitation from commercial wild harvesting or synchronous mortality from environmental or ecological disturbance. It is likely, therefore, that conservative management arrangements will be implemented in the short term to prevent serial depletion of existing clam beds by commercial harvesters.

Aspects of the age and size structure of *K. scalarina* in Tasmania suggest that the commercial fishery could only benefit from the development of cost effective hatchery techniques for producing cultured clam spat and the successful enhancement of wild clam beds. However, the growth rates obtained in this study are not encouraging.

5.13.6 ACKNOWLEDGMENTS

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5.13.7 REFERENCES

Beattie, J. H. 1992: Geoduck Enhancement in Washington State. *Bull. Aquacul. Assoc. Canada*, **4**: 24 pp.

Britton, W. 1991: Clam Cultivation Manual. *Aquaculture Explained*. **8**: 60 pp.

Capps, O., L. Shabman and J. W. Brown. 1989: Hard clam demand: Past determinants and future prospects. In: J.J. Manzi and M. Castagna (Editors), Clam Mariculture in North America. Developments in Aquaculture and Fisheries Science, **19**: Elsevier, Amsterdam, pp. 395-414.

Cranfield, H.J., Michael, K.P. and D. R. Stotter. 1993: Estimates of growth, mortality, and yield per recruit for New Zealand surf clams. *N.Z. Fish. Ass. Res. Doc.* **93/20**: 48 pp.

Cryer, M. 1996: Assessment of cockles on Snake Bank, Whangarei Harbour, for 1996. *N.Z. Fish. Ass. Res. Doc.* **97/2**: 20 pp.

Francis, R.I.C.C. 1988: Maximum likelihood estimation of growth and growth variability from tagging data. *N.Z. J. Mar. Freshwater Res.* **22**: 42-51.

Hancock, D.A. (Ed). 1992: The Measurement of Age and Growth in Fish and Shellfish, Australian Society for fish Biology Workshop, Lorne, 22-23 August 1990.

Bureau of Rural Resources Proceedings No. 12. (Bureau of Rural Resources, Canberra).

Nash, W.J. 1995: The Development of New Techniques for Assessing and Managing the Australian Abalone Fisheries. FR&DC Grant 88/94. Final Report to the Fisheries Research and Development Corporation, Canberra.

Paterson, K. J. and J.A. Nell. 1997: Effect of different growing techniques and substrate types on the growth and survival of the clams *Tapes dorsatus* (Lamarck) and *Katelysia rhytiphora* (Lamy). *Aquaculture Research*, **28**: 707-715.

Peterson, C. H. and R. Black. 1988: Density dependent mortality caused by physical stress interacting with biotic history. *Am. Nat*., **131**: 257-270.

Peterson, C. H., Irlandi, E.A. and R. Black. 1994: The crash in suspension-feeding bivalve populations (*Katelysia spp*.) in Princess Royal Harbour: an unexpected consequence of eutrophication. *J. Exp. Mar. Biol. Ecol*., **176**: 39-52.

Rice, M. A. and J. A. Penchenik. 1992: A review of the factors influencing the growth of the northern Quahog, *Mercenaria mercenaria* (Linnaeus, 1758).

J. Shellfish Res., **11/2**: 279-287.

Richardson, C.A. and P. Walker. 1991: The age structure of a population of hard-shell clam, *Mercenaria mercenaria* from Southampton Water, England, derived from acetate peel replicas of shell sections. *ICES J. Mar. Sci.,* **48**: 229-236.

Sheppard, R.A. 1984: Growth patterns in shells: methods of preparation and examination of transverse sections. *N.Z. J. Sci*., **27**: 117-122.

Thompson, I., Jones, D.S. and D. Dreibelbis. 1980: Annual internal growth banding and life history of the Ocean Quahog *Arctica islandica* (Mollusca: Bivalvia).

Mar. Biol., **57**: 25-34.

Walker, R. L., and P. B. Heffernan. 1994: Age, growth rate, and size of the southern surf clam, *Spisula solidissima similis (*Say, 1822). *J. Shellfish Res.,* **13/2**: 433-441.

5.13.8 TABLES AND FIGURES

Table 1. Best fit von Bertalanffy growth curve parameter estimates and asymptotic standard errors (parentheses) from age at length determinations for *K. scalarina* at each sample location, based on acetate peel readings.

Sample Location	n	L_∞	k	t_0	Size range of sample (mm shell length)	Range of age determinations (yr)
Ansons Bay	180	38.8 (0.92)	0.74 (0.13)	1.14	15.8-45.7 =34.6	2-20 =4.8
Little Musselroe Bay	152	37.1 (0.39)	0.40 (0.04)	-0.04	11.6-41.4 =33.8	1-22 =8.9
Cockle Creek	117	47.3 (4.37)	0.08 (0.04)	-8.89	26.3-47.3 =35.8	4-29 =10.5
Total/Mean	449	41.1	0.41	-7.79		

Table 2. Tag and recapture activities for *K. scalarina* at each of three sample sites. Dates and numbers in bold print represent total fishdown of sample sites. **R** denotes release of tagged clams.

Ansons Bay		Little Musselroe Bay		Cockle Creek	
Date	**Number of clams**	**Date**	**Number of clams**	**Date**	**Number of clams**
25/10/94	**1,000 R**				
9/1/95	310			**22/2/95**	**1,000R**
14/3/95	298	**2/5/95**	**1,000 R**		
31/5/95	290				
23/8/95	291	23/8/95	291	22/6/95	299
28/11/95	296	27/11/95	301	26/9/95	297
14/12/95	**784**			18/1/96	286
22/2/96	236	**9/5/96**	**662**	**29/8/96**	**475**
29/4/97	**361**	**30/4/97**	**428**	**17/12/97**	**670**

Table 3. Von Bertalanffy growth curve parameter estimates and asymptotic standard errors (parentheses) using Fabens' parameterization of the von Bertalanffy growth equation for recaptures of *K. scalarina* at each sample location.

Sample Location	Number of recaptures	Time at Liberty (yr)	L_∞ (mm)	L_{max} (mm)	k	Estimate of M (yr^{-1})	Size range of recaptures (mm shell length)
Ansons Bay	784	1.14	43.4 (0.25)	49.2	0.41 (0.01)	0.18	25.3-49.2
	361	2.51	44.2 (0.39)	47.2	0.32 (0.02)	0.24	29.1-47.2
Little Musselroe Bay	662	1.02	37.8 (0.23)	43.2	0.34 (0.02)	0.32	27.0-43.2
	428	2.00	38.0 (0.24)	42.9	0.26 (0.01)	0.28	25.7-42.9
Cockle Creek	475	1.52	39.4 (0.24)	49.0	0.26 (0.01)	0.34	27.2-48.7
	670	2.82	39.9 (0.16)	44.7	0.21 (0.01)	0.11	29.4-44.7

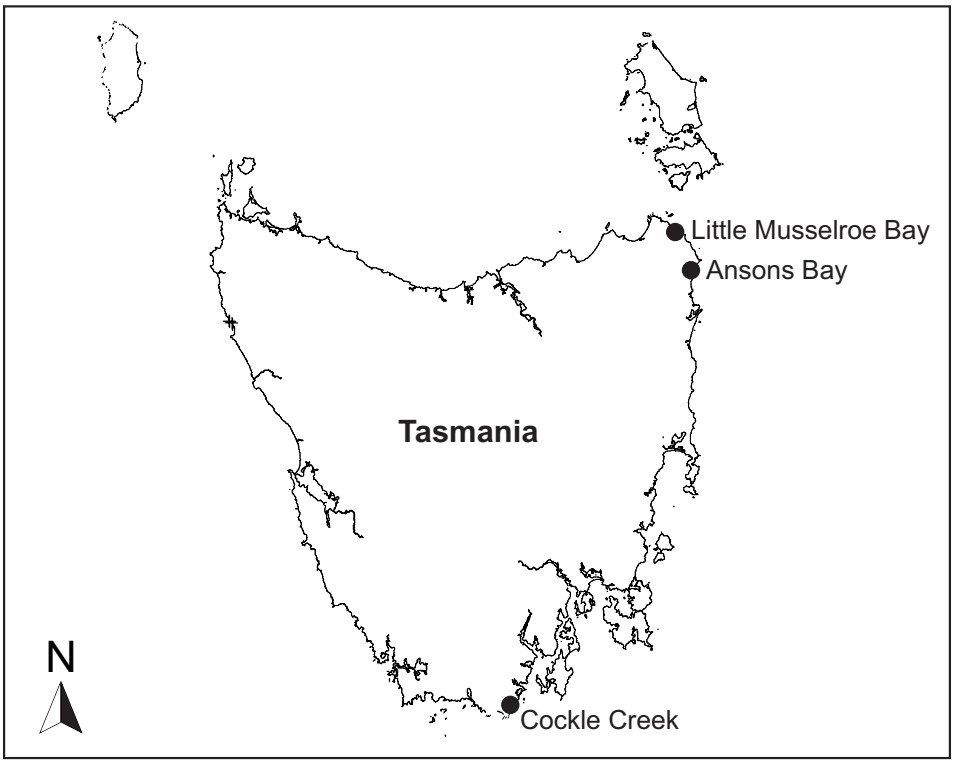

Figure 1. Map of Tasmania indicating collection sites of *K. scalarina*.

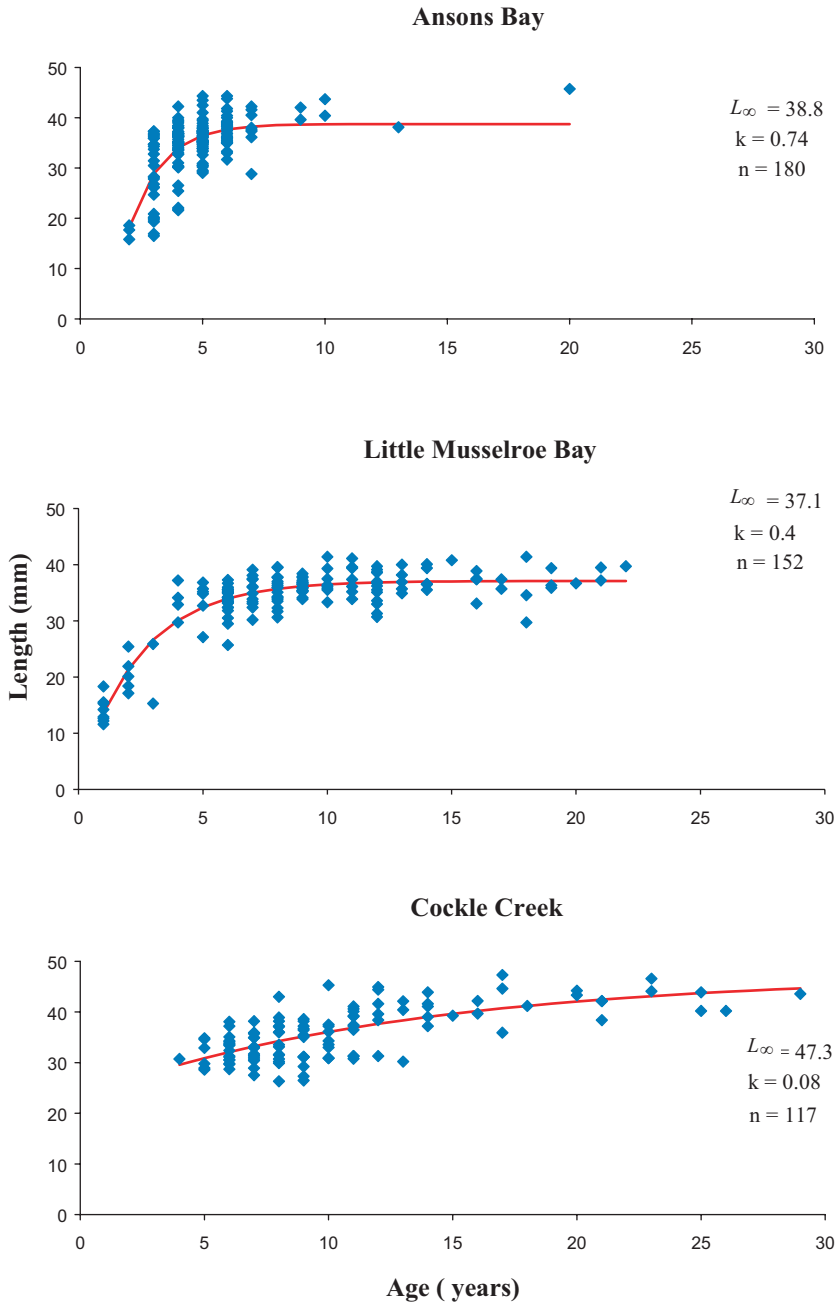

Figure 2. Age at length determinations for *K. scalarina* from acetate peel replicas of the transverse shell sections and best fit von Bertalanffy growth cirves for samples collected from Ansons Bay, Little Musselroe Bay and Cockle Creek.

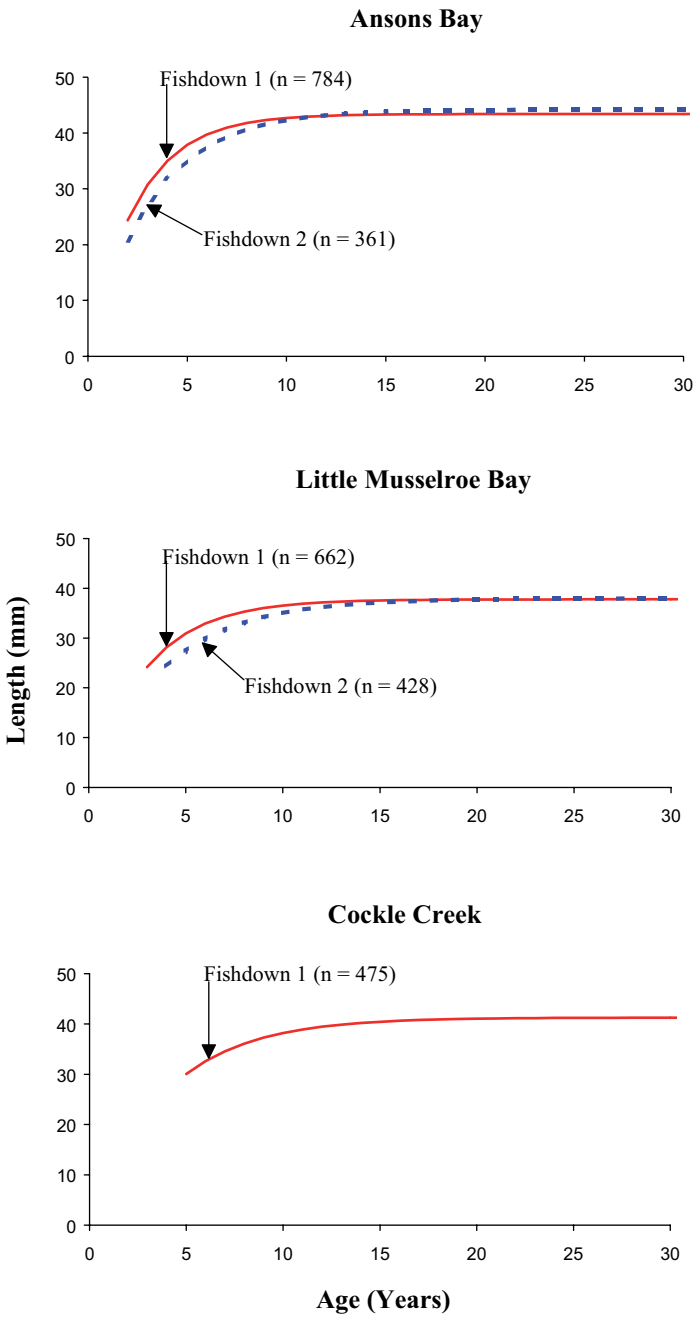

Figure 3. Von Bertalanffy growth curves using Fabens' (1965) parameterization of the von Bertalanffy growth equation for recaptures of *K. scalarina* at three separate geographical locations.

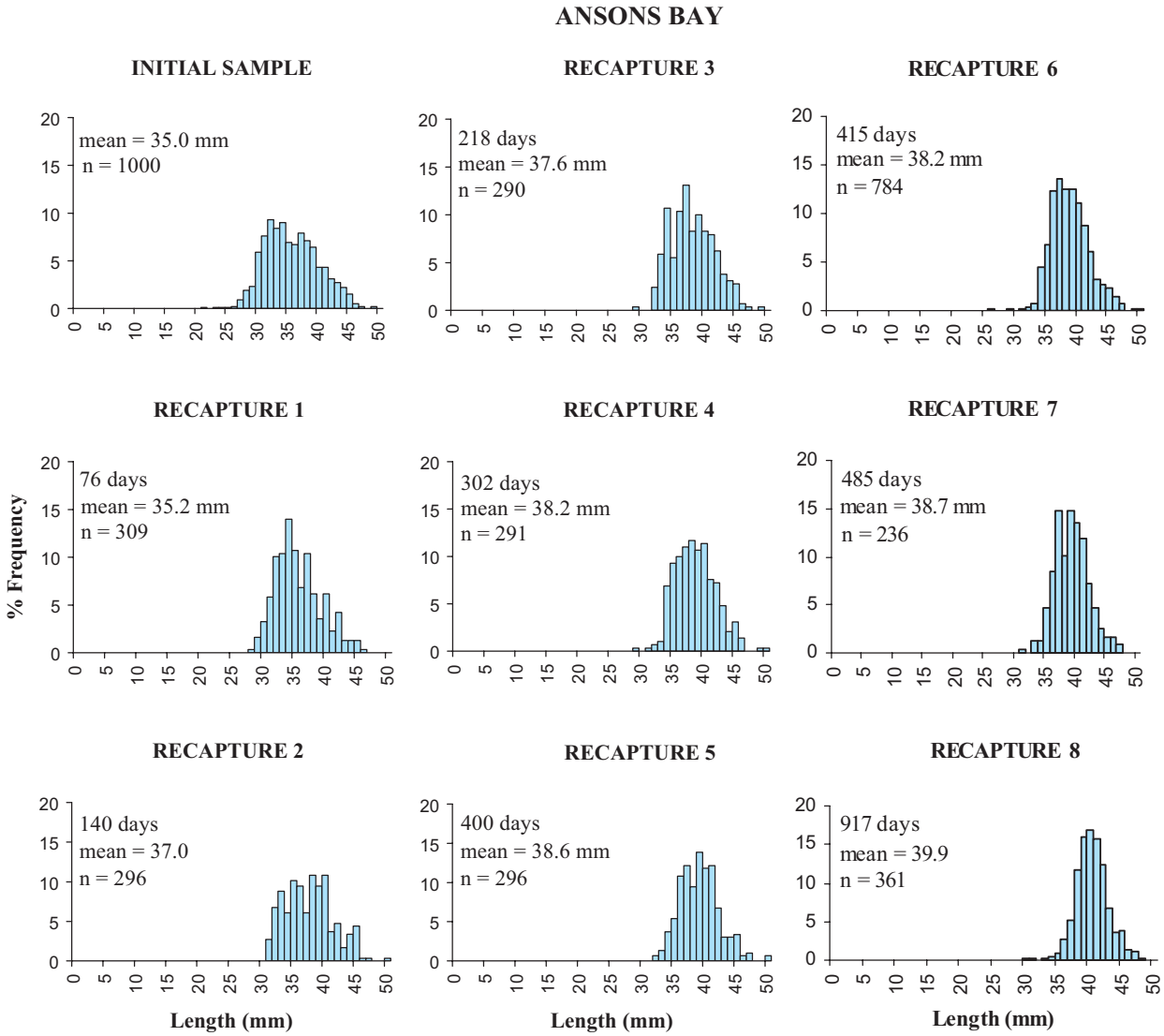

Figure 4. Length frequency histograms for recaptures of *K. scalarina* for specified times at liberty during tag and recapture trials at Ansons Bay, Little Musselroe Bay and Cockle Creek.

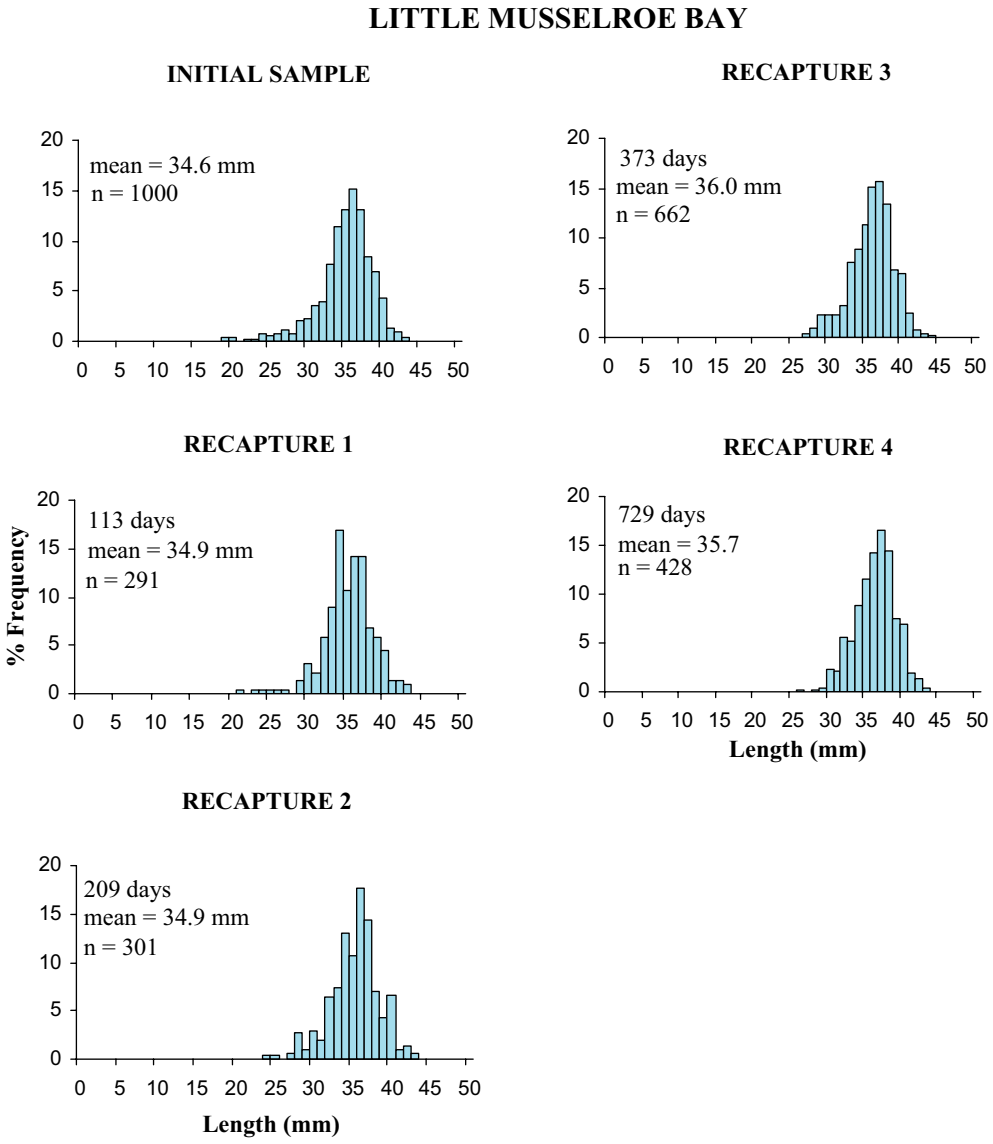

Figure 4 cont.

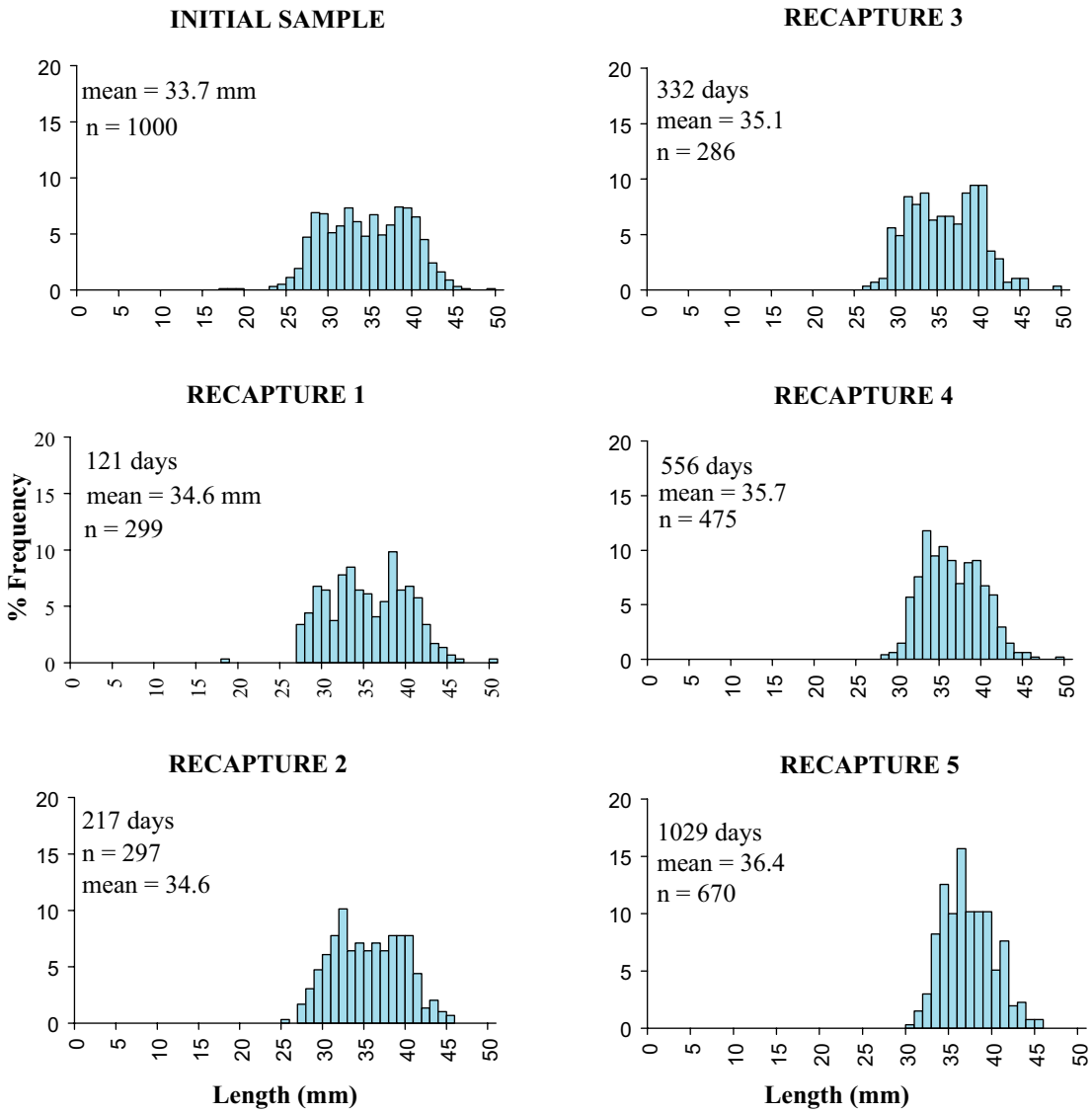

Figure 4 cont.

5.14 MANUSCRIPT 14

Clam harvesting and the conservation of wading birds in Tasmania.

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ABSTRACT

1. The purpose of this report is to investigate the possible effects of clam harvesting on populations of wading birds in Tasmania. Clam harvesting has the potential to affect wading birds adversely in a number of ways. Species that feed on clams may suffer directly by having the abundance and size distribution of prey available to them altered. Species that feed on other intertidal invertebrates may have their food supplies altered indirectly through the effects of disturbance to the sediment caused by harvesting operations and the ecological consequences of altering the density and size distribution within the populations of clams. All species may be disturbed away from feeding areas by harvesting operators. Should any species of wading bird be found to feed extensively on clams measures might be called for to exclude them from harvested areas thereby reducing the amount of feeding habitat available to the birds. Any impoverishment of feeding areas for wading birds could result in reduced carrying capacities of the affected areas and in reduced survival or productivity rates which may lead to decreases in population sizes.

The report reviews existing knowledge relevant to all aspects of these potential problems. This includes information on current clam harvesting procedures, information on the ecology of clams, and information on the numbers, distribution and conservation status of wading birds in Tasmania, their habitat preferences, feeding ecology and population ecology. Relevant overseas literature is also reviewed. An assessment is made of the adequacy of the current state of knowledge to address the problems and gaps in understanding are identified. Detailed research plans are provided to meet the shortfall in information.

2. The term "clam" is used to describe a group of bivalve molluscs in the family Veneridae. In Tasmania the stepped venerid, *Katelysia scalarina,* is the main harvested species, although individuals of the closely related *K. rhytiphora* are also included in some catches. Commercial harvesting of clams began in 1987 and at present exploratory harvesting licences have been issued to four operators each of whom is permitted to employ two assistants when engaged in harvesting operations. Harvesting is permitted at East and West Inlets (Stanley), Weymouth, Little Musselroe Bay, Anson's Bay, George's Bay, Little Swanport and Recherche Bay but is currently concentrated at Anson's Bay. A weekly quota of 300 kg, equivalent to approximately 16,500 individual clams, is permitted per licence holder. Quotas are not specific to individual populations and the total can be taken from a single population or spread over several. Up until 1994 harvesting was done with spades and forks to turn the sediment but following evidence of the damage done to clam populations by this procedure, harvesting is now permitted only by the use of fingers ("finger ploughing") to locate individual clams.

All aspects of the ecology of *K. scalarina* are inadequately understood. The species occurs intertidally in medium-grained sand sediment in sheltered estuaries and lagoons, but more precise details of its distribution and relationships with environmental variables are poorly known. The population dynamics of the species, including recruitment, growth and productivity, survival and movements are almost totally unstudied. *K. rhytiphora* occurs at lower tidal levels than *K. scalarina* in the same areas but even less is known of its

ecology. The present level of understanding of both species is inadequate to enable a scientifically based assessment of appropriate harvesting levels from natural populations and consequently, the impact of current harvesting levels on both *Katelysia* populations and on other components of the estuarine ecosystem cannot be determined. Research currently being undertaken jointly by the University of Tasmania and the Department of Primary Industry and Fisheries on *K. scalarina* will not provide the ecological information that is needed to determine appropriate harvesting levels from natural populations. This research concerns the culturing of clams and the enhancement of wild populations with hatchery-grown clams.

Relevant research is needed on all aspects of the ecology of wild, natural *Katelysia* populations. The strategy for harvesting clams from wild populations needs to be more carefully thought out and the relative roles of mariculture and wild harvesting need to be defined.

3. Twenty species of wading bird occur regularly in Tasmania of which 14 use estuaries and lagoons as their main feeding habitats or as seasonally important habitats. There is extensive overlap between the habitats selected by these species and the areas of present or potential clam harvesting and possible future clam "farming". The clam harvesting areas are particularly important habitats for pied and sooty oystercatchers. Both are scarce species in Australia and Tasmania supports about one-third of the total populations of each. Almost all clam harvesting sites along the northern and eastern coastline of Tasmania are within areas that meet the Ramsar Convention criteria for the identification of areas of international significance for the conservation of wading birds. At most of these sites, pied and sooty oystercatchers are the significant wader species.

4. Information concerning the diets and feeding behaviour of wading birds in Tasmania is examined. For most species there is either no detailed information or inadequate information. Pied oystercatchers were shown to include a high percentage of the clam *Katelysia scalarina* in their diet at a clam harvesting site (Anson's Bay) indicating the potential for serious conflict but more research is needed to quantify the extent of their dependence on this species and hence to predict the likely degree of competition between the birds and clam harvesting. Research is also needed into the diets of the other wader species that occur in clam harvesting areas. On the basis of present knowledge pied oystercatchers are highly likely to be adversely affected by clam harvesting and sooty oystercatchers may also be adversely affected. The likely impact on other species cannot be assessed from present knowledge.

5. Pied oystercatcher populations in Tasmania are characterised by exceptionally low productivity and high adult survival. They tend to be sedentary. Thus these populations do not have a high resilience to perturbations. They probably would be unable to cope with a significant decrease in adult survival as might be caused by a reduction in their food supplies or loss of feeding areas. Depleted populations would be slow to recover. Therefore, it is important to avoid problems before they occur rather than hope for recovery after damage is done. The same arguments probably apply to sooty oystercatchers but there is no detailed published information on any aspect of their population ecology,

6. Information relevant to the Tasmanian situation is reviewed concerning European and New Zealand oystercatcher populations. All species of oystercatcher have been shown to feed mainly on bivalve molluscs. Relationships between European oystercatcher populations and harvesting of the European cockle, *Cerastoderma edule* are reviewed. This cockle is

ecologically equivalent to *Katelysia* in Tasmania and the case history provides valuable insights into the kinds of interactions that are likely to be important in the Tasmanian case and assists in the formulation of appropriate methodology for subsequent research. The European studies demonstrated a direct and significant competition between the oystercatchers and the cockle harvesting industry.

7. Details are provided for research programmes that need to be undertaken to provide a full assessment of the impact of clam harvesting on wading bird populations. This includes research into the population ecology of the clams, the diet and feeding behaviour of the wading birds and the birds' responses to harvesting.

Layout of the Report

Chapter 5.14.1 This chapter summarises the background to clam harvesting in Tasmania and defines the areas of likely conflict with populations of wading birds. It specifies the questions that must be answered in order to resolve the issue.

Chapter 5.14.2 This contains a review of existing knowledge of the biology of clams, *Katelysia* spp., in Australia and provides details of *Katelysia* harvesting in Tasmania. Research programmes in progress relating to the ecology and harvesting of *Katelysia* are described. Information from these sources is used to assess the sustainability of current *Katelysia* harvesting and the adequacy of existing measures to guarantee sustainability.

The chapter also considers the indirect effects that the process of clam harvesting might have on other benthic invertebrate species that occur in the harvested areas and that might be prey species for wading birds. In particular it reviews the effects that might result from the mechanical disruption of the estuarine sediments during harvesting.

Chapter 5.14.3 This provides an analysis of existing knowledge of the numbers and distribution of wading birds in Tasmanian coastal habitats, identifies gaps in that knowledge and provides an assessment of the national and international conservation status of the species concerned.

Chapter 5.14.4 This chapter collates information on the diet and feeding behaviour of wading birds in Tasmania. It identifies gaps in existing knowledge that must be filled in order to make an assessment of the impact of *Katelysia* harvesting on wading bird populations.

Chapter 5.14.5 This chapter examines existing knowledge concerning the population dynamics of wading birds in Tasmania. It concentrates on pied and sooty oystercatchers, the species identified in chapter 5.14.4 as being at the greatest direct risk from clam harvesting.

Chapter 5.14.6 This examines a relevant case history concerning the diet and foraging behaviour of the closely related European pied oystercatcher and relationships between this species and harvesting of the European cockle, *Cerastoderma edule.*

Chapter 5.14.7 This chapter summarises the extent to which the information needed to make an assessment of the impact of clam harvesting on wading bird populations is met by existing knowledge. On the basis of this, it sets out a detailed plan of research that should be undertaken in order to resolve the issue.

5.14.1 INTRODUCTION

5.14.1.1 Background

The terms 'clam' and 'cockle' refer colloquially in Tasmania to several species of bivalve molluscs within the family Veneridae. The main target species of clam harvesting has been the

stepped venerid, *Katelysia scalarina,* which occurs mainly in the intertidal zone, although some of the closely related *K. rhytiphora* are also included in some of the catches. One other species of bivalve, *Venerupis largillierti,* which occurs mostly below low tide level has been harvested at one site only, George's Bay, St. Helens.

The commercial harvesting of clams in Tasmania began in 1987 and expanded rapidly in a number of estuaries and bays along the east and north coasts until 1993 when its further uncontrolled growth was restricted by the licensing of specified operators to harvest only in prescribed areas under the Sheltered Waters Clam Management Plan initiated by the Department of Primary Industries and Fisheries. The reported annual harvest increased four-fold from 15.6 tonnes in 1990 to 61.0 tonnes in 1993 (Department of Primary Industries and Fisheries 1994).

To date the harvest in Tasmania has been taken from the production of naturally occurring wild populations but increasing attention is being given to the possibility of clam culture in which hatchery reared immature clams may be 'planted out' among natural populations to enhance their productivity. Such approaches are being developed in Asia with other bivalve species (Manzi 1991) and research into the possibilities for *Katelysia* and *Venerupis* in Tasmania and Australia more generally are currently being undertaken at the Centre for Aquaculture, the University of Tasmania, Launceston under the direction of Dr. Greg Maguire.

Katelysia scalarina occurs mostly in sheltered bays and lagoons and normally lives within two to four centimetres of the sediment surface. The habitats in which it occurs and in which it is harvested are shared by a wide range of fauna including other benthic infauna such as molluscs, crustaceans and polychaete worms, and by their predators which include wading birds (Charadrii), sometimes referred to as 'shorebirds', wildfowl and various fish species. Thus the current harvesting of clams and its possible expansion to include new sites and new more intrusive methods could potentially have far reaching effects on these other components of the system.

The natural environment of Tasmania is one of the state's most important assets supporting a substantial tourism and recreation industry in addition to the traditional industries such as commercial fisheries. To secure the maximum benefit of this asset to the people of Tasmania and also to ensure that Tasmania meets its national and international obligations to sustainable development and conservation, it is essential that the wider implications of any changes in the existing uses of the environment be assessed fully. When harvesting of a resources such as clams is introduced it is essential that it is done in a way that guarantees the sustainability of the resource and of all components of the environment in which the resource occurs, including other species that depend on that resource at present. For wading birds in particular, Australia is signatory to a number of international agreements (CAMBA, JAMBA, Ramsar Convention - see chapter 5.14.3) under which it has promised to protect the habitats of these species.

The purpose of this report is to provide an initial assessment of the possible or likely environmental effects of clam harvesting particularly on populations of wading birds that occur in the same habitats as the clams. In doing so the report also assesses the sustainability of the existing clam harvesting programme. *The report is not intended to be a complete or final environmental impact assessment of the situation and should not be used as such.* Rather, it identifies the main questions that must be addressed to achieve a full assessment and examines the extent to which existing published and unpublished information from Tasmania and other Australian states can be used to answer these questions. Relevant information from similar situations overseas is examined and research programmes that need to be undertaken to achieve a full assessment are specified in detail.

5.14.1.2 Definition of the Problem

Wading birds use the coastal areas in which clam harvesting occurs mainly for feeding so any adverse effects of the harvesting are likely to operate mainly by altering trophic relationships within the system. This could occur directly, removal of clams could reduce the availability of food for any wader species that prey on clams. Most wading birds including those that eat molluscs show strong preferences for particular sizes of prey (see Chapter 5.14.4) so food availability for them must be considered in terms of the size distribution of individuals in the prey population in addition to their abundance. Thus the harvesting of clams might affect populations of wading birds by bringing about changes in the size distribution within the clam population and by changing the abundance of clams.

Potential effects of harvesting could also occur indirectly; changes to the density or size distribution of the clam populations caused by harvesting, or the disruption of the sediment caused by the mechanical process of harvesting, could alter the diversity and abundance of other invertebrate species of the benthic infauna such that the densities or size relationships of other potential prey populations for wading birds are altered. Thus wading birds that do not rely directly upon clams as food but that prey upon other invertebrates could also be affected by the harvesting.

It is also possible that clam harvesting could affect some or all species of wading bird by disturbing them away from their favoured feeding areas (Burger 1982, Mitchell, Moser and Kirby 1989, Pfister, Harrington and Lavine 1992). Many studies in Europe and North America have shown that wading birds may need to forage for all or nearly all of the suitable foraging time available to them over the low tide period when water depths are appropriate or substrates containing prey are exposed (e.g. Goss-Custard 1979). Thus even a small amount of disturbance at sensitive times could have a significant effect.

Reductions in the prey available to wading birds or in the time available for feeding at particular sites could result in food intake rates and overall daily rates of net energy gain being lowered. This could result in birds leaving the area and may cause increased densities in other areas. Lowered prey levels or increased densities could *affect* the birds adversely by reducing their survival or breeding performance or in the case of migratory species by reducing their ability to accumulate the reserves needed for successful migration to their breeding areas (e.g. Piersma 1987, Piersma and Jukema 1990). The cumulative effect could be to cause progressive reductions in the numbers of these birds. The implications of this would depend on the initial status of the species concerned and would be more serious for those that are scarce nationally or internationally and that depend significantly on the areas in which clam harvesting occurs.

There is one other aspect of the situation that could become significant. The intensive management of clam populations involving 'planting out' with hatchery reared immatures will result in local increases in the densities of clams. This could alter the feeding behaviour of any wading birds that prey on clams, causing them to concentrate their activity in these areas of 'enhanced' clam populations. In addition to consuming significant numbers of clams it is also possible that the birds could be part of natural parasite cycles involving clams as host species (Hulscher 1973, 1982; Goss-custard, West and Durell 1993). If these effects occurred it is inevitable that the wading birds would be regarded as pests and steps would be taken to solve the 'problem' by excluding the birds from the clam growing areas or even by demanding culls of their numbers. In the case of nationally rare wader species neither of these suggestions may be acceptable on conservation grounds and pursuit of them could lead to serious conflict. It would be better to anticipate that such difficulties are likely to arise well ahead of any developments and to undertake appropriate research to reach an arrangement that is acceptable.

The above points can be formalised as a set of specific questions that must be answered to allow an assessment of the impact of clam harvesting on wading birds populations.

1. Do any species of wading birds eat *Katelysia* such that the harvesting of *Katelysia* might have a direct effect on the availability of food for them? How important is *Katelysia* in the diets of these species and what other prey species are taken?

2. For each such species of wading bird that eats *Katelysia* does the harvesting of *Katelysia* adversely affect the birds' abilities to obtain their daily and seasonal food requirements? Consequently does harvesting reduce the birds' survival, breeding success or migration ability?

3. How many wading birds in the above category are likely to be affected by harvesting. and how do these numbers compare with the state, national and international population sizes of these species? Is it likely that *Katelysia* harvesting will adversely affect a significant number of nationally or internationally scarce, rare, vulnerable, threatened or endangered species?

4. Does the harvesting of *Katelysia* have an adverse impact on the diversity, abundance or size distribution of other species of benthic invertebrate infauna that occur in the harvested areas and that are likely to be the food source for wading birds that consume prey other than *Katelysia?* If so, what are the effects of such changes on the feeding behaviour and populations of these wading birds?

5. Does the process of harvesting disturb wading birds to such an extent that they move away from preferred feeding areas? If so, does this result in a reduction in the birds food intake rates?

6. Is it likely that any wading bird species could come to be regarded as a 'pest' of enhanced clam production? If so can appropriate management be devised that does not adversely affect populations of the wading birds?

5.14.1.3 General Methodology

This report is predominantly a review of existing published and unpublished knowledge relevant to the problem of *Katelysia* harvesting and the conservation of wading bird populations. In addition a limited amount of preliminary exploratory field work was undertaken to clarify a few important issues. Thus the methodology mainly involved literature searches and consultations with relevant experts.

Literature searches. Issues of the journals detailed below from 1960 to the present were searched for pertinent information on the ecology and management of *Katelysia* populations in Australia, the general effects of harvesting on the stability of estuarine ecosystems and the distribution, habitat preferences, status, diet and feeding ecology of wading birds in Tasmania and Australia. Information from similar situations overseas was also located.

Journals searched were: Australian Journal of Ecology; Australian Wildlife Research; Emu; Corella; The Stilt; An Occasional Stint; Tasmanian Bird Report; Notornis; Journal of Animal Ecology; Journal of Applied Ecology; Ecology; Ecological Monographs; Oecologia; Estuarine; Coastal and Marine Science; Netherlands Journal of Sea Research; Fisheries Investigations; London; Estuarine; Coastal and Shelf Science; Australian Journal of Marine and Freshwater Research; Canadian Journal of Zoology; Biological Conservation; Animal Behaviour; Behaviour; Ardea; Ibis; Ostrich; Ornis Scandinavica; Bird Study; Wildfowl; Auk; and Condor.

Consultations. Consultations concerning the ecology, harvesting and culturing of *Katelysia* in Tasmania were held with Mr. William Zacharin and Mr. Shaun Riely of the Tasmanian Department of Primary Industry and Fisheries and with Ms. Linda Bellchambers of the University of Tasmania, who jointly provided all of the previously unpublished information detailed in this report relating to *Katelysia* harvesting in Tasmania. This includes details of current co-operative research programmes between the University of Tasmania (under the direction of Dr. Greg Maguire) and the Tasmanian Department of Primary Industry and Fisheries.

Access to original data concerning the abundance and distribution of wading birds in Tasmania was provided by Mrs. Priscilla Park on behalf of the Tasmanian shorebird study group. She also provided details of her own unpublished observations on the diet of pied oystercatchers and of the long-term population study of pied oystercatchers in the Derwent/Pittwater area being conducted by Dr. Mike Newman and Mrs. Priscilla Park. Additional consultations concerning wader ecology were held with Mr. Bob Patterson of the Tasmanian shorebird study group and with Ms. Margaret Considine and Mr. Mark Weston of the Royal Australian Ornithologists' Union, Melbourne.

Site Visits and Fieldwork. Site visits were made to examine wading bird habitat at Bruny Island (Cloudy Bay Lagoon and Adventure Bay), Pipe Clay Lagoon, Derwent Estuary, Barilla Bay, Orielton Lagoon, Marion Bay, George's Bay, Anson's Bay, Great Musselroe Bay and Weymouth Bay. At Anson's Bay preliminary observations were made of the diet of pied oystercatchers in the *Katelysia* harvesting areas. Less detailed observations of oystercatcher diet were made at other sites. A limited number of counts of oystercatchers were undertaken during visits to some of the sites.

5.14.2 THE ECOLOGY AND HARVESTING OF CLAMS (*KATELYSIA*) IN TASMANIA

5.14.2.1 General Ecology of *Katelysia*

Distribution and Habitat. Three species of *Katelysia* occur in Tasmania; *K. scalarina, K. rhytiphora* and *K. peroni.* Harvesting is mainly confined to *K. scalarina* but individuals of *K. rhytiphora* are included in some catches. *K. scalarina* prefers relatively sheltered bays and estuaries and has been found around most of the Tasmanian coast including King Island and the Furneaux Group of islands (Richmond 1990). A filter feeder, it is found usually within 2 to 4 cm of the sediment surface (Nielson 1963). Nielson noted that its distribution was mainly intertidal in Victoria and this is supported by more detailed studies at Pipe Clay Lagoon and Moulting Lagoon in Tasmania and at Princess Royal Harbour, Western Australia (Woodward 1985, Bellchambers 1993, Wells and Roberts 1980). *K. rhytiphora* tends to occur in deeper water below low tide level but there is some overlap with *K. scalarina* at the lower end of the shore (Nielson 1963, Peterson and Black 1993). *K. peroni* has been little studied and there is no published information on its distribution or habitat preferences in Tasmania. In Western Australia it is apparently scarcer than the other species and occupies similar zonation levels to *K. rhytiphora* (Roberts 1984).

A number of studies have shown a distinct zonation in the distribution of *K. scalarina* within the intertidal area with a preference for lower tidal levels (Guiler 1950, Wells and Roberts 1980, Wells and Threlfall 1980, Wells and Roberts 1980, Woodward 1985). A possible explanation is that *K. scalarina* is susceptible to desiccation and thus has a limited tolerance of aerial exposure. In support of this Woodward (1985) found that the survival of caged small and medium sized individuals at Pipe Clay Lagoon increased when they were moved to lower tidal levels and *vice versa.* However this does not exclude other explanations and further work is required to test the significance of exposure more thoroughly.

Woodward found a relationship between the abundance of *K. scalarina* and sediment grain size at Pipe Clay Lagoon; there was a significant negative correlation with finegrained sands (63 - 125 μm) and a preference for medium-grained sands (125 - 250 μm). This agrees with Grange's (1977) general conclusion that suspension feeding bivalves tend to prefer areas of medium-grained sands.

In addition to density, size class distribution within populations of *K. scalarina* also varies in relation to position on the shore. Mean densities at Moulting Lagoon were highest just below mid-shore level on two out of three sample transects taken across the area. Recorded values were less than eight individuals per m^2 at the highest point sampled, increasing to around 48 per m^2 in the areas of maximum density lower on the shore. The average size of individuals in the population (measured as the anterio-posterior shell length) increased markedly towards low water level; at the highest levels most individuals were in the size range of 25 - 40 mm and at the lowest levels most were from 40 - 55 mm (Bellchambers 1993). At Pipe Clay lagoon the density and lengths of *K. scalarina* also increased towards low tide level (Woodward 1985) and similar trends were observed in Oyster and Princess Royal Harbours, Western Australia (Wells and Roberts 1980, Wells and Threlfall 1980).

The dispersion of individuals within populations of *K scalarina* has been shown to be non-uniform with a distinct tendency towards clumping (Nielson 1963, Bellchambers 1993) but the extent and scale of this clumping is unknown. Woodward (1985) found evidence that clumping was more prevelant during the main spawning period possibly suggesting a reproductive significance.

At Pipe Clay Lagoon there were significant relationships between the density of *K. scalarina* and the densities of other members of the invertebrate community (Woodward 1985). Densities of *K. scalarina* were negatively correlated with those of *Anapella cycladea.* This could be interpreted as evidence of competition for food or space (cf Peterson 1977) but other explanations such as differing microhabitat preferences are also possible. There were negative correlations between the abundances of all bivalve species including *K. scalarina,* and the deposit feeding gastropods, *Hydrococcus brazieri* and *Salinator fragilis.* This observation supports the trophic group amensalism hypothesis of Rhoads and Young (1970) in which it is proposed that the constant sediment reworking by deposit feeders, which produces a faecal - rich substrate that is easily resuspended, inhibits populations of suspension feeders by clogging their filtering apparatus or by limiting the establishment of their larvae. Densities of *K. scalarina* were positively correlated with the distribution of polychaete worm tubes. This may be related to the stabilising effect of the worm tubes on the substrate (Woodward 1986).

Feeding ecology. Members of the genus *Katelysia* are filter or suspension feeders (Coleman 1982, Roberts 1984). *K. scalarina* and *K. rhytiphora* have short siphons through which water bearing suspended food items is drawn into the mantle cavity and filtered through the gills (Nielson 1963). Precise details of feeding, including the identity of food items, tidal rhythms of feeding and the effects of density on feeding efficiency are unknown but are currently the subject of detailed study by Linda Belichambers of the University of Tasmania.

Population dynamics. In order to predict the sustainable yield possible from natural populations of clams, details of recruitment, growth, survival and movements need to be quantified. The extent of natural predation, including that from wading birds, needs to be known so that the outcome form varying amounts of combined predation from human harvesting and natural predators can be modelled. Little has been published on any of these population parameters and the current state of knowledge is therefore totally inadequate to address the problem. Studies

are currently being conducted jointly by the University of Tasmania (Dr. Greg Maguire) and the Department of Primary Industry and Fisheries which will progress towards and understanding of some of these aspects of *Katelysia* population dynamics but not all (Table 1). This research effort will not yield all of the information that is needed to manage the sustainable harvesting of natural populations of clams and it is implicit within the programme that harvesting will become based more on intensive mariculture techniques rather than on the production of natural populations. The research effort is targetted to this end. Thus there is considerable ambiguity about the intended futures of harvesting from natural production versus harvesting from enhanced production. The implications are considerable. The ecological understanding required for the two systems differs considerably and the environmental implications differ profoundly. A rigorous clarification is needed of the future roles of these two approaches before the adequacy of current research can be evaluated and appropriate research can be undertaken in the future. The following discussion summarises what is known at present of the dynamics of natural populations.

Recruitment and Productivity. Reproductive activity of K. *scalarina* has been shown to occur throughout the year in southern mainland Australia and at Little Swanport, Tasmania, but with distinct seasonal peaks (Nielson 1963, Coleman 1982, Greg Maguire unpublished). In southern Australia the main spawning season is in spring, from September to October, with a secondary peak around March and at Little Swanport the main season is from late winter through to summer but with some variation among years. A spring peak in spatfall was recorded at Pipe Clay Lagoon (Woodward 1985).

There is little information available on recruitment rates in Tasmanian clam populations. It is not known how spat production varies from year to year or from place to place, nor is it known if there is any relationship between spat production, recruitment and adult density. Significant relationships have been shown between density and recruitment in populations of the ecologically similar European cockle, *Cerastoderma edule* (Hancock 1971), and an understanding of such relationships is needed to allow modelling of the system and hence to predict the effects of differing harvesting regimes.

At present there is no method for the age determination of *K. scalarina* so the age structure of populations and age-specific growth rates and population productivity cannot be determined. Attempts are currently underway by the Department of Primary Industry and Fisheries to test an age determination method and to obtain data on growth rates from a marked population at Anson's Bay. The method depends on the identification of annual growth increments in radial shell cross-sections. Increments estimated in this way will be compared with the known annual growth of the marked animals. Results are not yet available and the usefulness of the method is not yet known.

Most growth in Tasmanian *Katelysia* populations occurs during spring and summer (Woodward 1985) and the average body condition (body mass per unit length) of the *K. scalarina* population at Little Swanport was significantly higher from August to December than during the rest of the year (Greg Maguire, unpublished). Mean lengths of *K. scalarina* differ among populations in different bays in Tasmania. At Weymouth most individuals in a sample of 700 taken in February 1994 ranged from 23 to 29 mm, whereas a sample of 700 from Little Musselroe Bay at the same time ranged from 26 to 40 mm and a sample of 650 from Anson's Bay ranged from 30 to 42 mm (Zacharin and Riely, unpublished). The reasons for these differences are unknown. None of these samples contained significant numbers of individuals less than 20 mm in length, possibly indicating an absence of recent recruitment. However it is also possible that the samples were

not taken randomly over the area and that localised recruitment was not sampled. Woodward (1985) recorded that recruitment did not occur uniformly over Pipe Clay Lagoon. Whatever, the data suggest a definite need for a much more thorough investigation of recruitment over all of these sites and at other sites in Tasmania where *Katelysia* populations occur.

Survival. There is no information on age-specific survival rates for Tasmanian clam populations and these data cannot be obtained until a reliable age-determination method becomes available. The causes of natural mortality are largely unknown and there is little information on mortality rates in relation to season, adult density or environmental variables. The significance of predation is unknown. Possible predators, in addition to wading birds, include crabs, fish and carnivorous gastropod molluscs. Woodward (1985) observed predation on *K. scalarina* by the gastropod *Nassarius pauperatus* at Pipe Clay Lagoon. In the preliminary fieldwork conducted in the preparation of this report 100 freshly dead *K. scalarina* were collected along a 75 m transect over the lower third of the beach at Anson's Bay in January 1995. The two valves were intact and undamaged in all of them. None had been bored by carnivorous gastropods. Freshly dead clam shells were abundant over most of the intertidal area indicating a substantial mortality and the intact nature of the shells is consistent with mortality arising from environmental factors such as excess aerial exposure or predation by oystercatchers. Predation by oystercatchers was observed at the site (see chapter 5.14.4).

At Pipe Clay Lagoon juvenile and medium-sized *K. scalarina* held in cages had significantly higher survival rates than non-caged individuals. Woodward (1985) suggested that this might have been caused by the shading effects of the cages but reduced predation is also a possible explanation. The greater survival of small and medium-sized *K. scalarina* held in cages lower on the shore compared with higher positions was noted earlier (see above). Woodward attributed this to the effects of exposure and desiccation but varying levels of predation again cannot be discounted. Individuals lower down the shoreline would be exposed to predation from wading birds for shorter periods at each tide and also over the spring/neap tide cycle so might have lower mortality rates. At Princess Royal Harbour, West Australia, Peterson and Black (1993) recorded survival of adult *K. scalarina* of around 90% over periods of approximately nine months. Their study plots were slightly below low water level so that the clams were covered by at least 5 cm of water at low tide on the most extreme spring tides. The population would have been exposed to potential predation from shorebirds for only brief periods and the authors noted that pied oystercatchers were infrequent feeders in the area.

Clams may be sensitive to exceptionally high temperatures. Numbers of moribund individuals were observed in water of about 5 cm depth at Anson's Bay on 25/10/94 when an extreme king tide with low during the middle part of the day coincided with temperatures between 30° and 35°C (Shaun Riely personal communication).

Dispersal. The horizontal dispersal movements of adult and juvenile *K. scalarina* have been studied at Moulting Lagoon. Individually marked animals on a 5 m x 4 m sample area, grided into I m^2 subplots, were recaptured at three-week intervals over a period of 12 weeks. Only four recaptures out of a total of 1368 adult recaptures and six out of 466 immature recaptures had moved into adjacent subplots indicating that short-term movements were infrequent and over only short distances. Similar results were obtained in a separate set of observations in which only a single recapture session was made after 12 weeks (Bellchambers 1993). Peterson and Black (1993) found more evidence of short distance movements at Princess Royal Harbour, West Australia with an average of nine immigrants per six months into four 1 m^2 unenclosed sample plots with average initial densities of 31.5 individuals per m^2, studied over 24 months.

Thus, short term movements within natural *Katelysia* populations seems to be limited but it is possible that the amount and lengths of dispersal movements in undisturbed populations may vary in relation to age, season and locality and there is a need for a more comprehensive study.

Bellchambers (1993) also examined the effects on *K. scalarina* movements of disturbance caused by harvesting. Three plots measuring 4 m x 4 m at low and mid-shore levels at Moulting Lagoon were harvested by "finger ploughing" which would have removed 95 - 100% of the original clam populations (Peterson l982). After a 12 week interval the plots were reharvested. In the low-shore plots the numbers of clams taken in the second harvest were from 46.5 to 61.5% of the original population densities and in the mid-shore plots values were from 33.7 to 57.6% of initial densities. It is clear that large numbers of individuals had moved into the harvested areas, but the distances moved by them are unknown and it is possible that they may only have come from immediately adjacent areas. The length distributions of the recolonising clams were not significantly different from those surrounding the experimental plots. These results suggest the possibility of density dependent movements within clam populations.

Table 1. Aspects of the biology and development of Katelysia and Venerupis currently under study in a joint programme of research between The University of Tasmania (Dr. G. B. Maguire and Ms. L. Bellchambers) and the Division of Sea Fisheries, Tasmanian Department of Primary Industry and Fisheries.

Subject	Species	Study Areas	Study Periods
Identify spawning seasons	*K. scalarina*	Little Swanport	1991-1995
	K. scalarina	Anson's Bay	1994-1996
	V. largillierti	George's Bay	1993-1996
Seasonal changes in body condition	*K. scalarina*	Little Swanport	1991-1994
	K. scalarina	Anson's Bay	1994-1996
Diet	*K. scalarina*	?	1994-1996
Age determination	*K. scalarina*	Anson's Bay	1994-1996
Habitat requirements	*K. scalarina*	?	1994-1996
Genetic variability	*K. scalarina*	Tasmania	Proposed for 1995
Develop hatchery, nursery and grow-out procedures	*K. scalarina*	Anson's Bay	1994-1996
		George's Bay	
	V. largillierti	Little Musselroe Bay	1994-1996
		Recherche Bay	

5.14.2.2 Clam Harvesting in Tasmania

Distribution of clam harvesting. Harvesting of clams is currently permitted under license at the following locations:- Anson's Bay, Little Musselroe Bay, George's Bay, Weymouth, East and West Inlets at Stanley, Little Swanport and Recherche Bay. Anson's Bay and Little Musselroe Bay have been the most consistently used sites. George's Bay, Weymouth and Little Swanport have not been exploited for *Katelysia,* although the latter is used as a study site and has been subject to regular sampling. Some harvesting has been done at Recherche Bay and at the East and West Inlets but harvesting at the latter areas is prohibited between October and March.

Katelysia scalarina occurs state-wide and further potential harvesting sites and also possible sites for farming of clams exceed those given above. A comprehensive list of such sites has not been prepared but the following have been identified as likely or possible candidates:- Port Davey, Cloudy Bay Lagoon (Bruny Island) and Great Musselroe Bay. There may be others in addition to those on this list (Will Zacharin and Shaun Riely, pers. comm.).

Venerupis largillierti is currently harvested only at George's Bay, St. Helens and there is no information on additional potential harvest or farming sites.

Number of harvesters. From 1993 to the present four clam harvesting licences (exploratory licences) have been issued. Currently three operators harvest *Katelysia* and the fourth harvests subtidal populations of *Venerupis.* Each operator is permitted two helpers each during harvesting operations.

Seasonal pattern of harvesting. Harvest data for *Katelysia* in the form of operators' catch returns were available only for Anson's Bay for the period from January 1992 to July 1993. At the time of writing (February 1995) no returns had been submitted to DPIF for the period from August 1993 to January 1995. However, harvesting in the earlier period was carried out throughout the year and although there was considerable variation from month to month there was no discernible seasonal pattern (Figure 1). Thus harvesting took place during the period of clam reproduction and spat settlement at rates similar to those at other times of year. The single operator harvesting subtidal *Venerupis* also harvested throughout the year with no significant seasonal variation.

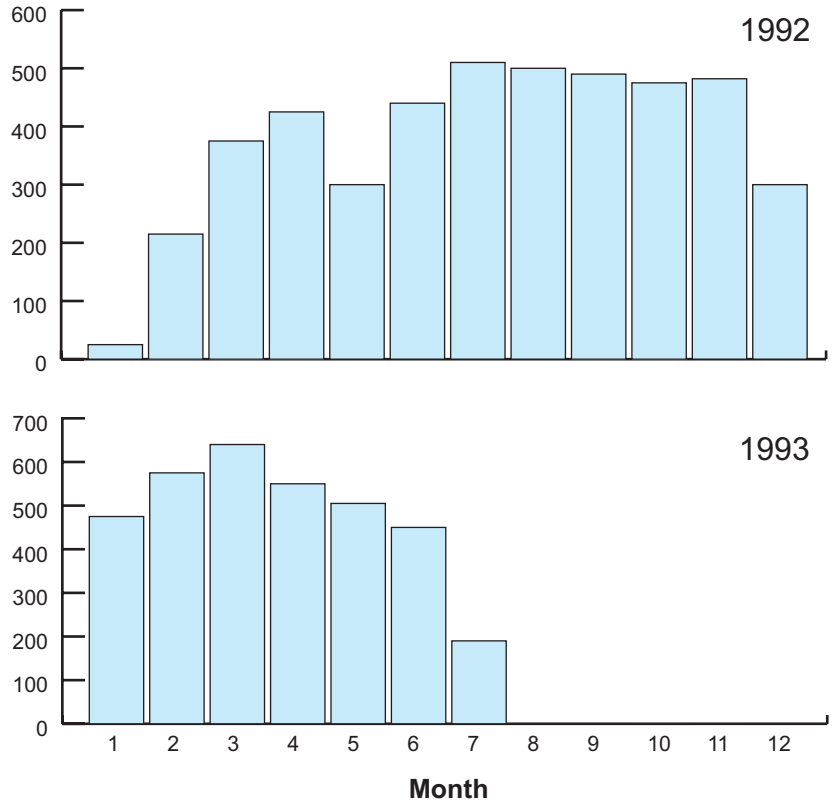

Figure 1. Seasonal pattern of reported clam harvest from Anson's Bay for the period from January 1992 to July 1993. These figures are the total reported returns from three operators combined. Data supplied by W. Zacharin, D.P.I.F., Hobart. No catch returns were available for the period from July 1993 to February 1995.

Clam harvesting methods. Harvesting for *Katelysia* was initially permitted by the use of forks or rakes to turn over the sediment containing the clams. This resulted in mounds of sediment being left on the surface which buried significant numbers of shellfish. The effect of this procedure on the survival of clams was examined experimentally at Moulting and Pipe Clay lagoons (Bellchambers 1993). In replicated trials, samples of previously marked *Katelysia* individuals within the size range of 25 to 40 mm were subject to disturbance using pitchfork and spade treatments to turn over the sediment, simulating the harvesting procedure. After an interval of three weeks the experimental plots and control plots also containing marked individuals were harvested to assess survival. No mortality occurred in the control plots but *Katelysia* in the experimental plots suffered mortality rates of around 40%. In a separate set of trials the effects of an alternative harvesting method, "finger ploughing", in which the fingers are dragged through the sediment to locate clams by touch, were assessed. Mortality rates of around only 5% were associated with the use of this technique. The high mortality from the use of pitchforks and spades was found to be at least partly a direct result of burial and failure to regain preferred depths. Clams buried to depths greater than 10 cm suffered significantly increased mortality with individuals buried to depths of 20 cm having a 55% mortality over a period of three weeks.

In response to these findings, harvesting has been restricted to use of the "fingerploughing" technique. In an experimental analysis Peterson (1982) showed that this method could result in the detection of almost all clams greater than about 10 mm in length. Harvesting by this method may therefore be expected to result in the almost complete depletion of adult clams from the areas that are harvested.

No assessment has been made of the effect of the finger ploughing method on spat settlement or on the survival of juvenile clams. Also, in practice the method more resembles a sorting through the sediment rather than simply raking the sediment and the above experiments may not have adequately simulated the procedure. Disturbance to the sediment may be considerably greater than suggested. Also in the experiments the clams were subject to only a single harvesting event and the results from repeated disturbance may be significantly different.

Harvesting for subtidal *Venerupis* at George's Bay is done using a rake. The effects of this procedure on the *Venerupis* population and other components of the subtidal infauna have not been examined.

Amount harvested. The recorded harvest levels are taken exclusively from the operators' own returns to the Department of Primary Industries and Fisheries under the terms of their exploratory licenses and there is no independent monitoring to assess the validity of these returns. Their accuracy could therefore be challenged. In practice a satisfactory policing of the industry would prove difficult. The operators state that only two harvesting sessions of about three hours each may be needed to achieve the weekly quotas and that they do not work to a fixed timetable. The existence of three licensed operators, each permitted to employ two assistants and to harvest over the same areas complicates the matter considerably.

Data from operator returns were available only up to July 1993 and not subsequently. No returns had been provided to DPIF from from July 1993 to February 1995 even though the licencees were required by the conditions of their licences 'to supply the Director (Sea Fisheries Division) with a complete and accurate record of all species of shellfish caught at the end of each month'. Since the start of 1994 the weekly quota has been set at 300 kg per licence holder. The quota is not specific to any site and can be taken from a single site or from several. One kilogram contains approximately 50 to 60 individual *Katelysia* (Will Zacharin, personal communication).

No information is available on either the standing crop at any time for any of the harvested sites or on the monthly or annual productivity of the clam populations at any of the sites. Thus it is not known how the permitted, reported or actual harvest levels might relate to either of these parameters. There is no information on any changes that may have occurred in the standing crop of clams at any site since harvesting began. Thus, on the basis of existing knowledge, the suitablity and sustainability of past and present harvest levels cannot be assessed for any of the harvesting sites by any accepted scientific criteria.

Provisions for the monitoring of the long-term impact of harvesting on coastal ecosystems. To date there has been no research into any of the possible effects of *Katelysia* harvesting on any other component of the estuarine ecosystems in which harvesting occurs, nor are there any specific plans for appropriate research programmes. At present there is no environmental monitoring of the possible impact of clam harvesting on coastal ecosystems.

The Effects of clam harvesting methods on the macrofauna of intertidal areas. Wading birds depend upon a wide range of macrofauna for their food supply including bivalve and gastropod molluscs, crustaceans and polychaete worms (see Chapter 5.14.4) and any activity that reduces the abundance and diversity of such prey in the sediment could have a deleterious effect on the birds. In addition to the removal of clams, harvesting affects intertidal areas by causing disturbance to the sediment and the consequences of this disturbance on all components of the macrofauna and subsequently on the bird populations must be considered.

There has been no research in Tasmania or elsewhere in Australia into the overall effect of harvesting using the finger ploughing technique on entire invertebrate communities. Specifically, there has been no assessment of its effect on immature *Katelysia* less than 25 mm in length nor on settlement and recruitment rates. There has been no assessment of its effect on other molluscs, polychaetes or crustaceans.

There is a considerable body of evidence from research in Europe and North America that any form of mechanical disturbance to the sediment of intertidal or subtidal areas is liable to cause extensive and in some cases extremely long lasting reductions in the abundance and diversity of macrofauna (e.g. Eagle 1975, Kaplan *et al.* 1975, Connor and Simon 1979, Jackson and James 1979, McLusky, Anderson and Wolf-Murray 1983, Aller 1989, Rice *et al.* 1989, Emerson *et al.* 1990). Thus there is a very strong case for research into the effects clam harvesting in Tasmania on the whole invertebrate communities of the harvested areas.

5.14.2.3 Conclusions

1. Current understanding of all aspects of the ecology of natural population of clams *(Katelysia scalarina* and *K. rhytiphora)* is inadequate. In particular, there is almost no information from Tasmania or elsewhere in Australia on natural variations in distribution and density, on reproduction, growth and productivity and on causes and extent of mortality. There is a need for research into all aspects of *Katelysia* ecology.

2. As a consequence of this lack of relevant information it is not possible at present to determine sustainable harvest limits for clam populations on Tasmania. Current limits set by DPIF are arbitrary and it is not possible to determine scientifically whether or not these harvesting levels are sustainable.

3. Research currently being undertaken jointly by the University of Tasmania (Centre for Aquaculture, Launceston) and DPIF into *Katelysia* is relevant only to the artificial culturing of the species and not to natural populations. This research will not provide the

information needed to determine sustainable levels of harvesting from natural populations of *Katelysia.*

4. At present there is no provision to determine what, if any, are the wider environmental effects *of Katelysia* harvesting. There has been no monitoring of the ecosystems in which *Katelysia* harvesting has occurred during the period of harvesting. No measures have been taken to ensure that *Katelysia* harvesting is sustainable or compatible with the conservation of the estuarine ecosystems in which it has taken place.

5. A joint program of research between the Centre of Aquaculture, University of Tasmania and DPIF is being undertaken which aims to develop the intensive farming or culturing of *Katelysia* in the natural environment. There is no provision in this research program to determine in advance or at any other time what the environmental effects of such developments might be.

5.14.3 HABITAT, DISTRIBUTION, NUMBERS AND CONSERVATION STATUS OF WADING BIRDS IN TASMANIA

5.14.3.1 Habitat Preferences of Waders in relation to Katelysia Harvesting

General habitat preferences. Twenty species of wading bird occur regularly in Tasmania, of which eight are resident breeding species, twelve breed in arctic or subarctic areas of Siberia and North America and spend the southern summer (September to April) in Australia and one, the double-banded plover, breeds in New Zealand spending the southern winter in Tasmania (Table 2).

The main feeding habitats of eleven of these species are estuaries, coastal lagoons and bays. This group includes the pied oystercatcher, Pacific golden plover, double-banded plover; red-capped plover, eastern curlew, whimbrel, greenshank, bar-tailed godwit, red knot, red-necked stint and curlew sandpiper. Three other species, the sooty oystercatcher, hooded plover and masked lapwing also feed in coastal lagoons and bays and although this is not their main habitat throughout the year it may be important or the main habitat at particular times. The remaining six species rarely make use of the lagoon or sheltered estuary habitat. Thus, there are 14 species that feed in the types of habitat in which *Katelysia* occurs and that could potentially be affected by *Katelysia* harvesting (Table 2).

Seasonal patterns of habitat use. Migrant species that breed in the arctic and subarctic areas make use of estuarine habitats in Tasmania mostly during the summer months although in some species, especially bar-tailed godwits, curlew sandpiper, red-necked stints, eastern curlew and greenshank, immature birds do not migrate and remain on the estuaries throughout the southern winter. The numbers of such individuals varies considerably from year to year, presumably in relation to breeding success in the previous breeding season (Thomas 1970). The availability of suitable estuarine habitats for these young birds will influence their pre-breeding survival rates and hence will be important in the population dynamics of the species.

Most of the adults of these northern breeding species return to Tasmanian estuaries during August and September and leave during late March and April. They therefore depend on the availability of estuarine habitats for approximately 7 months each year (Thomas 1968, 1970, Patterson 1989).

The double-banded plover migrates to Tasmania from New Zealand and occurs on Tasmanian estuaries during its non breeding season, form March to August (Thomas 1970, Pierce 1987).

Resident wader species depend upon estuarine habitats throughout the year but some show significant seasonal variations in their use of this habitat. The densities of pied oystercatchers in estuaries and lagoons during the late summer and winter months (mid February to August) are more than double those during spring and summer (Figure 2). This arises mainly from the movements of breeding adults: in early spring (late August - September) a proportion of those that have wintered in the estuaries and lagoons leave to establish territories along adjacent ocean beaches in which they later attempt to breed. Some pairs also remain to occupy territories around the estuaries but most of the individuals remaining in these habitats during summer are immature birds from one to five years of age and non-breeding adults, which tend to form loose flocks.

Immature and/or non-breeding adult sooty oystercatchers also tend to occupy estuarine habitats during the summer months but numbers increase substantially during the winter with adults moving in from rocky shore habitats (Thomas 1968, 1970, Hewish 1990, Newman and Park 1982). Immature birds may be excluded from rocky shore habitats by the territorial behaviour of the adults during summer but it is also possible that the specialist habit of the adults of eating species such as limpets and barnacles on these shores requires skills that are poorly developed in the young birds. The immatures may rely on prey in the estuarine areas that are easier to catch and manipulate while they gradually develop their skills. Adults may rely on the sheltered bays as feeding areas in winter when weather conditions render coastal feeding on rocky shores difficult or unprofitable. Whatever, it is likely that estuarine areas are important habitats for all age groups of sooty oystercatchers.

Table 2. Habitat preferences and residency status of wading bird species that occur regularly in Tasmania. Sources: Lane 1987, Marchant and Higgins 1993.

Habitats
LE lagoons and estuaries
CB coastal bays
1W inland water bodies, marshland
OB ocean beaches
RC rocky coasts
AL agricultural land, grassland

The main habitats of each species are shown in bold

Status
RB resident breeder
SM summer migrant - spends Australian summer in Tasmania
WM winter migrant - spends Australian winter in Tasmania

Table 2 cont.

Common name	Scientific name	Habitat preferences	Status
Pied Oystercatcher	*Haematopus longirostris*	**LE, CB,** OB, RC	RB
Sooty Oystercatcher	*Haematopus fuliginosus*	**LE, CB,** OB, **RC**	RB
Masked Lapwing	*Vanellus miles*	**LE, CB, IW, AL**	RB
Banded Plover	*Vanellus tricolor*	AL	RB
Grey Plover	*Pluvialis squatarola*	CB	SM
Pacific Golden Plover	*Pluvialis fulva*	**LE, CB**	SM
Hooded Plover	*Charadrius rubricollis*	CB, **OB**	RB
Double-banded Plover	*Charadrius bicinctus*	**LE, CB,** OB	WM
Red-capped Plover	*Charadrius ruficapillus*	**LE, CB,** OB, **IW**	RB
Black-fronted Plover	*Charadrius melanops*	LE, **IW**	RB
Ruddy Turnstone	*Arenaria interpres*	OB, **RC**	RB
Eastern Curlew	*Numenius madagascariensis*	**LE, CB**	SM
Whimbrel	*Numenius phaeopus*	**LE, CB**	SM
Greenshank	*Tringa nebularia*	**LE,** CB, **IW**	SM
Japanese Snipe	*Gallingp hardwickii*	**IW**	SM
Bar-tailed Godwit	*Limosa lapponica*	**LE, CB**	SM
Red Knot	*Calidris canutus*	**LE, CB**	SM
Sharp-tailed Sandpiper	*Calidris acuminata*	LE, CB, **IW**	SM
Red-necked Stint	*Calidris ruficollis*	**LE, CB,** IW	SM
Curlew Sandpiper	*Calidris ferrruginea*	**LE, CB,** IW	SM
Sanderling	*Calidris alba*	**OB**, CB	SM

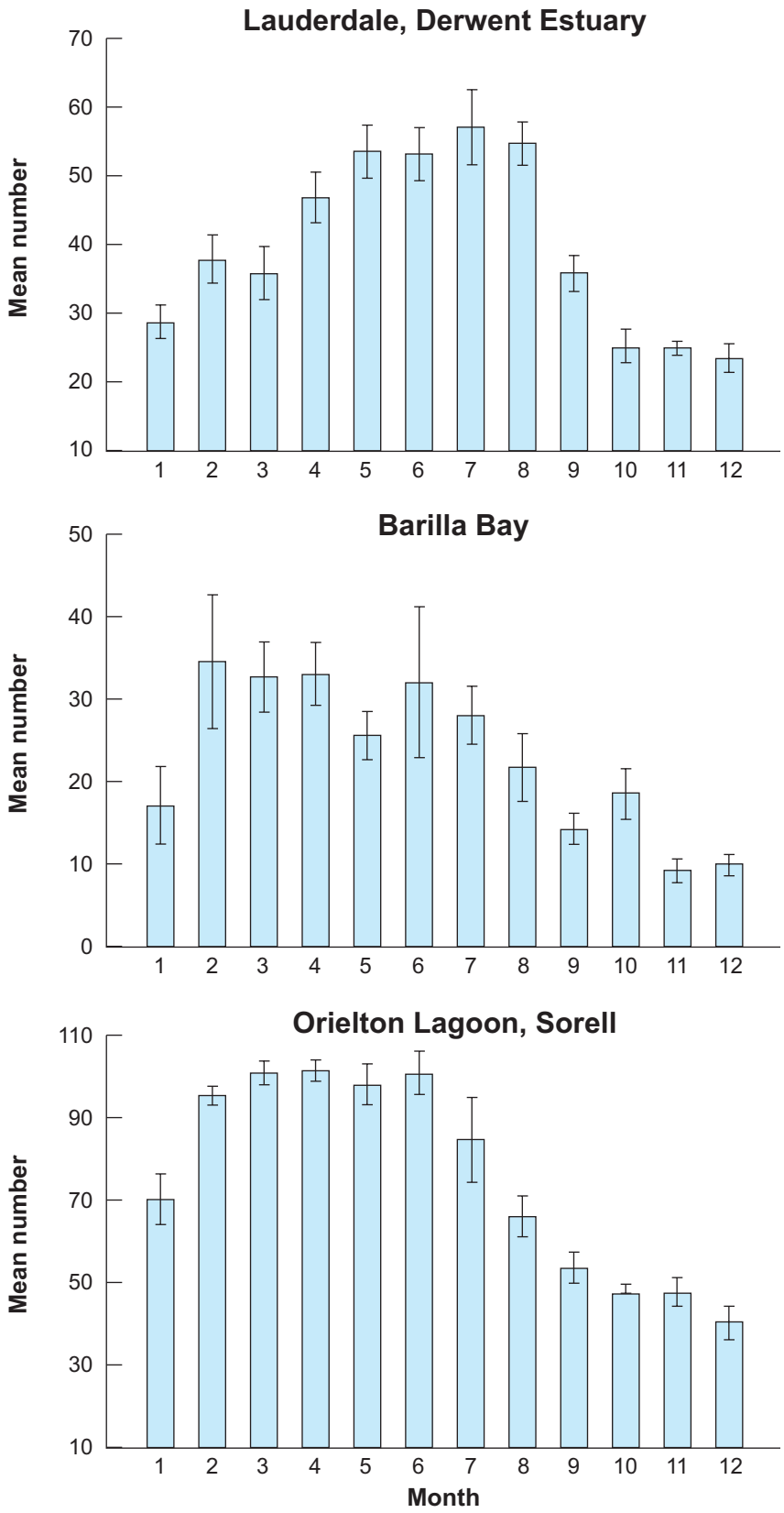

Figure 2. Seasonal changes in the numbers of pied oystercatchers in sheltered bay/estuary habitat types. The figures are mean numbers with standard errors from monthly counts between 1980 and 1985 undertaken by members of the Bird Observers Association of Tasmania Shorebird Study Group. Only those counts that covered the whole of the study area in question were included in the analysis. Counts were of adult and immature birds combined and did not include non-flying young birds.

5.14.3.2 Distribution and Numbers

S*ources of Information.* Most counts of wading birds in Tasmania have been organised by members of the Bird Observers Association of Tasmania Shorebird Study Group. From 1981 to 1985 a national project was undertaken by the Royal Australian Ornithologists Union to identify the main wader areas around Australia and to estimate the total numbers of individuals of each species. The B.O.A.T. Shorebird Study Group contributed to this project by visiting as many as possible of the potential wader habitats throughout Tasmania. This was a difficult task because of the limited number of fieldworkers in relation to the area covered and the remoteness of large parts of it. Most of the important areas were visited at least once and some areas, particularly the Derwent/Pittwater area were visited several times each month over the whole study period to gain an understanding of seasonal changes. The results of the national study (1981-85) were published by Lane (1987).

Many of the main wader areas in Tasmania have now been surveyed at least twice annually (July and February) since the early 1980s by members of the B.O.A.T. Shorebird Study Group. Other more remote or more extensive areas have been surveyed less frequently and in some cases recent counts have been more thorough than those done before 1985 (Schulz and Menharst 1984, Pierce 1987, Schulz 1990, 1991, Ashby 1992). Thus, although attempts have been made to achieve a high level of co-ordination in surveys, it has never proved possible to count all areas at exactly the same time every year. Nevertheless, it is evident from sites that have been surveyed many times over several years that there tends to be considerable short term stability in the numbers of many of the locally breeding species such as pied and sooty oystercatchers (Patterson 1989, Hewish 1990).

Results of surveys. The coastal areas of Tasmania have been subdivided into a number of zones to facilitate the analysis of regional variations in wading bird numbers (Figure 3). Counts undertaken during February are the most useful as these contain migrant as well as resident species and subsequent discussion concerns counts undertaken at this time.

The most important area of Tasmania for both diversity and total numbers of wading birds is the north west from Cape Grim to Stanley. This area regularly supports 20 species with estimated total numbers in excess of 10,000. Around 40% (4,200) of this total is made up of red-necked stints and curlew sandpipers but there are also substantial populations of Pacific golden plovers, red knot, eastern curlews and pied oystercatchers (Tables 3 and 4).

Three other areas of Tasmania support both high diversities and high total numbers of waders; the Derwent/Pittwater area with 15 species and a total of around 4,800 individuals, the Furneaux Islands with 14 species and 6000 individuals and the Cape Portland area with 15 species and about 4000 individuals. As with the north-west, red-necked stints and curlew sandpipers are the most numerous waders in all of these areas contributing in the order of 50 - 70% of the total individuals (Tables 3 and 4).

The Tamar Estuary and King Island also support high diversities with 15 and 17 species and substantial total numbers of around 1300 and 3000 respectively. Approximately 50 of the Tamar population again consists of red-necked stints and curlew sandpipers. On King Island, with its extensive stretches of rocky shore, around 48 % of the total numbers of individuals are ruddy turnstones (Tables 3 and 4).

All other areas of Tasmania support both lower diversities and lower total numbers of wading birds. With reference to *Katelysia* harvesting areas, almost all (with the exception of the East

and West Inlets, Stanley and Little Musselroe Bay) support relatively small numbers of waders. However, in nearly all cases these areas support significant numbers of pied oystercatchers and to a lesser extent sooty oystercatchers and are of international conservation significance for these species (see below).

The largest concentrations of both pied and sooty oystercatchers occur in the north-west from Cape Grim to Stanley and in the Furneaux Islands. The Derwent/Pittwater area supports a very high population of pied oystercatchers (Table 5). Most of the areas along the north and east coasts in which clam harvesting occurs or might occur in future support significant numbers of pied oystercatchers especially during the winter months from April to August. This includes the East and West Inlets at Stanley, Little and Great Musselroe Bays, Anson's Bay, George's Bay, Little Swanport, Moulting Lagoon and Cloudy Bay Lagoon on Bruny Island. Southport Lagoon is within this area but has not been surveyed for oystercatchers so its importance is unknown. In total, approximately 15 - 20% of the total pied oystercatcher population of Tasmania occurs in potential or actual clam harvesting areas (Table 5).

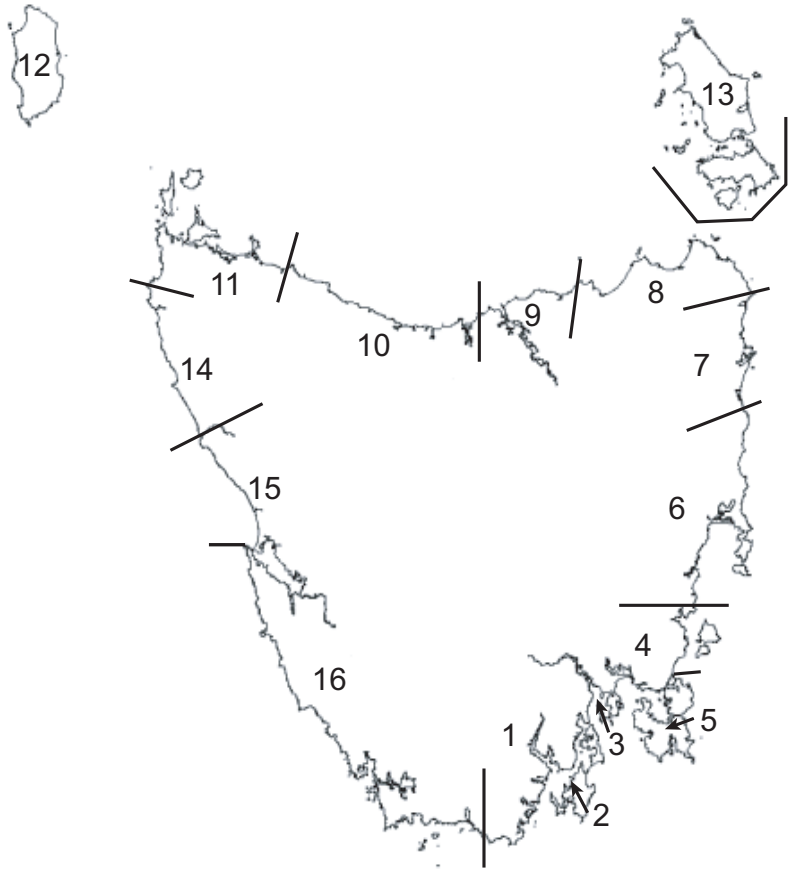

Figure 3. Subdivision of the Tasmanian coast into units used by the BOAT Shorebird Study Group. These units are referred to in Tables 3,4 and 5.

Units are as follows:

1. West Derwent South; 2. Bruny Island; 3. East Derwent/ Pitt Water; 4. Prosser; 5. Forestier/Tamar Peninsula; 6. Freycinet Coast; 7. St. Helens Coast; 8. Cape Portland Coast; 9. Tamar Estuary; 10. Central North East Coast; 11. Cape Grim Coast (including the Hunter Group); 12. King Island; 13. Furneaux Group; 14. Sandy Cape Coast; 15. Henty (Ocean Beach); 16. South West Coast.

Table 3. Distribution of Wading birds in Tasmania. The figures give the estimated total numbers of each species during summer (February) for each coastal subdivision (see Figure 3). Source of data: Watkins 1993.

Species codes: BaLa - Banded Lapwing; BarG - Bar-tailed Godwit; BfoP - Black fronted Plover; CurS - Curlew Sandpiper; DbPl - Double-banded Plover; EaCu - Eastern Curlew; Gank - Greenshank; GryP - Grey Plover; HooP – Hodded Plover.

Area	Zone	BaLw	BarG	BfoP	CurS	DbPl	EaCu	Gank	GryP	HooP
West Denvent South	1		0	0	0	5	2	0	0	
Bruny Is.	2		0	0	0	40	0	0	0	
E.Derwent/Pittwater	3		75	30	1300	360	150	75	0	
Prosser	4		15	10	0	40	12	0	0	
Tas. Peninsula	5		0	0	0	0	0	0	0	
Freycinet	6		0	5	140	30	13	75	0	
St. Helens Coast	7		20	0	0	50	11	5	0	
Cape Portland Coast	8		20	0	700	680	3	10	0	
Tamar	9		50	0	160	50	100	20	0	
Central North Coast	10		0	0	0	30	70	0	0	
Cape Grim Coast	11		38	0	1700	1465	250	34	120	
King Is.	12		0	20	100	370	1	15	0	
Furneaux Is.	13		80	0	2650	120	60	70	0	
Sandy Cape Coast	14		50	0	100	30	100	20	0	
Henty/Ocean Beach	15		0	0	0	170	0	0	0	
South-West Coast	16		0	0	0	0	0	0	0	
Unallocated Areas		1000	0	50	20	9000	0	25	0	1700
TOTALS		1000	348	130	6870	12440	772	349	120	1700

Table 3 (cont.)

Species Codes: PiOy - Pied Oystercatcher; PGoP - Pacific Golden Plover; ReKn - Red Knot; RcaP - Red-capped Plover; RenS - Red-necked Stint; RuTu - Ruddy Turnstone; San - Sanderling; ShtS - Sharp-tailed Sandpiper; SoOt - Sooty Oystercatcher.

Area	Zone	PiOy	PGoP	ReKn	RcaP	RenS	RuTu	Sand	ShtS	SoOt
SE/S Coast	1	8	0	0	5	0	0	0	0	0
Bruny Is.	2	260	0	0	120	120	0	0	0	45
E. Derwent/Pittwater	3	570	319	5	170	1300	0		20	40
Prosser	4	150	0	5	50	460	0	0	0	2
Tas. Peninsula	5	17	0	0	5	0	0	0	0	0
Freycinet	6	100	30	0	50	80	0	0	10	2
St. Helens Coast	7	250	5	0	100	500	5	0	0	30
Cape Portland Coast	8	143	223	10	200	1580	244	0	5	50
Tamar	9	167	25	5	20	480	159	0	0	50
Central North Coast	10	150	30	0	10	120	0	0	0	2
Cape Grim Coast	11	930	560	651	91	2500	771	0	5	663
King Is.	12	10	40	10	150	610	1252	5	50	88
Furneaux Is	13	500	20	0	80	1550	130	160	0	577
Sandy Cape Coast	14	24	15	0	40	310	100	20	0	30
Henty/Ocean Beach	15	14	0	0	100	650	0	330	0	0
South West Coast	16	80	0	0	0	0	0	0	0	60
Unallocated Areas		0	50	0	100	50	0	0	50	0
TOTALS		3373	1317	686	1361	10350	2656	515	145	1639

Table 4. The number of species and estimated total numbers of individual wading birds in each coastal zone in Tasmania. Data taken from Table 3.

Area	Zone	Number of species	Number of Waders
SE/E Coast	1	6	20
Brume Is.	2	7	585
E. Derwent/Pimvater	3	15	4414
Prosser	4	11	744
Tas. Peninsula	5	4	22
Freycinet	6	13	535
St. Helens Coast	7	12	976
Cape Portland Coast	8	15	3868
Tamar	9	15	1286
Central North Coast	10	9	412
Cape Grim Coast	11	20	10737
King Is.	12	17	2721
Furneaux Is.	13	14	5997
Sandy Cape Coast	14	14	839
Henty/Ocean Beach	15	7	1264
South West Coast	16	4	140

Table 5. Estimated numbers and distribution of pied and sooty oystercatchers in Tasmania. Sources: Schulz 1990, Schulz and Menkhorst 1990, Watkins 1993.

Area	Pied oystercatcher		Sooty oystercatcher	
	n	%	n	%
SE/S Coast	8	0.21	0	0
Bruny Island	260	7.7	45	2.8
East Derwent/Pittwater	570	16.9	40	2.4
Prosser	150	4.5	2	0.1
Tasman Peninsula	17	0.5	0	0
Freycinet	100	3	2	0.1
St. Helens coast	250	7.4	30	1.8
Cape Portland coast	143	4.2	50	3.1
Tamar estuary	167	5	50	3.1
Central north coast	150	4.5	2	0.1
Cape Grim coast	930	27.6	663	40.5
King Island	10	0.3	88	5.4
Funeaux Islands	500	14.8	577	35.2
Sandy Cape coast	24	0.7	30	1.8
Henly/Ocean Beach	14	0.4	0	0
Sout-west coast*	80	2.4	60	3.7
TOTALS	3373	100	1639	100

5.14.3.3 Conservation Status of Wader Species and their Habitats in Tasmania

Procedures for determining the conservation status of wetland areas and wading bird species. The wader populations of Tasmania include migrant species and Australian resident species and it is therefore necessary to recognise and list priority conservation areas according to both their international and national significances. Australia is a signatory to the Japan- Australian Migratory Bird Agreement (JAMBA) and to the China-Australia Migratory Bird Agreement (CAMBA) under which all parties have agreed to protect the habitats of migratory wader birds along the length of their migration routes and in their non-breeding areas (Table 6). The main internationally agreed procedure for identifying and conserving the World's most important wetlands is contained within the Ramsar Convention, to which Australia is also a signatory. The convention has specified two criteria for the identification of areas of **international** importance for wetland birds including wading birds:

a) Sites that regularly support 20,000 or more individual birds and/or

b) Sites that regularly support 1% or more of the individuals of one species or subspecies.

These criteria have been developed mainly from research on West European and American wading bird populations which are generally considerably larger than those of the Australia/ Asia area. Thus it has been suggested that a figure lower than 20, 000 may be more appropriate for Australia (Parish 1987). However, until the case for such a change has been accepted the higher figure remains in use for areas of international significance.

A similar set of criteria has been proposed for the identification of sites of **national** importance

but in this case the proposal to adopt a lower figure for total numbers has been included (Watkins 1993). The criteria for sites of national importance are;

a) Areas where 10,000 or more shorebirds have been recorded and /or

b) Areas where 1% or more of the individuals of the Australian population of a species or sub-species of shorebird have been recorded.

Within these criteria the terms "species and subspecies", "population", "regularly support" and "site (area)" need careful attention.

The importance of **sub-species** is recognised so that overall genetic diversity is conserved. However, existing definitions of sub-species may be liable to modification in the future as improved understanding of the genetic relationships among populations develops. Many of the migratory species that occur in Australia also have several geographically distinct breeding areas in Asia and with further research may also prove to have distinct nonbreeding areas in Australia (Lane 1987). Conservation policies should cater for these levels of diversity but in most cases there is inadequate information at present upon which to base decisions. This reinforces the need to try to protect as wide a range of sites as possible within Australia.

The term **population** refers in the case of Australian endemic species or subspecies to all individuals of that species or sub-species in Australia. For those species or subspecies that also occur outside Australia, the population is defined as all individuals of that species or sub-species occurring in the East Asian - Australasian Flyway (Parish et al. 1987). In all cases, minimum estimates of population size are used.

In the Ramsar Convention the term "regularly support" is used. However, this can be decided upon only when extensive information covering all seasons and over many years is available. In practice, for Australia, sites have been considered to be of importance if the specified criteria for numbers have been exceeded on at least one occasion (Watkins 1993). There are several reasons for this. For many areas extensive data sets of bird numbers do not exist, and in many cases only a single count has ever been undertaken. Also, Australian wetland systems tend to be more variable, especially in response to fluctuations in rainfall, than those of Europe and America upon which the RAMSAR Convention criteria were modelled. Sites that are used "regularly" by large numbers of wading birds will be important but specific sites may also be of immense importance if, for example, exceptionally large numbers of birds depend on them for even very brief periods during times of severe drought.

The Ramsar Convention identifies sites or areas of conservation significance. However, it is essential that such sites should not be delimited arbitrarily but should be natural ecological units. Wading birds tend to be more mobile than many other bird groups and may move among various components of a local wetland system according to season, tide or in response to disturbance. It is essential that entire functional units be identified and conserved as units. At present there is often inadequate information upon which to base such decisions with confidence and it is therefore important that conservation areas be defined conservatively until more information becomes available. It is essential that appropriate ecological research be done as soon as possible to help define these areas.

Shortcomings of Ramsar. The use of the criteria specified in the Ramsar Convention to conserve wading birds by protecting specific areas has a number of very serious shortcomings that must be borne in mind. As a conservation measure the Ramsar Convention is designed specifically to protect only the most important areas. This approach may be satisfactory under specific

conditions but not under others. It is particularly suitable for species that are highly clumped in their distribution so that large numbers occur at a few clearly defined sites. However, for species that are highly dispersed for at least part of their annual cycle, this method may fail totally to provide any degree of protection as there may be no specific areas that contain the required number of individuals to satisfy the criteria laid down in the convention. In such cases alternative approaches are needed.

There may also be problems in conserving sites that are important feeding areas for immature birds. First year individuals of many migrant species remain in Australia during the northern breeding season. Also, some Australian breeding species have delayed maturity and may not breed until they are up to six years of age. Because these immature individuals usually form a low percentage of any total population, sites that are used mainly by them and upon which they are totally dependent may not reach the criteria of numbers set by the Ramsar Convention and may not receive any protection.

A further complication concerns the use of sites as staging areas by migrant species. Such sites may only support relatively small numbers at any one time but because of a rapid turnover during migration may support a much larger total number of individuals (Smit and Piersma 1989). In the case of Tasmania this is unlikely to be a major concern as most wetland habitats are at the end of the migration routes for most species.

The Ramsar Convention also fails to address the need to consider conservation at a more local level. It is desirable that areas that are exceptionally important habitats for wading birds within the state of Tasmania should be given some degree of protection even if they fail to meet the very demanding criteria of the Convention. Only in this way can local biodiversity be maintained and also the recreational and tourism values of wildlife be secured. It is important that these areas of local significance be identified so that potential developments may be planned in such a way that those values are taken into account and not jeopardized.

In the cases of resident Australian species there is an additional complication. Most areas designated by the Ramsar criteria for such species are used by the birds for breeding as well as for feeding. A simple assessment of the value of a site based only on numbers may be inadequate if there are significant variations in breeding success among areas. An area may offer excellent habitat for feeding and may easily meet the requirements for numbers but the breeding performance of the birds in it may be below the level needed to maintain a viable population. Factors such as disturbance or predation which can lower productivity could act independently of food supply. The numbers of birds in the area may be maintained only by immigration. Such 'sink areas' which depend on immigration from neighbouring populations are known to occur in several bird populations and may be of common occurrence (Pulliam 1988, Newton 1991). Sites that are selected as important conservation areas for resident species must have the ability to maintain self supporting viable populations. This means that more detailed research other than a simple assessment of numbers is required in these cases.

When identifying priority conservation sites, it is also important to take into account the conservation status of individual species. Special consideration must be given to species that are endangered, threatened or nationally scarce.

Thus when deciding which wetland sites in Tasmania deserve particular consideration on the basis of their importance for the conservation of wading birds at least three pieces of information are needed: 1). The numbers of each species at a local level specific to particular areas of wetland; 2). The national totals for each species and; 3). The totals for each species in the entire

Australian/Asian Flyway.

Table 6. International conservation agreements relevant to the species of wading birds that occur in Tasmania. RAMSAR: Ramsar Convention on Wetlands of International Importance, Especially as Waterfowl Habitat; JAMBA: Japan - Australia Migratory Bird Agreement; CAMBA: China - Australia Migratory Bird Agreement.

Species	Relevant Conservation Agreements		
Pied Oystercatcher		Ramsar	
Sooty Oystercatcher		Ramsar	
Masked Lapwing		Ramsar	
Hooded Plover		Ramsar	
Red-capped Plover		Ramsar	
Black-fronted Plover		Ramsar	
Grey Plover	Ramsar	JAMBA	CAMBA
Pacific Golden Plover	Ramsar	JAMBA	CAMBA
Eastern Curlew	Ramsar	JAMBA	CAMBA
Whimbrel	Ramsar	JAMBA	CAMBA
Greenshank	Ramsar	JAMBA	CAMBA
Bar-tailed Godwit	Ramsar	JAMBA	CAMBA
Red Knot	Ramsar	JAMBA	CAMBA
Sharp-tailed Sandpiper	Ramsar	JAMBA	CAMBA
Red-necked Stint	Ramsar	JAMBA	CAMBA
Curlew Sandpiper	Ramsar	JAMBA	CAMBA
Sanderling	Ramsar	JAMBA	CAMBA

Conservation Status of Wader Species in Tasmania

Table 7 lists the Tasmanian, Australian and Australian/Asian Flyway minimum population estimates for wading bird species that occur in Tasmania. For most species the entire state of Tasmania supports considerably less than 5% of the total Australian populations. However, for three species, the pied oystercatcher, the sooty oystercatcher and the hooded plover, the state supports a very high percentage, of around 30 - 40% in each case, of the Australian and World total.

Pied and sooty oystercatchers are also the species that are most likely to be affected directly by clam harvesting (see Chapters 5.14.4 and 5.14.5). The minimum estimated population totals for Australia are not high for either species; 10,000 in the case of the pied oystercatcher and 4,000 for the sooty oystercatcher. These figures refer to the entire population including pre-breeding age immature birds and non-breeding adults so that the numbers of breeding pairs are likely to be only of the order of 3,000 - 4,000 for the pied oystercatcher and around 1,000 - 1,500 for the sooty oystercatcher. It is likely that the total Tasmanian breeding populations for these species are only around 1,000 and 500 pairs respectively. These are therefore nationally scarce species and Tasmania supports more of them than any other state. Thus their conservation in Tasmania requires special consideration.

Table 7. Estimated numbers of each wading bird species occurring in Tasmania expressed as a percentage of the total estimated Australian population. Estimated numbers in the entire Australian/ Asian Flyway populations are also shown. Figures are taken from Tables 3 and 4 and from Watkins 1993.

Species	Australian Population - minimum estimate	Asian-Australian population - minimum estimate	Tasmanian population - minimum estimate	Tasmanian population as of Australian population
Pied oystercatcher	10000	11000	3373	33.7
Sooty oystercatcher	4000	4000	1693	41
Masked lapwing	258000	287000	5000	1.9
Grey plover	12000	16000	120	1
Pacific golden plover	9000	90000	1317	14.6
Hooded plover	5000	5000	1700	34
Double-banded plover	30000	50000	11015	36.7
Red-capped plover	95000	95000	1361	1.4
Black-fronted plover	17000	17000	130	0.8
Ruddy turnstone	14000	28000	2656	19
Eastern curlew	19000	21000	772	3.5
Whimbrel	10000	40000	12	0.1
Greenshank	20000	40000	349	1.7
Latham's snipe	36000	36000	6000	16.7
Bar-tailed godwit	165000	330000	348	0.2
Red knot	153000	255000	686	0.5
Sharp-tailed sandpiper	166000	166000	145	0.1
Red-necked stint	353000	417000	12100	3.4
Curlew sandpiper	188000	250000	6870	3.7
Sanderling	8000	11000	515	6.4

Conservation Status of Wetland Areas in Tasmania

It is essential that areas nominated as sites of special conservation value be complete natural ecological units and failure to include important parts of such units that may be used seasonally or even less frequently may jeopardise the entire procedure. For both pied and sooty oystercatchers it has been shown earlier that at least two components of coastal wetland systems are essential. The sheltered and productive habitats of estuaries, bays and lagoons are essential feeding areas for immature and non-breeding birds throughout the year and for breeding adults in winter. These same habitats are also used by some pied oystercatcher pairs for breeding during spring and summer but significant numbers of them disperse to occupy territories along adjacent ocean beaches. Sooty oystercatchers disperse to breed on rocky islands. For pied oystercatchers natural conservation units consist of stretches of coast containing one or more estuarine or lagoon sites and the adjacent coastal beaches. For sooty oystercatchers both rocky islands and suitable estuarine areas must be conserved to provide for all of the species' requirements.

Around the coast of Tasmania, 14 areas fulfill the criteria set out by the Ramsar Convention as areas of international significance for wading birds (Table 8, Figure 3). The species involved are pied oystercatcher (12 sites), sooty oystercatcher (8 sites), hooded plover (5 sites), ruddy turnstone (2 sites), sanderling (2 sites), double-banded plover (1 site), eastern curlew (1 site) and curlew sandpiper (1 site). The same set of areas also contains sites of Australian national significance for a further three species, Pacific golden plover (3 sites), grey plover (1 site) and the red-necked stints (1 site). The most outstandingly important areas for conserving both diversity of species and total numbers of individuals are the north-west from Cape Grim to Stanley, the Furneaux Islands and the Derwent/Pittwater area.

Almost all of the current and possible future *Katelysia* harvesting sites fall within areas that have been demonstrated to be of international importance for the conservation of pied and sooty oystercatchers. This applies to the East and West Inlets at Port Stanley, Little and Great Musselroe Bays, Anson's Bay, George's Bay, Moulting Lagoon and Cloudy Bay Lagoon on Bruny Island. This emphasises the need to ensure that *Katelysia* harvesting in these areas is compatible with the conservation of these species. Significant conflict with the conservation of other wader species is only likely to occur if *Katelysia* harvesting is developed in the north east (Cape Grim to Stanley) on the Furneaux Islands or in the Derwent/Pittwater areas.

Table 8. Areas of International and National Importance for Wading Birds in Tasmania defined according to the criteria specified in the Ramsar Convention and in Watkins 1993. For locations of coastal zones, see Fig. 3. Sources of information on bird numbers: Watkins 1993, Pierce 1987, Patterson 1989, Schulz 1990 1992, Ashby 1992, Taylor unpublished (count of sooty oystercatcher numbers feeding on intertidal sand and mud flats, Bruny Island, January 1995) and Blackhall unpublished (counts of pied oystercatcher numbers on Moulting Lagoon).

Area	Species	Estimated number	Importance
Anderson Bay	Pied Oystercatcher	105	I
Bruny Island, including Cloudy Bay Lagoon	Pied Oystercatcher	260	I
	Sooty Oystercatcher	45	I
Cape Portland, including Little and Great Musselroe Bays	Pied Oystercatcher	143	I
	Sooty Oystercatcher	41	I
	Double-banded Plover	680	I
	Pacific Golden Plover	223	N
	Ruddy Turnstone	244	N
Derwent Estuary/Pittwater	Pied Oystercatcher	570	I
	Sooty Oystercatcher	40	I
	Pacific Golden Plover	319	N
	Double-banded Plover	367	N
Furneaux Islands	Pied Oystercatcher	500	I
	Sooty Oystercatcher	577	I
	Hooded Plover	250	I
	Sanderling	160	I
	Curlew Sandpiper	2640	I

Area	Species	Estimated number	Importance
King Island	Ruddy Turnstone	1252	I
	Hooded Plover	214	I
	Sooty Oystercatcher	88	I
	Double Banded Plover	370	N
Marion Bay	Pied Oystercatcher	125	I
	Hooded Plover	104	I
North-West (Cape Grim - Stanley) including East and West Inlets	Pied Oystercatcher	923	I
	Sooty Oystercatcher	663	I
	Ruddy Turnstone	1730	I
	Grey Plover	120	N
	Pacific Golden Plover	560	N
	Eastern Curlew	250	I
	Double Banded Plover	1465	I
Ocean Beach, Strahan	Hooded Plover	60	I
	Sanderling	450	I
Port Sorell	Pied Oystercatcher	153	I
South West National Park	Pied Oystercatcher	150	I
	Sooty Oystercatcher	62	I
	Hooded Plover	190	I
St. Helens Coast, including George's Bay and Anson's Bay	Pied Oystercatcher	255	I
Tamar Estuary	Pied Oystercatcher	167	I
	Sooty Oystercatcher	50	I
	Ruddy Turnstone	159	N
Moulting Lagoon	Pied oystercatcher	142	I

5.14.3.4 Conclusions

1. Twenty species of wading bird occur regularly around the coasts of Tasmania, eight of which are Australian residents and 12 migratory.
2. Eleven species feed mainly or exclusively in estuaries and sheltered coastal bays and could thus be affected by clam harvesting. However the main concentrations of most of these are not in the main clam harvesting areas.
3. Two species, the pied oystercatcher and the sooty oystercatcher depend on the habitats in which clam harvesting occurs at present or in which harvesting might be developed in the future. The main centres of pied oystercatcher distribution coincide with the main areas of

clam harvesting. Thus the pied oystercatchers is the species that is most likely to be affected significantly by clam harvesting. The same applies but probably to a slightly lesser extent with sooty oystercatchers.

4. Australia is signatory to a number of international agreements to conserve the habitats of migratory and resident wading birds. This includes the Japan Australia Migratory Birds Agreement (JAMBA), the China Australia Migratory Birds Agreement (CAMBA) and the Ramsar Convention. Fourteen areas around the Tasmanian coast fulfil the Ramsar Convention criteria for identifying conservation areas of International Importance. Twelve of these sites are internationally important for the conservation of pied oystercatchers and eight for sooty oystercatchers. Most of the current and possible future clam harvesting areas fall within these areas of international conservation importance. Australia has agreed to protect such areas from harmful development.

5.14.4 THE DIET OF WADING BIRDS IN TASMANIA

There have been few systematic and detailed investigations of the diets of wading birds in Australia. Most of the available information is anectdotal from casual observation. Prey types that have been recorded for species that occur in Tasmania are listed in Table 9. The remainder of the text concentrates on those species that are known to feed in the types of habitat in which *Katelysia* harvesting has occurred or might occur in the future.

Table 9. Summary of main prey types recorded in Australia of waders that occur in Tasmania. The table lists the main groups of prey that have been recorded from direct observations or the analysis of gut contents for those species of waders that spend at least part of their time foraging in sheltered bays or estuaries in Tasmania.

Species	Recorded Prey groups in Australia	Sources
Pied Oystercatcher	Bivalve and Gastropod Molluscs, Polychaete worms, Crustaceans, Echinoderms.	Evans 1975, Thomas 1986, Marchant and Higgins 1993, Weston 1991, Park (this report), Taylor (this report).
Sooty Oystercatcher	Bivalve and Gastropod Molluscs, Polychaete worms, Crustaceans, Echinoderms, Acidians.	Parry 1977, Considine 1979, Chafer 1994.
Masked Lapwing	Bivalve and Gastropod Molluscs, Annelida, Crustacea, Insects, Plant matter.	Marchant and Higgins 1993.
Hooded Plover	Bivalve and Gastropod Molluscs, Polychaete worms, Crustaceans, Insects, Plant seeds.	Marchant and Higgins 1993.
Double-banded Plover	Gastropod Molluscs, Crustaceans, Insects, Plant seeds.	Thomas 1986.

Species	Recorded Prey groups in Australia	Sources
Red-capped Plover	Gastropod Molluscs, Polychaete worms, Crustaceans, Insects.	Thomas 1986, Marchant and Higgins 1993.
Black-fronted Plover	Gastropod Molluscs, Crustaceans, Insects, Plant seeds.	
Grey Plover	Gastropod Molluscs, Polychaete worms, Crustaceans, Insects, Plant seeds.	Marchant and Higgins 1993.
Pacific Golden Plover	Gastropod Molluscs, Polychaete worms, Crustaceans, Insects, Plant seeds.	Baker and Vestjens 1989, Marchant and Higgins 1993.
Eastern Curlew	Crustaceans	McLennan 1917, Thomson 1935, Lea and Gray 1935, Hindwood and Hoskin 1954, Daan, In Lane 1987, Baker and Vestjens 1989.
Greenshank	Bivalve and Gastropod Molluscs, Crustaceans, Insects, Fish, Amphibians.	Wheeler 1955, Vestjens 1977, Marchant and Higgins 1993.
Bar-tailed Godwit	Polychaete worms, Bivalve Molluscs, Crustaceans.	Evans 1975, Taylor 1994.
Red Knot	Gastropod Molluscs, Crustaceans.	Marchant and Higgins 1993.
Red-necked Stint	Bivalve and Gastropod Molluscs, Polychaete worms, Crustaceans, Insects, Plant seeds.	Thomas and Dartnall 1971, Marchant and Higgins 1993.
Curlew Sandpiper	Bivalve and Gastropod Molluscs, Polychaete worms, Crustaceans, Insects, Plant seeds.	Thomas and Dartnall 1971, Marchant and Higgins 1993.
Sanderling	Polychaete worms, Insects.	Lea and Gray 1935.

5.14.4.1 Pied oystercatcher

The oystercatcher genus *Haematopus* is widespread and there are ecological equivalents of the Australian pied oystercatcher in New Zealand, Asia, Africa, Europe and North America. These are very similar in morphology, size and behaviour to the Australian species and occupy a similar range of habitats. The diet and foraging behaviour of several of them has been studied in detail and throughout their range they have been shown to depend mainly upon bivalve molluscs for food which they take from both rocky areas and from soft substrates. When feeding over soft substrates they take shallow-burrowing bivalves and often forage while wading in shallow water so that they are able to locate feeding molluscs by the activity of their siphons. They are then able to insert their bill between the valves and remove the flesh using a scissor-like action. When feeding over substrates that have become exposed at low tide they are able to locate buried, resting bivalves just below the surface by tactile means and are then able to hammer and prize open the closed-up shell using blows of their specially modified bill. The lengths of bivalves taken normally ranges up to about 40 to 50 mm. Molluscs less than about 20 mm are generally swallowed whole (see chapter 5.14.6).

A more detailed discussion of the relationships between these other species of pied oystercatcher and shellfish populations is provided in chapter 5.14.6. However it is important to point out at this stage that from what is already known of these species and from the close similarity in anatomy and behaviour shown to them by the Australian pied oystercatcher, it is to be expected that the Australian species will feed on the same types of prey using similar methods. Thus, any shallow-burrowing bivalve within the size range of up to 50 mm is likely to be potential prey. Bivalves of the genus *Katelysia* meet these specifications exactly so the possibility that they are important prey for pied oystercatchers in Tasmania must be considered to be high.

Diet of pied oystercatchers from previously published and unpublished reports

There has been no extensive, systematic and quantitative study of the diet of pied oystercatchers in Tasmania or in any other part of Australia. Available information is mainly anecdotal, from the casual observation of foraging birds and from the analysis of the gut contents of a small number of dead birds.

For Australia generally, pied oystercatchers have been shown to take bivalve and gastropod molluscs, polychaete worms, crustaceans, echinoderms and insects (Table 9). The recorded list of bivalve molluscs taken on mainland Australia includes mussels *(Cockadia spp.* and *Mytilus edulis),* cockles (Cardidae), the common pipi *(Donax deltoides)* and venerids (Veneridae).

In Tasmania, pied oystercatchers have been observed taking edible mussels *(M. edulis),* Reddish Trough Shells *(Mactra rufescens),* the Smooth-toothed Triangle *(Anapella cycladea)* and the stepped venerid *(Katylesia scalarina). Anapella cycladea* were identified in the gut contents of a single dead bird (Table 10). The observations of birds taking *Katylesia scalarina* were clear and unambiguous. The birds were watched by P. Park at Oaks Pt., Port Sorrell removing bivalves from the substrate and taking them to firmer ground where they were opened and the flesh consumed. The shells in question were collected and subsequently identified by E. Turner of the Tasmanian Museum.

Table 10. Recorded prey of the pied oystercatcher in Tasmania. These records are from casual observations not from systematic study. They therefore do not necessarily represent the relative importance of the species shown nor is the list complete.

Prey species	Observation	Gut Contents	Details	Source	Identification verified
Smooth-toothed triangle (*Anapella cycladea*), crabs (Graspidae)	-	Yes		Thomas 1986	
Conical sand shell *(Polinices conicus)*		Yes	25/10/87, Dead -juvenile,Norfolk Bay	P.Park, (unpubl.)	E. Turner, Tas. Museum, Hobart
Conical sand shell (*P. conicus),* Bubble shell *(Philine angasi)*		Yes	13/10/83 Dead adult, Orielton Lagoon	P.Park, (unpubl.)	E. Turner
Conical sand shell		Yes	20/12/84, dead adult, East Marsh Lagoon, Lauderdale	P.Park, (unpubl.)	E. Turner
Stepped venerid *(Katylesia scalarina)*	Yes		10/9/83, Oaks Pt. Sorell, 2 adults feeding, shell remains collected.	P.Park, (unpubl.)	E. Turner
			9/1/95, Ansons Bay, extensive observations of 8 adults feeding and collection of eaten shells.	I.R. Taylor, this report (see Table)	from Richmond 1990
Mussels *(Mytilus edulis)*	Yes		29/12/84, Shelly Beach, South Arm, 1 adult feeding, shell remains collected	P.Park, (unpubl.)	E. Turner

Prey species	Observation	Gut Contents	Details	Source	Identification verified
Reddish trough shell (*Mactra rufescens*)	Yes		19/1/93, Seven Mile Beach, 2 adults feeding, shells collected	P.Park, (unpubl.)	from Richmond 1990
			10-24/1/95, Seven Mile Beach, 8-12 adults, 18 shells collected	I.R. and S.G. Taylor, this report (see Table)	from Richmond 1990
Smooth-toothed Triangle (*A. cycladea*)	Yes		Frequently observed, Lauderdale, South Arm, Five Mile Beach and Bruny Is.	P.Park, (unpubl.) and I.R. Taylor, this report	from Richmond 1990
Narrow wedge shell (*Paphies elongata*)	Yes		Ocean beaches, Marion bay, Tasman Peninsula, Stumpys Bay. Observations of numerous foraging birds	I.R. Taylor, this report	from Richmond 1990
Polychaete worms	Yes		Frequently obsevered at several sites along entire coast	I.R. Taylor, this report	
Amphipods (Crustacea)	Yes		From beach-cast kelp, Cresent Bay, Stumpys Bay. Observations of foraging birds.	I.R. Taylor, this report	

Diet of pied oystercatcher from preliminary fieldwork in this study

The preliminary field work done during the preparation of this report included a limited, initial assessment of the pied oystercatcher's diet at a number of sites on the east coast of Tasmania, especially at Anson's Bay, primarily to confirm or otherwise the significance of *Katelysia* as a prey item. Eight separate foraging adult birds were observed from a flock of 45 individuals at Anson's Bay in January 1995 and a total of 69 prey items were recorded. Of these, 42 (60.9%) were positively identified *as K. scalarina* (Table 11). The birds caught these visually, by wading slowly through water up to 90 mm deep. They made quick stabs into the water and if successful lifted the clams clear, holding them by their siphons. Presumably, the birds detected feeding clams by the activity of their siphons.

When the birds caught the clams in water deeper than about 30 mm, they walked quickly to shallower water, placed the clams onto the substrate without releasing their grip and removed the flesh with a scissor-like action of the bill. When the clams were lifted clear of the water the distinctive patterning and shape identifying them as *K. scalarina* could be seen clearly. Also, 11 freshly attacked shells were collected from the feeding area and their identity verified as *K. scalarina.* All of the clams taken by the birds in this way were within the length range of 20-40 mm, estimated by comparison with the birds' bill length and from measurement of collected shells. In addition to these positively identified clams, the birds were observed taking a total of 17 bivalves (24.6% of total observed prey items) that could not be identified. Six of these were white and less than about 20mm in length and were swallowed whole. In the remaining 11 cases the birds were wading in water about 30 mm deep and attacked bivalves without removing them from the water. They used exactly the same scissors action that was used when attacking the *K. scalarina* that were positively identified as such and the amount of flesh removed was similar. *K. scalarina* was the only bivalve of the appropriate size found during searches of the areas where these birds had been feeding so it is probable that this additional unidentified category of bivalves were also *K. scalarina.* If this were the case *K. scalarina* would have comprised 77% of the prey items taken at Anson's Bay during the observation period. The birds were also observed taking polychaete worms on three occasions (4.4% of prey items) and a total of seven items (10.1%) could not be identified even to the phylum level. These were all less than 20 mm and a clear view of them was not obtained during the observations either because they were swallowed too quickly or the birds turned their back to the observer during swallowing.

Observations were also made of feeding oystercatchers at Lauderdale Bay and Pipe Clay lagoon in the Derwent area. Identified prey items included the smooth toothed triangle *Anapella cycladea* and polychaete worms. The substrate in the areas where these birds were feeding was examined and no *K. scalarina* individuals were found, although this species was found in other parts of these bays.

Pied oystercatchers feeding along ocean beaches at Marion Bay (Tasman Peninsula) and in Stumpys Bay (Mt. William National Park) were observed to take crustaceans, especially amphipods around beach-cast kelp, polychaete worms and the narrow wedge shell *Paphies elongata.*

From these limited observations it is clear that the diet of pied oystercatchers in Tasmania varies considerably according to habitat, probably in relation to the range of suitable prey available. The observations at Anson's Bay indicate that clams are likely to be a highly important prey item in some and perhaps many sheltered bay habitats.

Table 11. The diet of pied oystercatchers recorded at Anson's Bay, January 1995. Data from observations of 8 adult individuals.

Prey Type	*Katelysia scalarina*			Unidentified bivalves		Polychaetes	Unidentified Type
Length	<20 mm	20-40 mm	> 40 mm	<20 mm	>20 mm		
Number (% of total items)	0	42 (60.9)	0	6(8.7)	11(15.9)	3(4.4)	7(10.1)

5.14.4.2 Sooty oystercatcher

There has been no study of the diet of the sooty oystercatcher in Tasmania. On mainland Australia there have been three detailed studies, in Victoria (Considine 1979) and in New South Wales (Parry 1977, Chafer 1994). All three concentrated on the diet of sooty oystercatchers feeding on rocky shore habitats with only very limited information from ocean beach habitats. No information was collected for the sheltered bay and estuarine habitats types in which sooty oystercatchers feed in Tasmania and in which *Katelysia* might be harvested. Thus these studies provide no data that might be directly applicable to the situation in Tasmania. Nevertheless, they do provide information on the general types of prey taken by sooty oystercatchers which is worth examining.

At all study sites molluscs were the most important prey. This included a wide range of gastropod species including *Cellana tramoserica, Nerita atramentosa, Patella spp., Turbo undulatus, Dicathias orbita* and a long list of others. Bivalves, including *Mytilus edulis, Trichomya hirsuta* and *Austromytilus rostratus,* were taken at all sites but in lesser quantities than the gastropods. The birds tackled the opening of these bivalves in much the same ways as do pied oystercatchers, by hammering and prising apart the valves. Thus they possess both the behavioural and morphological adaptations that would enable them to prey upon bivalves such as *Katelysia* in sheltered habitats. Other significant prey of sooty oystercatchers included crustaceans, especially barnacles and amphipods, sea urchins (echinoderms) and ascidians. On sandy beach habitats they were recorded to take amphipods (sand hoppers) and polychaete worms.

5.14.4.3 Pacific golden plover

No detailed study of the diet of the pacific golden plover has been undertaken in Australia and available information is anecdotal from casual observations and examination of the gut contents of a small number of dead individuals. Golden plovers forage by picking small prey items from the surface or by probing just below the surface of mud and sand flats and in some areas from pasture. They detect their prey mainly by visual means. Recorded prey in Australia include polychaete worms, gastropod molluscs, crustaceans, insects and arachnids (Thomson 1935, Domm and Recher 1973, Evans 1975, Barker and Vestjens 1989).

5.14.4.4 Red-capped Plover

Red-capped plovers forage mainly by sight, picking prey items from the surface of mud and sand flats. There has been only one study of their diet in Tasmania which involved the analysis of the gut contents of four individuals collected for the purpose (Thomas 1986). They were taken from the Hobart area but the exact locations and habitats were not recorded. Prey remains removed from the birds' guts were not identified to the species or genus level so the results were

somewhat superficial. Gastropod molluscs, crustaceans, insects and plant seeds were identified (Table 12).

There have been no detailed studies elsewhere in Australia and only anecdotal information is available which agrees generally with the results from Tasmania. Recorded items have included plant matter, gastropod molluscs, crustaceans and a wide range of adult and larval insects (Hall 1974, Poore et al. 1979, Lane and Jessop 1983, Barker and Vestjens 1989). The relative importance of these items is unknown as is any variation in relation to habitat or season.

Table 12. Prey items identified in the gut contents of four red-capped plovers (n = 4) collected in the Hobart area (Thomas 1986).

	Insects		**Crustaceans**			**Molluscs**		**Seeds**
	Adult	Larvae	Ostracods	Crabs	Isopods	Bivalves	Gastropods	
Number of guts	2	3	1	0	0	0	3	1

5.14.4.5 Double-banded Plover

Double- banded plovers forage mostly by sight, taking items from the surface of mud and sand flats but will also forage over closely cropped pasture and tilled land (Ambrose et al. 1990).

In Tasmania, there has been one study of their diet, involving the analysis of the gut contents of six individuals collected in the Hobart area (two from Clear Lagoon and four from Barilla Bay). The guts of four individuals contained the remains of adult insects and three contained insect larvae. The species were not determined. Four guts contained the remains of graspid crabs and one, the remains of isopods. Gastropods were found in two guts and plant seeds in one (Table 13., Thomas 1986).

Table 13. Prey items identified in the guts of six double-banded plovers (n= 6) collected in the Hobart area (Thomas 1986).

	Insects		**Crustaceans**		**Molluscs**	**Seeds**
	Adult	Larvae	Crabs	Isopods	Gastropods	
Number of guts	4	3	4	1	2	1

5.14.4.6 Curlew Sandpiper

There has been one study of the diet of curlew sandpipers in Tasmania which involved the analysis of the gut contents of 58 individuals collected in the Derwent/Pittwater area from September 1967 to April 1968 (Thomas and Dartnall 1971). Precise details of locations and habitats were not recorded but the birds were reported to have fed mainly on mud and muddy sand habitats in the areas where they were collected. The data were presented as the percentages of guts containing particular prey types which provides only a limited idea of the relative importance of different prey. The overall range of items was highly diverse including gastropod and bivalve molluscs, amphipods, polychaete worms, adult and larval insects and a variety of plant matter. Gastropods and insect larvae were the most frequently recorded items but it cannot necessarily be assumed from this that they were the most important prey. Prey items recorded in gut contents analysis may not be an accurate representation of the proprtions consumed because soft bodied items may be digested more rapidly and leave fewer trades. Thus items

such as polychaete worms may be seriously under-represented. Bivalve molluscs, which were not identified, occurred in 26% of the guts examined so must also have been significant prey (Table 14). Their sizes were reported to be within the range of 3-5 x 1-3 mm, and could have included the very young stages of several species.

The only other study of the diet of curlew sandpipers in Australia, conducted at Western Port, Victoria found polychaete worms to be important prey (Daan 1987).

Table 14. Prey items identified in the guts of curlew sandpipers (n = 58) collected in the Derwent/ Pittwater area (Thomas and Dartnall, 1971).

	Polychaetes	**Amphipods**	**Insects (diptera larvae and pupae)**	**Bivalves**	**Gastropods**	**Plant matter**
Percentage of guts	12	17.2	58.6	25.9	67.2	39.6

5.14.4.7 Red-necked stint

The diet of red-necked stints has been investigated in Tasmania by the examination of the gut contents of a sample of 59 individuals from the Derwent/Pittwater area (Thomas and Dartnall 1971). These birds were collected at the same time and in the same habitats as the curlew sandpipers reported above and the analytical methods and potential biases were the same as those described for the curlew sandpipers. The stints took the same range of prey types as did the curlew sandpipers, including bivalve and gastropod molluscs, amphipod and ostracod crustaceans, adult and larval diptera, polychaete worms and plant seeds (Table 15). The remains of amphipod and ostracod crustaceans were found more frequently in the stint guts than in the curlew sandpipers and molluscs were found less frequently. While it is probable that these represent real differences in diet, given the potential biases in collection and analysis, this cannot be taken to be the case with absolute confidence.

Table 15. Prey items identified in the guts of red-necked stints (n = 59) collected in the Derwent/ Pittwater area (Thomas and Dartnall, 1971).

	Polychaetes	**Amphipods**	**Ostracods**	**Insects (diptera larvae and pupae)**	**Bivalves**	**Gastropods**	**Plant matter**
% of guts	3.3	38.9	18.6	46.3	4.9	32	13.5

5.14.4.8 Foraging behaviour of wading birds in. Tasmania

In order to assess the degree to which *Katelysia* harvesting might affect populations of wading birds it is necessary to go beyond a simple evaluation of the diets of these birds. An understanding of the behavioural and energetic relationships between the birds and their food supply must be obtained so that the effects of *Katelysia* harvesting on their daily time and energy budgets can be assessed. The effects of *Katelysia* harvesting at different intensities on the density, size distribution and body condition (biomass and energy content) of *Katelysia* and other invertebrates that share the same environment and that might be preyed upon by the birds must be quantified. Relationships between the diets (species and size composition) of the birds and the range of prey available to them has to be quantified as must be the relationships between their daily time budgets and the rates at which they are able to obtain food (expressed

in terms of biomass and caloric value) and the densities and size distributions of potential prey available to them.

At present information of this kind is not available for any species of wader in Tasmanian environments nor have equivalent studies been undertaken in any other part of Australia for the species in question. Thus the above questions cannot be answered. On the basis of its known diet in Tasmania, the pied oystercatcher is the species most likely to be affected directly by clam harvesting. Although studies of its foraging behaviour of the kind detailed above have not been made in Australia extensive equivalent studies have been made of the closely related European pied oystercatcher. These provide valuable insights into what might occur in Tasmania and for this reason are reviewed in Chapter 5.14.6.

5.14.4.9 Conclusions

l. The current state of knowledge concerning the diets of wading birds in Tasmania is inadequate. At best only preliminary studies have been done usually involving small samples and at specific, restricted localities at particular times of year. Relevant studies have not been undertaken elsewhere in Australia. There is a need for detailed research in which the diets of all species are quantified over a representative range of sites selected across Tasmania but particularly in relation to clam harvesting areas. Such studies must sample tidal, diurnal and seasonal variations in diet and also variations in relation to the age or breeding status of the birds.

2. From the information that is available at present pied oystercatchers are most likely to be affected directly by *Katelysia* harvesting. The birds have been observed eating *Katelysia* at a number of sites and at Anson's Bay *Katelysia* was the most frequently taken prey. The birds took clams within the same size range as those harvested by humans. Sooty oystercatchers feed in some of the same areas as pied oystercatchers and and it is likely that they aslo include *Katelysia* in their diet and may thus also be directly affected by *Katelysia* harvesting.

3. It is unlikely that any other wader species take *Katelysia* within the same size range as those harvested. However, several species (e.g. curlew sandpiper) include small bivalve molluscs in their diets which could include immature *Katelysia.* More research is needed to clarify this.

4. Although most wader species other than the oystercatchers probably do not rely on *Katelysia* they depend on other invertebrate prey such as polychaete worms, other bivalve molluscs and gastropod molluscs and crustaceans the populations of which could be affected adversely in various ways by *Katelysia* harvesting.

5.14.5 POPULATION ECOLOGY OF WADING BIRDS IN TASMANIA

Clam harvesting in estuaries and lagoons may result in an impoverishment of these habitats as feeding areas for wading birds and, if this results in a significant reduction in the birds' abilities to obtain food, it is then likely that their population numbers would be adversely affected. Thus to assess fully the overall effect of clam harvesting it is necessary to have a thorough understanding of the population ecologies of the species concerned. Information is needed on productivity, survival rates, movements and population trends. Ideally, it would be desirable to be able to model the population dynamics of each species so that any effects on population size of changes to the values of these population parameters caused by clam harvesting can be predicted. Such modelling would be difficult to achieve for the migratory species that nest in arctic and subarctic Siberia and migrate south to spend the southern summer in Tasmania. Their

breeding success and the causes and extent of the mortality suffered along the migration route are inadequately known. Thus the effect, if any, of clam harvesting on their population ecology would be impossible to predict from existing knowledge and it is unlikely that the required information would become available in the near future. However, it is more important in the first instance to investigate the effects of clam harvesting on populations of resident species that have been shown in earlier sections of this report to rely more heavily on the habitats where clam harvesting might develop. The species that are most likely to be affected are the pied oystercatcher and the sooty oystercatcher and the following discussion focuses on these species, reviewing what is known of their population ecology in Tasmania and Australia generally, and in the case of the pied oystercatcher including information from the closely similar European pied oystercatcher.

5.14.5.1 Pied oystercatcher population dynamics

A long term population study of pied oystercatchers in the Derwent/Pittwater area was begun in 1977 by Dr. M. Newman, which will provide data on productivity, survival and dispersal. The study is ongoing and most of the analysis remains to be done. Nevertheless valuable preliminary results are available and most of the information summarised here is from that study.

Australian pied oystercatchers are strongly territorial during the breeding season and breeding pairs are highly faithful to their territories form year to year. At Mortimer Bay 14 out of 15 individually colour-banded breeding birds present in the 1989/90 breeding season occupied the same territories as in the 1988/89 season (Newman 1992).

The density of territorial breeding birds averaged 2.5 pairs per km of shoreline in the Derwent/Pittwater area in 1991/1992 (Newman and Park 1992). This area included estuarine and lagoon habitats as well as ocean beach habitats. On the Tasman Peninsula, which is mostly ocean beaches, density was 1.2 pairs per km in 1985 (Newman 1985), and in north-east Tasmania, again an ocean beach habitat, density was 1.3 pairs per km in 1994/95 (Taylor and Taylor, unpublished). Thus densities appear to be higher on the more productive estuarine areas.

Most laying of first clutches occurs during October and November and mean first clutch size in 1989/90 at Mortimer Bay was 1.9. Second clutches and in some instances third clutches were laid to replace lost first clutches and the mean clutch size for all clutches was 2.1. Hatching success was only 29.6% (all clutches) and only 7.4% of eggs laid resulted in fledged young, at an average of 0.25 young per pair (Newman 1992). Over the whole of the Derwent/Pittwater area during the 1991/92 breeding season only 21% of 110 breeding pairs were successful, producing on average only 0.27 fledged young per pair (Newman and Parks 1992).

In a separate study on Flinders Island, 27% of 67 pairs bred successfully but no details were given of the number of young produced (Lauro and Nol 1992). Known causes of failure in this and the above studies included predation, disturbance by humans and their livestock, erosion of sand-dune - nesting places and inundation by storm-driven, exceptionally high tides. The birds' habit of nesting in exposed locations on sandy beaches, close to the high tide level leaves them exceptionally vulnerable to the increasing human use of these areas for recreation. Increasing disturbance that causes the birds to leave the nest repeatedly and for long periods may increase the rate of desertion and also of predation from gulls and ravens. Chick mortality is increased through highly intrusive activities such as horse riding and the use of four-wheeled drive vehicles and also by the uncontrolled behaviour of domestic stock and dogs. Productivity of Tasmanian pied oystercatchers can be expected to decrease as pressure from these sources increases. Newman (1991) has suggested that productivity in the Derwent/Pittwater area, where these kinds of disturbance are now frequent, may already not be high enough to replace annual losses.

Dispersal distances form natal sites to breeding sites have not been published but most sightings of birds colour-banded in the Derwent/Pittwater area have been within 50 km of hatching sites (Newman 1982, 1984). Breeding adults tend not to change breeding areas between successive breeding seasons (Newman 1992).

The average age of first breeding has not been determined but most individuals in the Derwent/Pittwater area were at least 5 years old before they began to breed and many were known to be considerably older (Newman 1984, 1992, unpublished).

Details of annual survival rates have not yet been published but preliminary results suggest very high survival rates in all years of life. The average age of the breeding population at Mortimer Bay was over 9 years in 1989/90 with 6 out of 10 individuals known to be over 10 years of age. The oldest individuals were known to be at least 18 years old. This suggests an annual adult survival of around 90%. Newman (1991) has suggested that only about 20% of young reared (and colour-banded) in the Derwent/Pittwater area survive to reach breeding age, but survival rates for each separate year between fledging and breeding age have not been published.

The size of the breeding population in the Derwent/Pittwater area is probably limited by the territorial behaviour of breeding pairs. Flocks of non-breeding birds are present during the breeding season at several sites throughout the area (see chapter 5.14.3). These contain young birds that have not yet attained breeding age but also significant numbers of older individuals that presumably could breed given the opportunity. Newman (1992) has observed birds being recruited from the non-breeding flock to breed with territory owning birds following the loss of mates during the breeding season.

These population characteristics for the Tasmanian pied oystercatchers are similar in every respect to those described for the closely related European pied oystercatcher. European birds are also strongly territorial during the breeding season. Productivity was also low for all populations studied although not as low in most cases as in the Tasmanian population. Clutch sizes averaged 2.7 - 2.8 in most studies (Harris 1967, 1969, Heppleston 1972) and the percentage of eggs resulting in fledged young varied from 16 to 40.0% in four population studies (Dickenson 1932, Harris 1967, Greenhalgh 1969, Heppleston 1972). Birds breeding in estuarine and ocean beach habitats in eastern Scotland, similar to those in which Tasmanian birds breed, produced 0.56 fledged young per pair (n = 102 pairs, Heppleston 1972). In another study of birds breeding on a rocky shore area, a poor quality habitat for pied oystercatcher, only 0.16 fledged young were produced per pair (Safriel 1967).

European pied oystercatchers usually breed first when in their fourth year (Harris 1967). Survival rates for all age groups from the first year onwards have been estimated to be between 80.0% and 96.0% (Goss-Custard et al. 1982).

Adults are highly faithful to both breeding and wintering areas. Flocks of non-breeding birds which include adults of breeding age occur during the breeding season suggesting that the size of the breeding population is limited by the territorial behaviour of breeding pairs. This has been confirmed by experiments in which breeding adults removed from their territories were replaced by previously non-breeding birds (Harris 1970, Heppleston 1972).

5.14.5.2 Sooty oystercatcher population dynamics

There are no published accounts of any aspect of the population ecology of sooty oystercatchers in Tasmania or elsewhere in Australia. Productivity, survival rates and movements are unknown. A colour-banding study currently in progress in Victoria may in time provide information on some of these (Minton 1990).

5.14.5.3 Conclusions

1. Populations of pied oystercatchers in Tasmania are characterised by extremely low productivity and high annual survival rates. Movements are mostly local so that emigration and immigration are unlikely to be important in the species' population dynamics. Local populations probably depend mostly on their own productivity. It seems likely that natural productivity is now being lowered significantly especially in south east.

   Tasmanians populations, but perhaps also those in other areas, suffer from human disturbance in the breeding areas and generally increased predation levels from domestic and feral animals. Populations in the south east may already be approaching the point where productivity no longer replaces losses through mortality. Thus it is possible that pied oystercatcher populations throughout most of eastern Tasmania are particularly vulnerable to any increase in mortality rates.

2. The number of breeding pairs in populations of pied oystercatchers is probably limited by territorial behaviour and as a consequence there are at present flocks of non-breeding birds, including breeding-age adults, during the breeding season at many coastal lagoon and estuary sites. The existence of these birds, along with the very high annual survival rates of adults means that the effect of a small but nevertheless significant increase in mortality (i.e. reduction in survival) would not result in an immediate reduction in the size of the breeding population. There would be a lag period during which previously nonbreeding birds would be recruited to the breeding population and this would be followed by a slow decline. This decline would be difficult to detect with any degree of statistical certainty until the downward trend had continued for several years. This means that the monitoring of oystercatcher breeding populations on its own would not be an appropriate method to gauge any impact of clam harvesting on the birds. If there was an adverse effect it may be many years (perhaps even as much as 10 years) before it could be detected by which time further serious damage would undoubtedly occur before remedial action could be taken. Monitoring of the numbers of *non-breeding* adults in the population and also the rate of turnover of birds within the breeding population would be a much more sensitive indicator of any change. This could be achieved only with the use of individually colour-banded birds.

3. Pied oystercatcher populations have a very low natural productivity and as a consequence, a population that had become depleted would be very slow to recover. This stresses the need to avoid any damage before it is done rather than rely on recovery afterwards.

4. Having identified that pied oystercatcher populations may already be vulnerable, there is clearly a need for a much more rigorous quantification of all population parameters to determine how real this risk is. There is especially a need for a detailed analysis of the data already collected relating to survival in the Derwent/Pittwater population study, and there is a need for other similar studies to be conducted in clam harvesting areas.

5. At present we know nothing about the population dynamics of sooty oystercatchers in Tasmania, although it is likely that they are broadly similar to the pied oystercatcher in having low productivity and high survival rates. It is not possible at present to predict the effects on their population size of any changes in population parameters that might arise from clam harvesting or other forms of coastal development and there is a clear need for a large scale long-term population study of the species.

5.14.6 RELEVANT OVERSEAS CASE HISTORIES

5.14.6.1 European pied oystercatchers and edible cockle (Cerastoderma edule) populations

The species of wading birds that are most likely to be affected directly by the clam harvesting industry in Tasmania are the oystercatchers. Both pied and sooty oystercatchers could be affected significantly but it is likely that the pied could be more adversely affected as the main clam harvesting areas are also important feeding areas for the species throughout the year and it has been demonstrated in the preliminary studies that clams are significant prey for them.

An exactly parallel situation involving the European pied oystercatcher *Haematopus ostralegus* and harvesting of the cockle *Cerastoderma edule* in Britain and The Netherlands has been studied in great depth over some 30 years. The European pied oystercatcher is similar to the Australian species in morphology, habitat selection and feeding behaviour and the cockle *C. edule* is also ecologically very similar to *Katelysia scalarina.* It is therefore of considerable value to review these similarities in morphology and behaviour and to review the case history to identify aspects that help in the understanding of the Tasmanian situation and in the planning of future research into the problem.

There is also some relevant information for the closely similar New Zealand species of oystercatcher.

Morphological Comparisons fo Australian, European and New Zealand Pied Oystercatchers

Australian pied oystercatchers are morphologically similar to European pied oystercatchers. Their plumage characteristics are almost identical and their body proportions are similar. They have the same bill shapes (Cramp and Simmons 1983, Marchant and Higgins 1993). The only significant difference is that the Australian species has a considerably larger overall body size (Table 16). The New Zealand South Island pied oystercatcher *H. finschi* and variable oystercatcher *H. unicolor* also resemble the Australian and European species closely although their body sizes, especially that of *H. finschi,* are closer to that of the European species (Table 16). On the basis of their closely similar anatomical adaptations it is to be expected that all of these species of oystercatcher should feed in similar ways and on similar prey types.

Table 16. Comparison of weights and measurements of Australian, European and New Zealand South Island pied oystercatchers. Body mass data are for birds during the breeding season. 1) Data for Tasmanian birds. 2) Data for Dutch birds. Sources: Cramp and Simmons 1983, Marchant and Higgins 1993.

	Australian pied oystercatcher[1] *H. longirostris*	European pied oystercatcher[2] *H. ostralegus*	South Island pied oystercatcher *H. finschi*
Body mass (g) male	641.7 (6)	531.0 (107)	517.0 (52)
(Breeding season) female	710.0 (5)	(sexes combined)	554.0 (63)
Wing length (mm) male	277.0 (7)	254.0 (61)	257.0 (103)
female	279.3 (6)	255.0 (47)	261.0 (51)
Bill length (mm) male	67.0 (8)	69.6 (62)	80.9 (103)
female	73.9 (8)	78.4 (43)	90.0 (51)
Tarsus length (mm) male	55.1 (8)	50.1 (65)	49.9 (103)
female	56.4 (8)	51.5 (51)	51.0 (51)

Habitat Selection of European and New Zealand pied oystercatchers.

European pied oystercatchers occupy mainly coastal habitats and show seasonal changes in distribution. During the breeding season territorial pairs occupy a wide range of habitats including coastal beaches, bays and estuaries and in some areas also occur along inland rivers and lakes and on farmland. In winter there is a concentration into flocks which occupy mainly estuaries and sheltered bays but the birds are also able to feed in adjacent areas of farmland (Dare 1966, 1970, Heppleston 1971, Goss-Custard 1994). Thus their habitat selection and dependence in winter on sheltered bays is similar to that shown earlier (see Chapter 5.14.3) for the Australian pied oystercatcher in Tasmania, with the important exception that the Tasmanian birds do not forage on farmland and consequently are more dependent on the coastal habitats.

Of the two New Zealand species, *H. finschi* shows a marked preference for estuarine habitats with soft substrates whereas *H. unicolor* prefers more rocky areas (Baker 1974 a, b). The former is therefore ecologically equivalent to the European and Australian pied oystercatchers.

Diet of European and New Zealand pied oystercatchers.

Prey species. There have been eleven large scale intensive studies of the diet of the European oystercatcher in Britain and the Netherlands (Table 17) mostly undertaken in estuarine habitats where the predominant substrate types were medium-grained or finegrained sands. Some of these studies also examined the oystercatchers' diet when feeding on mussel beds growing on harder substrates. Thus the study situations included habitats that were similar to the sheltered bays and lagoons in Tasmania where Australian pied oystercatchers and the clam, *Katelysia* occur.

In all of these European studies the oystercatchers depended mostly on bivalve molluscs. On soft substrates the cockle *Cerastoderma edule* was the main prey in almost all areas. Other bivalves such as *Macoma balthica, Tellina tenuis* and *Scrobicularia plana* were usually subsidiary species as were polychaete worms such as *Nereis diversicolor* and occasionally crustaceans such as the crab *Carcinus maenas. C. edule is* a shallow burrowing, suspension feeding bivalve with relatively short siphons and is in many ways behaviourally and ecologically equivalent to *K. scalarina.*

Oystercatchers feeding on mussel beds consumed mainly the mussel *Mytilus edulis* and smaller quantities of other bivalve and gastropod molluscs and polychaetes.

The New Zealand South Island pied oystercatcher *H. finschi* and variable oystercatcher *H. unicolor* also both depend heavily upon bivalve molluscs including the pipi *Paphia australe,* the cockle *Chione stutchburyi,* the large wedge shell *Macomona liliana,* the ribbed venus shell *Protothaca crassicosta,* mussels *Mytilus* spp. and several others. Their diets also include a range of gastropod molluscs, polychaete worms and crustaceans. The South Island pied oystercatcher tends to specialise on the burrowing, suspension feeding bivalves, whereas the variable oystercatcher because of its preference for rocky areas is less dependent on them (Baker 1974a,b).

Table 17. The diet of the European oystercatcher, *Haematopus ostralegus,* at various study sites in Britain and the Netherlands. Throughout, the oystercatchers depend mainly upon bivalve molluscs, especially the cockle, *Cerastoderma edule,* and the mussel, *Mytilus edulis.*

Study Area	Habitat Type	***Main Prey Species***	***Subsidiary Prey***	Source
Ythan Estuary, Scotland	sheltered estuary sand/mud flats	*Macoma balthica*	*Mytilus edulis,* polychaetes	Heppleston 1971
	mussel beds	*Mytilus edulis*	*Littorina spp.*	
Strangford Lough. N. Ireland	sheltered bay sandflats	*Cerastoderma edule*	polychaetes, *M. edulis*	O'Connor and Brown 1977
Morecombe Bay, England	sheltered bay sandflats	*C. edule*	*M. balthica, Tellina tennuis,* polychaetes, *Carcinus spp.*	Dinnan 1957, Dare and Mercer 1973
	mussel beds	*M. edulis*	*C. edule, M balthica, T. tennuis*	Dare and Mercer 1973
Anglesey, Wales	sheltered bay sandflats	*C. edule*	polychaetes, *Scrobicularia plana*	Sutherland 1982b
Burry Inlet, Wales	sheltered estuary sandflats	*C. edule*		Davidson 1967
Ribble Estuary, England	sheltered estuary sandflats	*C. edule*	*S. plana, M. balthica,* polychaetes	Greenhalgh 1975
The Wash, England	extensive bay sand flats mussel beds	*C. edule M. edulis*	*M. balthica*	Goss-Custard et al. 1977.
Exe Estuary, England	sheltered estuary sand/mud flats	*S. plana, polychaetes*	*C. edule, Carcinus maenas*	Goss-Custard and Durrell 1983
	mussel beds	*M. edulis*	polychaetes, *Littorina spp., S. plana*	Goss-Custard and Durrell 1983
Wadden Sea, Holland	extensive bay sand flats	*C. edule M. edulis*	*M. balthica,* polychaetes	Hulscher 1982, 1983

Specialist Feeders. Several studies have shown that individual European oystercatchers observed over short periods tend to concentrate their predation on particular prey types or that shot samples tend to have mainly one prey type in their guts (Heppleston 1971, Dare and Mercer 1973, Dare 1977, Sutherland 1982b). This suggests that some or most individuals in these oystercatcher populations tend to specialise on particular prey but given that *C. edule* and *M. edulis* are by far the dominant prey in most situations, most individuals obviously concentrate on these species. The existence of specialists has been confirmed in an extensive study of colour-banded individuals on the Exe Estuary, Devon, England (Goss-Custard and Durell 1983, Boates and Goss-Custard 1993). Some adults were found to specialise on particular prey and it was also shown that there was a change in feeding behaviour with age. Juveniles initially did not consume the larger bivalves such as *C. edule* but relied upon smaller bivalves such as *Scrobicularia plana* and polychaetes when feeding on the estuary. They also fed more in adjacent agricultural land than did the adults where they took earthworms (Lumbricidae) and leatherjackets (Tipulidae). Feeding on the larger bivalves developed gradually as they aged up to their fourth year. The birds were shown to take several years to become as proficient as adults in handling and eating mussels (Norton-Griffiths 1968). These findings are important as they show that the juveniles in the population relied upon a different prey spectrum from the adults.

It is likely that oystercatchers in Tasmania will behave similarly to these European birds and that any effects of *Katelysia* harvesting on other components of the benthic infauna such as polychaetes could have a severely adverse effect on the foraging efficiency and survival of juvenile oystercatchers.

Foraging Methods European oystercatchers use a variety of specialist and nonspecialist feeding techniques depending upon prey type, habitat and time of day. There are also differences in relation to the birds' ages, discussed above. When feeding in coastal habitats the birds search for prey both by wading in shallow water and by walking slowly over exposed substrates. Prey are detected by sight and also by touch. The latter is made possible by the presence of Herbst's corpuscles in the distal 15 mm of the bill (Safriel 1967, Bolze 1968, Norton-Griffiths 1968). Thus they are able to search for prey during darkness as well as during daylight (Greenhalgh 1975, Hulscher 1976), although they tend to forage more on moonlit nights suggesting that visual foraging may also be used at night (Heppleston 1971b). When feeding by sight the birds probably are able to detect feeding bivalves from the action of their siphons and usually catch their prey by seizing the siphons. When foraging by touch they make repeated and rapid probes into the sediment with the tips of their bills and remove bivalves by grasping the entire shell.

When feeding on bivalves European oystercatchers employ two basic techniques to gain access to the shell and extract the flesh; hammering and stabbing. Hammering consists of breaking one valve by a series of short thrusting blows then inserting the bill and cutting the adductor muscles. This method is usually employed to open shells that are closed at the time of capture. Stabbing consists of inserting the bill between the gaping valves of feeding or moribund bivalves and then severing the adductor muscles. The flesh is pulled free once the muscles are cut (Norton-Griffiths *1967)*. Stabbing is most often used for bivalves caught when the birds forage by wading whereas hammering is usually used for shells dug from exposed, dry substrates at low tide or removed form mussel beds (Drinnan 1957, Hulscher 1964). Small shells less than 10-20 mm may be swallowed whole (Norton-Griffiths 1968, Evans 1975). Some oystercatchers obtain part of their food by stealing from conspecifics (Ens and Goss-Custard 1984).

The New Zealand oystercatchers use the same prey location and prey handling techniques as the European species (Baker 1974a,b).

Preferences for Prey Species and Lengths. Most studies of European oystercatchers feeding on soft substrates equivalent in type to those of the *Katelysia* harvesting areas in Tasmania have shown that the cockle C. *edule* is consistently by far the dominant prey of adult birds, taken more frequently than their relative availablity in comparison with other bivalve species. Other bivalves and polychaetes are taken in smaller quantities and there is evidence that these are not preferred prey. For example, in the Waddensee, The Netherlands, the birds foraged mainly on *C. edule* in most years but in 1988, 1989 and 1990 there was a failure in recruitment of the cockles while human harvesting of them continued. The abundance of cockles fell drastically to very low levels and the oystercatchers switched their predation to *Macoma balthica* and juvenile *Mya arenaria,* the populations of which were severely reduced as a consequence (Beukema 1993). By early 1991 the oystercatchers feeding on these bivalves were in poor condition and high numbers were found dead (Camphuysen 1993). These observations suggest that *C. edule* was the preferred prey for energetic reasons and that the oystercatchers were unable to survive on the alternative prey species. The oystercatcher is a relatively large wader and on energetic grounds would be expected to select relatively large prey species.

Oystercatchers in Europe show a strong selection for size within populations of their preferred prey and this selection behaviour can be explained on the basis of their energy requirements and the relative profitability of prey of different size classes. With the cockle *C. edule* the birds show a strong preference for individuals between 20 and 40 mm in length (Goss-Custard, Jones and Newbery 1977, O'Connor and Brown 1977, Hulscher 1982). Their preference in this length range was mainly for second year cockles and they avoided first year individuals with lengths of 15 mm and less (Horwood and Goss-Custard 1977). Young *Macoma* less than 10 mm were also rejected and individuals in the length range of 10 - 20 mm were preferred (Goss-Custard, Jones and Newbery 1977, Hulscher 1982). In the Waddensee oystercatchers preferred *Mya arenaria* individuals in the range of 15 - 40 mm and rejected those less than 15 mm (Zwartz and Wanink 1984).

When feeding on mussels oystercatchers also showed significant selection for size which varied seasonally and also according to the techniques used to open the shells. Most of the selection behaviour was explicable in terms of the profitability of different size classes in relation to the energetic and time costs involved in handling them (Cayford and GossCustard 1990).

These results are of immediate relevance to the exploitation of *Katelysia* populations in Tasmania. It is to be expected that the oystercatchers would select *Katelysia* within a similar size range to that selected from the European cockle populations, i.e. from about 20 to 40mm, and this is supported form the initial observations made at Anson's Bay (see Chapter 5.14.4). Thus this includes the main length range of *Katelysia* currently harvested in Tasmania. It is unlikely on energetic considerations that adult oystercatchers would be able to switch to alternative prey of lower body mass without incurring a significant reduction in their foraging efficiency. Switching to alternative prey would also probably result in the serious depletion of those prey perhaps with further consequences in the estuarine ecosystem.

Daily Food Consumption. The daily food requirements of pied oystercatchers in Europe have been estimated by two methods; from feeding trials of captive birds and from observations of wild birds. There are difficulties with both approaches. The first involves inactive individuals and extrapolations have to be made to give estimates for wild birds which involves making a number of assumptions. The second approach involves the quantification of prey capture rates and the reward per capture and multiplying by the amount of time spent feeding. However, the birds also feed at night when these assessments are difficult to make so assumptions again have

to be made. Energy needs depend upon ambient temperatures, the body mass of individual birds and their activity at the time. Extrapolations are therefore needed to cater for these variations.

From field observations of wild birds in the Burry Inlet, Wales, Davidson (1967) estimated an average consumption during winter equivalent to 363 second year cockles *C. edule* per 24 hours. O'Connor and Brown (1977) estimated an intake of 827 cockles per 24 hours at Strangford Lough, N. Ireland. In this they assumed that the birds fed at night at the same rates as during the day but Greenhalgh (1975) has shown that they feed at much slower rates at night. This estimate is therefore too high. From a combination of field observations of birds eating mussels and data from a captive individual which was fed mussels, Heppleston (1971) estimated an intake of 480 mussels per 24 hours in October and 521 in December. The October figure was equivalent to 354 kcal/24 hours. For oystercatchers in the Waddensee, Netherlands, Hulscher (1982) estimated a daily requirement per individual in winter (November to April) of 55 g AFDW (ash-free dry weight) and 40 g AFDW for the rest of the year. The AFDW per individual bivalve of standardised length varies according to season, position on shore and in relation to density (Sutherland 1982a). For example the AFDW of individual mussels, *M edulis, of* 45 mm length was 629 mg in an area of low density (<50 adult mussels per m^2) but only 484 mg in an area of high density (>500 adult mussels per m^2, Goss-Custard, West and Durell 1993). Thus daily requirements expressed as numbers *of* bivalves will vary considerably according to circumstances. Nevertheless, it is clear that individual oystercatchers need to consume large numbers *of* bivalves each day.

Relationship between food intake rates and prey density. Relationships between the rates at which oystercatchers caught and consumed cockles *C. edule* and the densities of cockles available have been studied in Wales (Sutherland 1982b) and on the Wash, Eastern England (Goss-Custard 1977). In the Welsh study the birds' prey capture rates increased from about 2 cockles per minute at cockle densities of 20 per m^2 to around 8 per minute at densities of 250 per m^2, but then showed little change over densities up to 600 per m^2. However, the size and body mass of the cockles was related to their density so that the profitability of the birds' feeding, expressed as AFDW consumed per minute was greatest at densities of between 25 and 150 cockles per m^2.
On the Wash the relationship between the numbers of cockles eaten per minute and cockle densities was almost the same as that found in Wales but as density increased the birds selected larger individuals so that their ingestion rate or profitability (biomass/min) continued to increase over a wider range of densities than in Wales.

It is clear that the birds' feeding rates and ingestion rates are affected by the density of their prey but that relationships are complex and variable. Thus changes to the density of bivalves such as *Katelysia* through harvesting or enhancement can be expected to affect the birds' feeding rates although the exact nature of the relationship cannot be predicted from existing information.

Relationships between the density of feeding birds and the density of prey. Several studies have shown that European oystercatchers tend to concentrate their feeding activity and hence to occur at the highest densities in areas that have high densities of cockles (e.g. Goss-Custard 1977, O'Connor and Brown 1977). At the Burry Inlet, Wales highest densities of birds occurred in the areas where their foraging was most profitable, at intermediate cockle densities (Sutherland 1982b). In this study area the main predation by oystercatchers occurred overwinter from October to March and cockle densities declined progressively overwinter as a result of this predation and from the harvesting by humans. The densities of cockles recorded in November varied greatly over the study area but by the following May there was little spatial variation in

density. This suggests the existence of a lower threshold of cockle density to which all parts of the population had become reduced and below which it was not energetically profitable for the birds to feed (Horwood and Goss-Custard 1977).

Similar principles will almost certainly apply to interactions between Australian pied oystercatchers and *Katelysia* populations. Increasing the densities of clams available to the birds by the enhancement of natural stocks may result in the birds congregating in those areas. The harvesting of natural clam populations, if not carefully regulated, has the potential to lower their densities to levels below those at which the birds are able to feed profitably. This may force the birds to leave the area. The precise numerical relationships will differ from those in the European oystercatcher/cockle interactions and would have to be quantified for the Tasmania situation.

Relationships between European oystercatcher populations and the cockle C. edule harvesting industry. In most areas of Europe, oystercatchers consume second year cockles preferentially although they also take lesser quantities of other age classes. The heaviest predation from the oystercatchers occurs overwinter, during the cockles' second winter, when the largest numbers of oystercatchers concentrate in coastal bays and estuaries. The cockles become sexually mature during their second winter and spawn mostly in May. Commercial harvesting concentrates mostly on third year cockles although significant numbers of second year individuals are also taken, especially when the size of the third year age group is low (Davidson 1967, Hancock 1971, 1973). Very few cockles in these harvested populations survive beyond three or four years of age whereas in nonharvested populations individuals up to ten years old are found (Cole 1956, Hancock and Urquart 1965, Brown and O'Connor 1974).

Hancock (1971,1973) concluded that there could be direct competition between oystercatchers and commercial harvesting operations and that in certain circumstamces harvesting would reduce the availability of cockles to oystercatchers and *vice verca.* Evidence for direct competition under particular cicumstances was also obtained in the Netherlands (Buekema 1993, Camphuysen 1993). Problems have arisen particularly when fixed quota harvesting levels have been pursued regardless of variations in recruitment rates.

5.14.6.2 Conclusions

1. European pied oystercatchers are morphologically and behaviourally similar to Australian pied oystercatchers. They occur in the same habitat types and feed in the same ways on the same types of prey. It is therefore highly probable that general principles concerning relationships between the European birds and their food supply that have been elucidated in several detailed studies will be applicable to the Tasmanian birds.

2. European oystercatchers are highly specialised in their predation showing distinct preferences for particular bivalve species within particular size ranges. They have highly developed feeding behaviour, to process these prey, which takes several years to learn. Their prey preferences can be explained in terms of the profitability of their foraging. The size range of *Katelysia* taken at Anson's Bay by Australian pied oystercatchers is similar to the preferred size range of bivalves taken by European oystercatchers. Thus it is likely that any change in the size range of *Katelysia* available to the birds caused by harvesting could reduce the profitability of their feeding.

3. Studies of European oystercatchers have shown complex relationships between the density of bivalves available to them and their rate of food intake. Relationships were not linear,

so that some reduction in bivalve densities could take place without reducing the birds' food intake rates. However, below particular critical densities the birds' intake rates were reduced below maintainance levels.

4. Positive relationships have been demonstrated for European oystercatchers between densities of bivalves and densities of foraging birds. Birds concentrate in areas of high bivalve density and conversely are forced to abandon areas where bivalves are reduced below particular threshold levels. It is probable that this will also apply to Australian pied oystercatchers feeding on *Katelysia.* Overharvesting may render areas unsuitable for the birds. Enhancement of natural populations of clams by restocking may result in large numbers of birds concentrating in the areas concerned. Given the very large numbers of bivalves that can be consumed daily by the birds, this may result in a serious conflict between the interests of clam harvesting and conservation of the birds.

5.14.7 ENVIRONMENTAL IMPACT OF KATELYSIA HARVESTING – A STATEMENT OF RESEARCH NEEDS AND DETAILED RESEARCH PROPOSALS

5.14.7.1 Research Needs

Effects of *Katelysia* harvesting on wading bird populations

Harvesting from natural populations of *Katelysia* has the potential to bring about significant changes in the density and size distribution of *Katelysia* available to wading birds and through the effects of sediment disturbance, to alter the availability of other invertebrates that may be important prey species for the birds. Thus the result could be a wide reaching decrease in overall prey availability. It is also possible, should any of the wader species prove to be significant predators of *Katelysia,* that they could come to be regarded as constraints on the harvesting procedure and attempts may be made to limit their access to the harvested areas which will have the effect of reducing the extent of suitable foraging areas for them. This is especially likely to occur should there be a significant development in the practice of enhancing natural clam populations with hatchery-grown individuals. Harvesting operations may also disturb wading birds away from the harvested areas, again having the effect of reducing the extent of foraging areas available to them. As a consequence of these effects the birds may be forced to feed in areas of reduced or lower food abundances or at higher than normal densities in areas that are unaffected by clam harvesting. The short-term result of these changes may be to reduce the birds' foraging efficiency resulting in a lowering of their daily food intake. The long-term effects of reduced food intake levels may be reduced survival, reduced breeding performance or in the case of migratory species, reduced migratory ability. All could result in declining population sizes.

Our current state of knowledge and understanding of the issues described above are totally inadequate to provide answers to any of them. However, the information that is available suggests strongly that pied oystercatchers and probably also sooty oystercatchers, both of which are primarily mollusc predators, may rely heavily or partly on *Katelysia* for food. Both pied and sooty oystercatchers are nationally uncommon species with populations in the order of 4,000 and 1,500 breeding pairs respectively. Almost all of the current and potential clam harvesting sites fall within areas that are of international significance for the conservation of wading birds as defined by the criteria set out in the Ramsar Convention to which Australia is a signatory and in most cases the important shorebird species in these areas are the oystercatchers (see chapter 5.14.3).

Thus there is a fully justified and urgent need for research into the relationships between clam harvesting and wading bird populations, particularly concentrating on the oystercatchers.

5.14.7.2 Research Plans

Effects of *Katelysia* Harvesting on pied and sooty oystercatchers.

Feeding Behaviour Studies

A. Assessment of the birds' diets

Objectives. The aims of this part of the research programme should be to quantify the importance of *Katelysia* in the diets of both species of oystercatcher and to identify and quantify all other prey types in their diets. The study must concentrate on the habitat types in which *Katelysia* populations are known to occur and must include areas that have been or are being harvested as well as areas that are not. The study must explore the subject fully so must specifically sample a range of sites to determine the extent of variation among sites. It must investigate the possibility of seasonal, tidal or diurnal variations, differences in relation to the age of the birds and the possibility of specialists within the bird populations.

Methods:

1. ***Selection of study sites.*** Study sites should be selected to sample the range of areas in which *Katelysia* harvesting is or could be developed. Probably five sites is the maximum that could be studied in an initial project although this number could profitably be increased if resources were available. It would prove useful to select three sites that are likely to be ongoing harvesting sites and two sites that contain harvestable *Katelysia* populations but that have not and will not be harvested during the research period. This will enable aspects of the research to be done in these latter areas free from any possible constraints of harvesting operations and would also allow direct comparisons of harvested and non-harvested areas. It would prove valuable to co-ordinate studies of the bird populations with those of the *Katelysia* populations, discussed later, so at least two of the bird study areas should be the same as the *Katelysia* study areas. An initial list of suitable study sites could include East or West Inlets at Stanley, Great Musselrbe Bay, Anson's Bay, George's Bay, Little Swanport, Moulting Lagoon and Cloudy Bay Lagoon, Bruny Island.

2. ***Seasonal variation in diet among sites.*** Comparisons of the birds' diets over the selected range of sites should be continued over a period of at least 24 months. The study should be divided into two-month sample periods so that there are six such sample periods in each calendar year. Data from each site should be collected during each sample period. At least two full days of fieldwork should be undertaken at each site for each bi-monthly sample period.

3. ***Tidal variation in diet.*** At each bi-monthly sample period, observations should be timed to cover entire tidal cycles so that any tidal variations in diet are identified and quantified. Samples of diet should be obtained for each hour of the tidal cycle during which the birds are observed to feed. It is unlikely that the birds would be able to feed over high tide so these observation periods would probably cover 6 - 8 hours.

4. ***Age-related differences in diet.*** Feeding behaviour, including prey species selection might change as the birds age. During observations of feeding birds a record should be made of the birds' ages whenever possible. In a long-term study this could be done by having populations of individually colour-banded birds. Such a population already exists in the Derwent/Pittwater area but not in any of the main *Katelysia* harvesting areas. In an initial

study age comparisons could be restricted to first year birds and birds older that first year. First year birds can be distinguished on plumage characteristics.

5. ***Diurnal changes in diet.*** The birds will probably feed at night as well as during the day and may select different prey. Conventional observation methods will not be possible at night. Attempts should be made to hire night-viewing equipment (image intensifier) to test for such possible differences.

6. ***Observation and recording procedures.*** Preliminary studies conducted at Anson's Bay suggest that the major items in the birds' diets can be identified by the direct observation of feeding birds at close range using a telescope. This method should therefore be employed initially and other approaches considered only if it proves to be inadequate. Observer skill improves with practice and the researcher(s) should undertake an initial training period in which potential prey items taken from the substrate are identified at appropriate distances. These may be attached to the bills of mounted or model oystercatchers to simulate the real situation. A series of doubleblind tests should be conducted to test the accuracy of the observer(s) before the direct observations of wild birds are begun.

   At each observation period an individual bird from a known age class (see below) should be selected as randomly as possible from the population. The individual's precise location should be recorded on a map of the study area so that any spatial variations that might exist within study sites can be examined. It should then be followed until it has been observed to eat 10 to 20 prey items. The identity of all food items should be recorded when possible and any that cannot be identified should be recorded in a separate "unidentified" category. The lengths of each prey item should be recorded as precisely as possible by reference to the length of the bird's bill. Tests should be done of observer accuracy in estimating the size of prey items (see Goss-Custard et al. 1977). When the series of such observations has been completed on one individual another individual should be selected and so on until a high percentage (say at least 20%) of the total population of birds feeding in the area has been sampled. This is to ensure that the data obtained on diet is representative of the population and not just of a few individuals.

   Proper attention must be paid to total sample sizes of prey items to ensure that they are adequate for appropriate statistical testing of any observed variations in diet. Any shortcomings in this detected from analysis of the first 12 months work should be made good in the subsequent 12-month study period.

B. Assessment of Daily Activity Budgets, Feeding Rates and Daily Food Intake Levels

Objectives. *A* comparison is needed of the birds' daily food consumption and of the effort and time expended to obtain it between natural, non-disturbed situations and areas that are subject to *Katelysia* harvesting. The same set of information is also needed to calculate the numbers and biomass *of Katelysia* removed by oystercatcher populations from harvested and non-harvested *Katelysia* populations. When used in conjunction with data on the densities and productivity of the *Katelysia* populations, this will enable calculations to be made of the percentage of both standing crop and productivity removed by the birds over particular periods of time. This information can then be used to asses the likliehood and extent of competition between the birds and the harvesters.

The birds' food requirements may vary seasonally according to their activity and according to environmental conditions. Major differences are likely to occur in relation to temperature, especially comparing winter and summer and in relation to the birds' breeding season.

The number of prey items consumed per day can be calculated by multiplying the amount of time spent foraging per day (daily activity budget) by the rate at which prey items are caught. The amount of each prey species can be calculated from this using the data collected above on diet composition (see above). Daily energy consumption can be calculated by multiplying the number of items of each species eaten by the calorific value of each item. To calculate the total numbers of *Katelysia* removed by entire populations of the birds, daily consumption by individuals must be multiplied by the total number of birds in each particular area (see 4, below).

The birds' activity budgets and prey capture rates may vary according to tide, time of day and season. All of these potential variations must be quantified.

Methods:

1. ***Simultaneous recording of data on activity budgets and prey capture rates.*** It will be possible to record both activity budgets and prey capture rates during the same observation sessions. Both should be quantified in two-monthly sample periods over 24 months. Observation periods should be organised so that possible variations in relation to tide, time of day and season are investigated.

2. ***Quantifying daily activity budgets.*** Select observation positions at each study site from which a high percentage or preferably all of the bird population can be seen simultaneously. At each study site, activity budgets should be sampled to coincide with the two-monthly sampling programme for diet determination (see above). Activity budgets should be quantified at least once per month so that data are replicated within each twomonthly sample period. Data should be recorded over a full low-tide cycle to cover the entire period for which the birds feed. Subdivide the tidal cycle into one-hour sample periods before and after low tide. At 20-minute intervals during each tide-hour, scan the study area and record the number of birds feeding and the number not feeding (the "not feeding" category could be further subdivided into preening, sleeping, resting etc. if a more detailed analysis of activity is preferred). Attempt to subdivide the data according to the birds' ages (first year birds and those older than one year) so that any differences in activity budgets in relation to age can be detected and quantified. It is possible that there might also be differences in relation to the sex of the birds, so if possible a record should be kept of their sexes (determined from bill proportions). However, this should not be regarded as a priority at this stage unless initial observations suggest large differences at particular times of year. Compute average values of the three 20-minute samples in each hour to give the average percentage of the population feeding each hour and use the same procedure to give equivalent values for entire tidal cycles. Assuming that all individuals of the same age category tend to spend about the same amount of time feeding, these figures will be equivalent to the amount of time spent feeding by the average individual. Calculate such values separately for first year birds and older than first year birds.

   It is possible that the birds may feed differently depending upon the time of low tide during daylight hours and also upon the spring/neap tide cycle. Their behaviour may also be different during darkness. At the start of the study a period of intensive sampling should be undertaken to determine the extent, if any, of such differences and subsequent sampling procedures should be decided from the results of this. The end result should be a calculation of the average amount of time spent foraging during a 24 hour period for each of the two-monthly sample periods throughout the year.

3. ***Quantifying prey capture rates:*** The same sampling periods and sessions should be used as those used for quantifying activity budgets, so that the two sets of data can be combined. Sampling of prey capture rates can be done between scans of the population to determine activity budgets.

   Individual birds should be selected at random, their ages (first year or older) recorded and if possible their sex, and they should be followed for one-minute sample periods during which the number of prey items caught and consumed should be recorded. It may also be possible to record the identity of each prey item during these observations and, if this proves possible, the data obtained can be added to the data set for diet (see above). This may remove the need to have separate observation periods for recording diet. Also, if possible, the number of steps taken by the birds could be recorded to give a measure of the effort expended during foraging in addition to the amount of time used. As far as possible a new individual should be selected after each one-minute sample period to ensure that as many birds as possible in the population are sampled.

   The possibility of variations in prey capture rates in relation to time of day and the spring/neap tide cycle should be investigated in the same way as is done for daily activity budgets so that the number of prey items consumed by the average bird per 24 hours can be calculated.

4. ***Quantifying the total number of birds in the study area.*** The total number of birds using the entire intertidal area for each of the study sites should be assessed for two complete low tide periods each month. The pattern of behaviour of the birds might vary according to local circumstances and preliminary intensive studies at each site could improve the efficiency of the methodology adopted. At some sites, especially those with no other potential foraging areas close-by, the whole population of oystercatchers may remain at the feeding area throughout the low tide period. It is unlikely that they will forage non stop for the entire time but non foraging birds will probably rest in small groups on the flats close to the feeding areas rather than return to the high tide roost site. Where the birds behave in this way a small number of counts of their total numbers each low tide will be adequate. However there may be more complex systems where the birds are able to move more widely over more extensive intertidal areas so that their distribution varies according to the state of tide. In these situations the numbers of birds feeding in the specific areas under investigation should be assessed each hour of low tide. Preferably three counts of the area should be made each hour and mean values taken for each hour.

C. Seasonal Changes in Body Mass and Energy Content of Prey Species

The body condition of *Katelysia* has been shown to vary seasonally (see chapter 5.14.2) and it is likely that the condition and energy content of other prey species will also vary seasonally. Thus the number of prey items consumed by the birds to obtain the same energy intake will probably also vary and it will therefore be important to quantify the seasonal changes in condition (body mass per length) and energy content per gram of dry tissue of the bird's main prey species.

Methods:

1. Select at least two study sites, one in which *Katelysia* are harvested and one in which they are not. These study areas should be the same as two of the areas selected for the bird foraging behaviour studies.

2. Conduct a preliminary survey at each study site to determine if there are any variations in body condition in relation to position on the shore. At each site, the beach should be

subdivided into zones with respect to the position of low tide. The width of each zone will have to be decided according to the characteristics of each site individually. Within each zone individuals of all major prey species, including *Katelysia* should be sampled. For each individual animal obtain a measure of length and its oven-dry body mass. Using analysis of variance, test the data set for significant variation body condition (body length/dry body mass) in relation to zone up the beach profile.

3. Sample populations of major prey species, including *Katelysia,* at each study site at two-monthly intervals. For each sample period determine dry body mass/body length relationships for each species. Use the results of 2 immediately above to determine if the sampling needs to be stratified according to location on the beach profile. Also use the data set from 2 in order to determine appropriate sample sizes. These should be adequate to define 95% confidence intervals within 20% of mean values. Using the oven-dry samples of each species determine the calorific content per gram dry weight at two monthly intervals.

4. It would prove valuable to test for differences in body condition among prey populations, especially *Katelysia* and other bivalve populations, more widely over a range of bays and lagoons so that the general applicability of the results from the intensive study areas is known. Once the range of variation in relation to position up the shore and season is understood from the intensive study sites, samples should be taken from comparable situations in a wider range of bays.

D. Density Dependent Relationships in Feeding Behaviour

Objectives. One of the main effects of harvesting *Katelysia* populations may be to alter the density relationships between the birds and their prey and thus it is important to understand the nature and extent of these relationships. In the studies of European oystercatchers feeding on the cockle *Cerastoderma edule* (chapter 5.14.6), it was shown that the birds fed at higher densities in areas of high cockle densities. This suggests that if *Katelysia* densities in Tasmania were increased locally by the enhancement of natural stocks, the oystercatchers would respond by increasing their densities in these areas leading to an increase in their predation levels on the clams. In the European study the prey capture rates of individual birds were not linearly related to cockle density but were asymptotic. This suggests that a limited reduction in the density of *Katelysia* by the harvesting of natural populations could possibly be done without a consequent reduction in the oystercatchers' prey capture rates.

Deliberate attempts to exclude oystercatchers from harvested areas or accidental exclusion through disturbance may cause the birds to concentrate in other areas at densities above those that would occur naturally.

Thus there are two main types of question to be answered in relation to density.

1. How do populations of the birds respond to variations in the density of their prey, including *Katelysia?* Is the natural density of the birds related to the density of their prey and would artificial increases in *Katelysia* density result in increases in the densities of the birds? Do the birds' prey capture rates vary in relation to the density of their prey?

2. How do populations of the birds respond to artificial, enforced increases in their own densities? Are increased densities sustained or are densities limited by the birds' behaviour such that some individuals eventually become excluded from the feeding area? If increased densities are sustained is there a negative effect on the birds' prey capture rates and daily food intake rates?

Methods:

These questions are technically difficult and time consuming to answer convincingly in the field. Nevertheless answers to them would be extremely valuable in predicting the possible consequences of *Katelysia* harvesting to both bird populations and *Katelysia* populations. In theory they could be tackled experimentally by altering the size of feeding areas available to the birds or by altering the densities of *Katelysia* populations but both of these approaches would be extremely expensive to set up. A more realist approach would entail an initial set of observations to determine if natural variations in *Katelysia* density were associated with variations in oystercatcher density and prey capture rates and if short term natural increases in bird density were associated with increased levels of aggression or decreased prey capture rates.

Assessment of Oystercatcher numbers at *Katelysia* harvesting sites and throughout Tasmania.

Objectives: At present, detailed up to date information concerning the numbers of pied and sooty oystercatchers is available only for a limited number of sites around the coast of Tasmania. For many of the actual or potential *Katelysia* harvesting sites there is either inadequate or limited recent information and for that reason it is not possible to assess fully the importance of these areas to the oystercatcher populations. A much more up to date and more extensive series of population counts is needed for the areas in question. Data for sites in the Derwent/Pittwater area show clearly that the numbers of birds using the sheltered bay/ lagoon habitat type varies seasonally, with substantially more birds in winter than summer. Thus separate counts will have to be done for at least these two periods of the year.

More detailed information is also needed for the distribution of the birds in each bay to test if their distribution in all cases overlaps with that of the potential *Katelysia* harvesting areas.

Methods:

1. Identify all sites that may be potential *Katelysia* harvesting areas.

2. At two-monthly intervals over a period of at least 24 months visit each site at low tide and count all of the birds present, including both those feeding at the time and those not feeding. One count should coincide with the peak of the birds' breeding season, preferably in November. At this count as well as those in September and January an attempt should be made to distinguish between locally breeding territorial pairs and non-breeding flock birds. Where possible in the January and March counts an attempt should be made to count the number of first year birds as a measure of the productivity of the local population.

3. For each site visited at low tide plot the positions of all foraging birds onto a suitable scale map of the site. Also plot the limits of *Katelysia* distribution at these sites.

4. Compare the distributions of the foraging birds at each site with the distribution of *Katelysia.*

Long-term Monitoring of oystercatcher populations and the establishment of refence sites

Objectives: Long-term monitoring of oystercatcher numbers would be a valuable check on the possibility of adverse effects arising from clam harvesting. However, **monitoring on its own should definitely not be considered an adequate total response to assessing the impact of**

clam harvesting. As an approach to management monitoring is necessarily reactive. By the time it has been established beyond doubt that there is a significant negative trend in bird numbers it may take too long to determine its cause and implement appropriate remedial actions. There are no guarantees that the situation may be recoverable. A proactive approach is needed so that potential problems are anticipated and so avoided. Nevertheless, monitoring is an important back-up to ensure that objectives are being met and to provide for the possibility of detecting any problems that were unforeseen in the initial impact analysis. Thus a long-term monitoring programme of oystercatcher populations in *Katelysia* harvesting areas should be established. It is essential also that number of reference or control sites should be maintained where there is no *Katelysia* harvesting. Such refence sites can be multipurpose and can be used for long-term research into natural *Katelysia* populations and other environmental studies of the sheltered bay/lagoon habitat type, in addition to the necessary monitoring of oystercatcher poulations against which to compare any changes that might occur in the harvested sites. To cater for the natural variability that occurs around the Tasmanian coast and to provide for replication that is necessary for valid scientific methodology, a minimum of at least three such reference sites should be nominated and maintained free of all *Katelysia* harvesting.

Methods:

1. Select at least 3 sites (which should be entire bays or lagoons) at which *Katelysia* harvesting is undertaken and at least 3 reference sites where *Katelysia* harvesting is not permitted. These should be representative and comparable and should be widely distributed, especially along the east and north coasts of Tasmania.

2. Counts at these sites should be undertaken twice per year every year. The counts should be done in November/December to coincide with the peak of the breeding season and in May to coincide with the middle of the non-breeding season.

3. Counts should be undertaken at low tide at comparable times of day and on days of comparable tide heights, preferably on spring tides.

4. On the November/December counts attempts should be made to assess separately the numbers of territory owning oystercatcher pairs and the number of non-breeding flock birds. On the May counts, attempts should be made to assess separately the number of first year birds and the numbers older that first year to give an annual index of local productivity.

5. The results of the monitoring programme should be analysed and assessed annually so that significant changes can be detected as soon as possible.

The Effects of Disturbance on Wader Feeding Behaviour

Objectives. The presence of humans involved in harvesting could disturb wading birds away from their feeding areas, reducing the amount of time spent feeding or forcing them to feed in areas of poorer food supplies where they may suffer a reduced daily food intake. The response is likely to vary among species and perhaps also at different times of the year in relation to breeding and migration. It is also possible that some species may habituate or adapt their behaviour to the regular and predictable activity of harvesters. This possibility of habituation makes it difficult to quantify the effects of disturbance fully. However, a set of preliminary observations should be done to gain an idea of the significance of disturbance and should it prove important these should be followed up by a more elaborate set of trials or experimental manipulations. The results of these may also provide useful information for the management of disturbance.

At a preliminary level, observations could be conducted at areas where harvesting has been done for some time and where habituation would have had time to develop and at sites where there was no previous history of disturbance. It must be remembered that some species that may be extremely sensitive to disturbance may already have abandoned areas where harvesting has occurred regularly so observations in non-harvested areas are essential. Two approaches to the study are possible, 1) the observation of birds at harvested sites, quantifying responses to harvesters and comparing days when harvesting is done with days when no harvesting is done, 2) experimental manipulation of disturbance at harvested and non-harvested sites, recording the birds' responses. Recording of the responses of all wading bird species, including oystercatchers, could be done at the same observation sessions.

Methods:
Harvested Areas

1. Select a study site where harvesting has been done for at least 6 months and where the major part of the area can be observed from a single observation point. Anson's Bay is probably suitable.

2. Record the following responses of all birds present to the activity of harvesters:

a) flight distances (i.e. the distances at which birds move away from approaching harvesters).

b) the distances moved by the fleeing birds.

c) for birds that were feeding prior to disturbance the amount of time between fleeing and resumption of feeding.

d) compare the feeding rates of birds at various time intervals before and after being disturbed.

3. Compare the distributions and densities of feeding birds on days when harvesters are present and days when no harvesters are present. For this, a grid of markers could be set up and the position of birds plotted onto similarly grided maps. Should there prove to be significant changes in distribution of the birds and consequently increases in local densities, feeding rates could be compared with and without disturbance to determine if the birds' food intake rates are reduced by them being forced into less profitable areas or having to feed at higher densities.

Non-harvested areas

1. Select one or more areas that have not been harvested and that can be observed clearly from vantage points.

2. Repeat the types of observations done in harvested areas but using researchers to take the role of harvesters. Attempt to record the responses of as many wading bird species as possible. Comparisons of responses such as flight distances between the harvested and non-harvested sites should give an indication of any development of habituation at the harvested sites.

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5.14.9 REFERENCES

Ashby, R. 1992. Waders of far north-west Tasmania. *The Stilt,* **19,** 44-49.

Baker, A. J. 1974. Prey specific feeding methods of New Zealand oystercatchers. *Notornis,* **21**, 219-233.

Baker, A. J. 1974. Ecological and behavioural evidence for the systematic status of New Zealand oystercatchers. *Life Sciences Contributions, Royal Ontario Museum,* **96**, 1-34.

Barker, R. D. and Vestjens, W. J. M. 1989. The food of Australian birds. Non passerines. CSIRO Div. Wild. and Ecology., Canberra.

Boates, J. S. and Goss-Custard, J. D. 1989. Foraging behaviour of oystercatchers *Haematopus ostralegus* during a diet switch of worms *Nereis diversicolor* to clams *Scrobicularia plana. Can. J. Zool.,* **67**, 2225-2231.

Bolzem, G. 1968. Anordnung and Bau der Herbstschen Korperchen in Limicolenschnabeln im Zusammenhang mit der Nahrungsfindung. *Zool. Anz.,* **181**, 313-355.

Bellchambers, L. 1993. Aspects of the ecology of *Katelysia scalarina* (Bivalva: Veneridae), a commercially exploited cockle. Unpublished BSc thesis, University of Tasmania.

Beukema, J. J. 1993. Increased mortality in alternative bivalve prey during a period when tidal flats of the Dutch Wadden Sea were devoid of mussels. *Neth. J. of Sea Res.,* **31**, 395-406.

Brown, R. A. and O'Connor, R. J. 1974. Some observations on the relationships between oystercatchers, *Haematopus ostralegus L.,* and cockles, *Cardium edule L.,* in Strangford Lough. *Irish Nat. Journal,* **18,** 73-79.

Camphuysen, C. J. 1993. Zeevogelstrandingen op de Nederlandse Kust: 26 jaar een vinger aan de pols (1965-199 *1). Limosa,* **66,** 1-16.

Cayford, J. T. and Goss-Custard, J. D. 1990. Seasonal changes in the size selection of mussels, *Mytilus edulis,* by oystercatchers, *Haematopus ostralegus*: an optimality approach, *Anim. Behav.,* **40**, 609-624.

Cole, H. A. 1956. A preliminary study of growth-rate in cockles *(Cardium edule L.)* in relation to commercial exploitation. *Journal du Conseil.* Conceil permanent International pour l'Exploration de la Mer, **22**, 77-90.

Coleman, N. 1982. Population density and biomass of the bivalves *Tellina mariae* and *Katelysia rhytiphora* from a seagrass bed in Western Port, Victoria. *Journal of Malacological Society of Australia,* **5***,* 141-149.

Connor, W. G. and Simon, J. L. 1979. The effects of oyster shell dredging on an estuarine benthic community. *Estuar. Coast. Mar. Sci.,* **9**, 749-758.

Considine, M. 1979. The feeding behaviour of the sooty oystercatcher (*Haematopus fuliginosus*) on rocky shores in Victoria. Unpubl. Hons Thesis, Dept of Zoology, Monash Univ., Clayton, Victoria.

Cramp, S. and Simmons, K. E. L. (Eds) 1983. Handbook of the Birds of Europe, the Middle East and North Africa: the Birds of the Western Palearctic. Vol. 3. Oxford University Press, Oxford.

Daan, P. 1987. The feeding behaviour and ecology of shorebirds. In Lane, B.A. *Shorebirds in Australia.* Nelson, Melbourne.

Dare, P. J. 1966. The breeding and wintering populations of the oystercatcher *(Haematopus ostralegus* Linnaeus) in the British Isles. *Fishery Invest., London.* Ser. II, **25**(5), 1-69.

Dare, P. J. 1970. The movements of oystercatchers, *Haematopus ostralegus L.,* visiting or breeding in the British Isles. *Fishery Invest., London.* Ser. II, **25**(9), 1-137.

Dare, P. J. 1977. Seasonal changes in body-weight of oystercatchers *Haematopus ostralegus. Ibis,* **119**, 494-506.

Dare, P. J. and Mercer, G. J. 1973. Foods of the oystercatcher in Morecombe Bay, Lancashire. *Bird Study,* **20**, 173-184.

Davidson, P. E. 1967. A study of the oystercatchers *(Haematopus ostralegus L.) in* relation to the fishery for cockles *(Cardium edule L.)* in the Burry Inlet, South Wales. *Fishery Invest., London.* Ser. II, **25** (7), 1-28.

Dircksen, R. 1932. Die Biologie des Auternfischers, der Brandseeschwalbe and der Kustenseeschwalbe. *Journal fir Ornithologie,* **8**, 427-521.

Drinnan, R. E. 1957a. The winter feeding of the oystercatcher *(Haematopus ostralegus)* on the edible cockle *(Cardium edule). J Anim. Ecol.,* **26**, 441-469.

Drinnan, R. E. 1957b. The winter feeding of the oystercatcher *(Haematopus ostralegus)* on the edible cockle *(Mytihus edulis)* in the Conway Estuary, North Wales. *Fishery Invest., Lond.* (Ser. II), **22** (4), 1-15.

Eagle, R. A. 1975. Natural fluctuations in a soft bottom benthic community. *Journal of Marine Biological Association of U.K.,* **55,** 865-878.

Ens, B. J. and Goss-Custard, J. D. 1984. Interference among oystercatchers, *Haematopus ostralegus L.,* feeding on mussels, *Mytilus edulis L.,* on the Exe estuary. *J Anim. Ecol.,* **53,** 217-232.

Emerson, C. W., Grant, J. and Rowell, T. W. 1990. Indirect effects of clam digging on the viability of soft-shell clams *Mya arenaria. Neth. J. Sea. Res.,* **27**, 109-118.

Evans, P. R. ***1975.*** Notes on the feeding of waders on Heron Island. *Sunbird*, **6**, 25-30.

Goss-Custard, J. D. 1977. The Ecology of The Wash. III. Density-related behaviour and the possible effects of a loss of feeding grounds on wading birds (Charadrii). *J. Anim. Ecol.,* **14**,721-739.

Goss-Custard, J. D. 1979. Effect of habitat loss on the number of overwintering shorebirds. *Studies in Avian Biology, **2**,* 167-177.

Goss-Custard, J. D., Cayford, J. T., Boates, J. S. and Durrell, S. E. A. le V. dit. 1987. Field tests of the accuracy of estimating prey size from bill length in oystercatchers, *Haematopus ostralegus,* eating mussels, *Mytilus edulis. Animal Behaviour,* **35**, 1078-1083.

Goss-Custard, J. D., Durrell, S. E. A. le V. dit. 1983. Individual and age differences in the feeding ecology of oystercatchers *Haematopus ostralegus* wintering on the Exe Estuary, Devon. *Ibis,* **125**, 155-171.

Goss-Custard, J. D., Durrell, S. E. A. le V. dit, Sitters, H. P. and Swinfern, R. 1982. Age-structure and survival of a wintering population of oystercatchers. *Bird Study,* **29**,83-98.

Goss-Custard, J. D., Jones, R. E. and Newbery, P. E. 1977. The ecology of The Wash. I. Distribution and diet of wading birds (Charadrii). *J. Appl Ecol.,* **14**, 681-700.

Goss-Custard, J. D., West, A. D. and Durrell, S. E. A. le V. dit. 1993. The availability and quality of the mussel prey *Mytilus edulis of* oystercatchers *Haematopus ostralegus. Neth. J. Sea Res.,* **31**, 419-439.

Grange, K. R. 1977. Littoral benthos-sediment relationships in Manakau Harbour, New Zealand. *N. Z. J. Mar. Freshw. Res.,* **11**, 111-123.

Greenhalgh, M. E. 1975. Studies of the foods and feeding ecology of wading birds. Unpublished PhD thesis, Liverpool Polytechnic.

Guiler, E. R. 1950. The intertidal ecology of Tasmania. *Papers and Proceedings of the Royal Sociey of Tasmania,* **1949**, 29-41.

Guiler, E. R. 1951. The intertidal ecology of Pipe Clay Lagoon. *Papers and Proceedings of the Royal Society of Tasmania,* **1950**, 29-4 1.

Hancock, D. A. 1971. The role of predators and parasites in a fishery for the mollusc *Cardium edule L. Dynamics of Populations* (ed. P.J. den Boer & G.R. Gradwell), pp 419-439. Proc. Adv. Study Inst. Oosterbeck, 1970, Centre for Agric. Publication and Documentation, Wageningen.

Hancock, D. A. 1973. The relationship between stock and recruitment in exploited invertebrates. *Rapp. P.-v Reun. Cons. int. Explor. Mer,* **164**, 113-131.

Hancock, D. A. and Urquart, A. E. 1965. The determination of natural mortality and its causes in an exploited population of cockles *(Cardium edule L.). Fishery Invest., Lond.* Ser. II, **24** (2), 1-40.

Harris, M. P. 1967. The biology of oystercatchers, *Haematopus ostralegus,* on Skokholm Island, S. Wales. *Ibis,* **109,** 180-193.

Harris, M. P. 1970. Territory limiting the size of the breeding population of the oystercatcher, *Haematopus ostralegus:* a removal experiment. *J. Anim. Ecol.,* **39**, 707-713.

Heppleston, P. B. 1971. The feeding ecology of oystercatchers, *Haematopus ostralegus* L. in winter in northern Scotland. *J Anim. Ecol.,* **40**, 651-672.

Heppleston, P. B. 1971. Feeding techniques of the oystercatcher *(Haematopus ostralegus). Bird Study,* **18**, 15-20.

Heppleston, P. B. 1972. The comparative breeding ecology of oystercatchers *Haematopus ostralegus L.* in inland and coastal habitats. *J. Anim. Ecol.,* **41,** 23-51.

Hewish, M. 1990. The winter 1989 population monitoring count: pied and sooty oystercatchers at monitored sites. *The Stilt,* **17**, 5-16.

Hindwood, K. A. and Hoskin, E. S. 1954. The waders of Sydney (county of Cumberland), New South Wales. *Emu,* **4***,* 217-55.

Horwood, J. W. and Goss-Custard, J. D. 1977. Predation by the oystercatcher, *Haematopus ostralegus (L.),* in relation to the cockle, *Cerastoderma edule (L.),* fishery in the Burry Inlet, South Wales. *J. Appl. Ecol.,* **14**,139-158.

Hulscher, J. B. 1971. Localisation of cockles *(Cardium edule) by* the oystercatchers *(Haematopus ostralegus L.)* in darkness and daylight. *Ardea,* **64**, 292-310.

Hulscher, J. B. 1982. The oystercatcher as a predator of the bivalve, *Macoma balthica* in the Dutch Wadden Sea. *Ardea,* **70**, 89-152.

Hulscher, J. B. 1964. *Levende Nat.,* **67**, 80-85.

Hulscher, J. B. 1993. Burying depth and trematode infection in *Macoma balthica. Neth. I Sea Res.,* **6**, 141-156.

Jackson, M. J. and James, R. 1979. The influence of bait digging on cockle, *Cerastoderma edule,* populations in North Norfolk. *J. Appl. Ecol.,* **16,** 671-679.

Kaplan, E. H., Welker, J. R., Krans, M. G. and McCourt, S. 1975. Some factors affecting the colonisation of a dredged channel. *Mar. Biol.,* **32**, 193-204.

Lane, B. 1987. *Shorebirds in Australia.* Nelson, Melbourne.

Lauro, B. and Nol, E. 1993. The effect of prevailing wind direction and tidal flooding on the reproductive success of pied oystercatchers *Haematopus longirostris. Emu,* **93**, 199-202.

Lea, A. M. and Gray, J. T. 1935. The food of Australian birds, Part *1. Emit,* **34**, 275-92

Manzi, J. J. 1991. Clams, cockles and arkshells. *Production of Aquatic Animals* (Ed C.E. Nash). Elsevier, Amsterdam/Oxford.

McLennan, W. 1917. Northern Australian Birds. *Emit,* **16**, 63-98.

McLusky, D. S., Anderson, F. E. and Wolfe-Murphy, S. 1983. Distribution and population recovery of *Arenicola marina* and other benthic fauna after bait digging. *Marine Ecology -Progress Series,* **11**, 173-179.

Marchant, S. and Higgins, P. J. (Eds) 1993. *Handbook of Australian, New Zealand and Antarctic Birds. Volume 2: Raptors to Lapwings.* Oxford University Press, Melbourne.

Newman, 0. M. G. 1982. Dispersal of pied oystercatchers in the Hobart area. *An Occasional Stint,* **1**, 51-58.

Newman, O. M. G. 1984. Survival of pied oystercatchers banded as pulli. *An Occasional Stint, **3**,* 45-51.

Newman, O. M. G. 1985. Pied oystercatchers on the Tasman Peninsula. *Tasmanian Bird Report, **14**,* 9-11.

Newman, O. M. G. 1991. Forecast of population decline in oystercatchers based on breeding disturbance. *Tasmanian Bird Report,* **20**, 21-24.

Newman, O. M. G. 1992. Pied oystercatcher breeding at Mortimer Bay, Tasmania. *Emu,* **92**, 87-92.

Newman, O. M. G. and Park, P. 1992. Pied oystercatcher breeding success in the *1991/92* summer. *Tasmanian Bird Report,* **21**, 13-22.

Newton, I. 1991. *Bird Population Studies. Relevance to conservation and Management.* (Eds. C.M. Perrins, J.D. Lebreton & G.J.M. Hirons). Oxford University Press, Oxford.

Norton-Griffiths, M. 1967. Some ecological aspects of the feeding behaviour of the oystercatcher, *Haematopus ostralegus,* on the edible mussel, *Mytilus edulis. Ibis,* **109**, 412-424.

Norton-Griffiths, M. 1968. *The feeding behaviour of the oystercatcher* Haematopus ostralegus. PhD Thesis, University of Oxford.

O'Connor, R J. and Brown, R. A. 1977. Prey deletion and foraging strategy in the oystercatcher. *Oecologia,* **27**, 75-92.

Parish, D. 1987. Conservation of wader habitats in East Asia. *The Conservation of International Flyway Populations of Waders.* (Eds. N.C. Davison & M.W. Pienkowski). Wader Study Group Bulletin. No. **49.** *Suppl.* IWRB Special Publication No. **7**, 4-14.

Parish, D., Lane, B., Sager, P. and Tomkovich, P. 1987. Wader migration systems in East Asia and Australasia. *The Conservation of International Flyway Populations of Waders.* (Eds. N.C. Davison & M.W. Pienkowski). Wader Study Group Bulletin. *No.* **49**. *Suppl.* IWRB Special Publication No. **7**, 4-14.

Patterson, R. M. 1989. Twenty-five years of wader research in south-east Tasmania. *The Stilt,* **15,** 7-8.

Peterson, C. H. 1979. Predation, competitive exclusion and diversity in the soft sediment benthic communities of estuaries and lagoons. *Ecological Processes in Coastal and Marine Systems* (Ed. R.J. Livingston). Plenum Press.

Peterson, C. H. 1982a. The importance of predation and intra- and interspecific competition in the population biology of two infaunal suspension-feeding bivalves, *Protothaca staminea* and *Chione undatella. Ecological Monographs,* **52**, 437-475.

Peterson, C. H. 1982b. Clam predation by whelks *(Busycon spp.):* experimental tests of the importance of prey size, prey density and seagrass cover. *Marine Biology,* **66,** 159-170.

Peterson, C. H. and Black, R 1993. Experimental tests of the advantages and disadvantages of high density for two coexisting cockles in a Southern Ocean lagoon. *J. Anim. Ecol.,* **62**, 614-633.

Pierce, R. 1987. Observations on distribution and numbers of double-banded plovers in Tasmania. *The Stilt,* **11**, 32-35.

Piersma, T. 1987. Hop, skip or jump? Constraints on migration of arctic waders by feeding, fattening and flight speed. *Limosa,* **60,** 185-191.

Pulliam, H. R. 1988. Sources, sinks and population regulation. *American Naturalist,* **132**, 652-701.

Rice, M. A., Hickok, C. and Zehra, I. 1989. Effects of intensive fishing effort on the population structure of quahogs, *Mercenaria mercenaria* in Narragansett Bay. *Journal of Shellfish Research,* **8**, 345-354.

Roads, D. C. and Young, D. K. 1970. The influence of deposit-feeding organisms on sediment stability and community trophic structure. *J. Mar. Res.,* **28**, 150-178.

Roberts, D. 1984. The genus *Katelysia* (Bivalvia: Veneridae) in southern Australia. *Journal of Malacological Society of Australia,* **6**, 191-204.

Schulz, M. 1990. Repeat survey of the waders of south-west Tasmania. *The Stilt,* **16,** 52-54.

Schulz, M. 1990. Notes on the waders of Flinders and King Island, Bass Strait. *The Stilt,* **17**, 40-43.

Schulz, M. and Menkhorst, K. A. 1990. A survey of the waders of south-west Tasmania. *The Stilt*, **5**, 21-24.

Seed, R. and Brown, R A. 1975. The influence of reproductive cycle, growth and mortality on population structure in *Modiolus modiolus (L), Cerastoderma edule (L)* and *Mytilus edulis L.* (Mollusc: Bivalvaia). *Proceedings of the 9th European Marine Biology Symposium, pp 257-274.* Aberdeen University Press, Aberdeen.

Sutherland, W. J. 1982a. Spatial variation in the predation of cockles by oystercatchers at Traeth Melynog, Anglesey. I. The cocke population. *J Anim. Ecol.,* **51**, 481-489.

Sutherland, W. J. 1982b. Spatial variation in the predation of cockles by oystercatchers at Traeth Melynog, Anglesey. II. The pattern of mortality. *J. Anim. Ecol.,* **51**, 491500.

Thomas, D. G. 1968. Waders of Hobart. *Emu,* **68**, 98-125.

Thomas, D. G. 1970. Flucuation of numbers of waders in south-eastern Tasmania. *Emu,* **70**, 79-85.

Thomas, D. G. 1986. Gut contents of four small waders. *The Stilt,* **9**, 30-31.

Thomas, D. G. and Dartnall, A. *L.* 1971. Ecological aspects of the feeding behaviour of two calidritine sandpipers wintering in south-eastern Tasmania. *Emu,* **71**, 20-26.

Thomson, D. F. 1935. *Birds of Cape York Peninsula.* Government Printer, Melbourne.

Vestjens, W. J. M. 1977. Status, habitats and food of vertebrates at Lake Cowal. CSIRO Division of Wildlife Research, Technical Memorandum No. *12.*

Watkins, D. 1993. A *National Plan for Shorebird Conservation in Australia.* Australasian Wader Studies Group, Royal Australian Ornithologists' Union and World Wide Fund for Nature. RAOU Report No. *90.*

Wells, F. E. and Roberts, D. 1980. Molluscan assemblages on an intertidal sandflat in Princess Royal Harbour, Western Australia. *Aust. J. Mar. Freshw. Res.,* **31**, 499-507.

Wells, F. E. and Threfall, T. J. 1980. *A* comparison of the molluscan communities in intertidal sandflats in Oyster Harbour and Peel Inlet, Western Australia. *J. Moll. Stud,* **46,** 300-311.

Wheeler, W. R. 1955. Charadriiformes at the Laverton salt works, Victoria 1950-1953. *Emu*, **63**, 8-13 .

Weston, M. A. 1991. *Foraging strategies of the pied oystercatcher Haematopus longirostris, in the intertidal environment.* Unpublished Honours Thesis, University of Melbourne.

Woodward, I. 0. 1985. *The structured dynamics of a tidal mollusc community.* Unpublished PhD. Thesis, University of Tasmania.

Zwartz, L. and Wanink, J. 1984. How oystercatchers and curlews successively deplete clams. *Coastal Waders and Wildfowl in Winter.* (Eds. P.R.Evans et al.). pp. 69-83, Cambridge University Press, Cambridge.

5.15 MANUSCRIPT 15

Genetic studies of the venerid clam genus *Katelysia*

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5.15.1 ABSTRACT

Two samples of *Katelysia scalarina*, one of K. *rhytiphora* and one of *K. peronii* were compared for 16 allozyme loci. Diagnostic loci were identified, one of which (*SOD**) provided unambiguous discrimination of all three clam species. *Katelysia rhytiphora* and *K. peronii* were found to be the most similar pair ($D = 0.34$). Variability levels were high, with average observed heterozygosities per locus ranging from 0.257 to 0.314, and percent polymorphism from 62.5 to 81.3. Six samples of *K. scalarina* (three from Tasmania and one each from Victoria, South Australia and Western Australia), examined for six variable loci, revealed three distinct groups: the three Tasmanian samples; the Victorian and South Australian samples; and the Western Australian sample. Differentiation between the Tasmanian and mainland subpopulations was striking, especially for the *PGM** locus. Coastal gene flow mediated by stepping-stone migration between adjacent subpopulations may account for the similarities between subpopulations on the same land mass, whereas the 10 to 12 day larval period is likely to hinder gene flow across the Bass Strait between Tasmania and the mainland. It is suggested that any future Tasmanian hatchery production should use Tasmanian rather than mainland broodstock, and vice versa, to prevent the introduction to native populations of possibly ill-adapted genotypes.

Keywords: *Katelysia*, clam, allozyme, diagnostic loci, stock-structure, gene flow.

5.15.2 INTRODUCTION

The world-wide aquaculture production of clams, cockles and ark shells is on a par with that of oysters: about 1.25 million tones in 1994, compared with 1.1 million tons for oysters (FAO 1996). Total production is substantially higher - in 1992 only about 40% of mollusc production came from aquaculture (FAO 1995). In Australia, mainly wild clam populations are harvested, but the sustainability of this practice is uncertain and so aquaculture production is being considered.

Clams of the genus *Katelysia* are among those most heavily exploited in the temperate waters of southern Australia. The three species - *K. scalarina, K. rhytiphora,* and *K. peronii* - can be difficult to distinguish morphologically (Roberts 1984, Lamprell and Whitehead 1992). The first two species have very similar distributions, ranging from Western Australia to New South Wales and including Tasmania, whereas the less-abundant *K. peronii* is absent from New South Wales (Nielsen 1963, Lamprell and Whitehead 1992). *Katelysia* species are frequently sympatric, occupying the soft-bottoms of intertidal and shallow subtidal zones of sheltered bays and estuaries.

In Tasmania, the main clams harvested are *Katelysia* (principally *K. scalarina*) and *Ruditapes largillierti* (thought to have been introduced from New Zealand). Both genera inhabit soft-bottomed habitats: *Katelysia* in the intertidal and shallow subtidal zone of sheltered bays and estuaries, *Venerupis* in the subtidal zone. The total Tasmanian clam catch rose from 15.56 tonnes in 1990 and 14.00 t in 1991 to 51.44 t in 1992 and 60.97 t in 1993 (Anon. 1994). From 1994 to

1996, between 23 and 45 t per year of *K. scalarina* alone were harvested (S. Riley pers. com.). Most are exported live to fish markets in Melbourne and Sydney.

With the increased utilization of the *Katelysia* resource, a hatchery-based industry to supplement or replace the wild fisheries is being considered. Experimental hatchery production in New South Wales of *K. rhytiphora* to metamorphosis has been reported (Nell et al. 1994), and similar work with *K. scalarina* is under way in Tasmania (Kent et al. 1998). In Asia, "almost all of their cockle and clam landings involve some aspect of culture in the grow-out process" (Manzi 1991).

While there has been considerable ecological research into these species (Nielsen 1963, Coleman 1982, Roberts 1983, 1984, Lamprell and Whitehead 1992, Bellchambers and Richardson 1995), there have been very few biochemical genetic studies. Nielsen (1963) carried out some preliminary chromatography research but was unable to differentiate *Katelysia scalarina* from *K. rhytiphora*. Roberts (1984) examined protein-stained isoelectric focusing gels, and found some apparent species differences but did not assess within-species variation.

This paper investigates the allozyme genetics of *Katelysia*. The first part examines the genetic relationships of the three taxa, principally to find diagnostic allozyme markers to assist in species identification. The second part examines the genetic population structure of *K. scalarina* in Tasmania and the mainland of Australia, principally to assist fisheries management and any future aquaculture operations. A knowledge of the genetic diversity of wild stocks can help minimise any deleterious effects of introducing hatchery-bred animals or translocating stock (Allendorf and Waples 1996, Rhymer and Simberloff 1996).

5.15.3 MATERIALS AND METHODS

Samples of *Katelysia scalarina* (Fig. 1) were collected from six sites: three in Tasmania and one each in Victoria, South Australia, and Western Australia (Fig. 2). Samples of *K. peronii* and *K. rhytiphora* (Fig. 1) were collected from the same site in South Australia (Table 1). The clams were transported live to the laboratory in cool-boxes with ice-packs. On arrival, adductor muscles and digestive gland tissues were dissected and stored at -80˚C in 2 mL microcentrifuge tubes. The siphon tissue of small individuals was pooled with their adductor muscle tissue.

Genetic analysis

Before electrophoresis, tissues were homogenised manually with 2 to 4 drops of distilled water and centrifuged for 5 min at 10 000 rpm; the supernatants were used for electrophoresis (Table 2). Either starch or cellulose acetate gels were used (Table 2). Starch gels used 9% Connaught starch with a discontinuous histidine-citrate buffer system run at 100 V for 4.5 h (gel buffer 0.005M histidine HCl pH 7.0; electrode buffer 0.41M trisodium citrate pH 7.0); the cellulose acetate gels were Helena Titan III plates run at 150 V for 1 h (gel and electrode buffer 0.075 M tris and 0.025 M citric acid, pH 7.0). Staining techniques followed Richardson et al. (1986) and Hebert and Beaton (1989). Heterozygote banding patterns were consistent with known quaternary structures (Ward et al. 1992).

Sixteen loci were examined in the between-species comparisons (Table 3), and six (Table 6) were examined in the *Katelysia scalarina* stock-structure analysis. Five of the latter loci were chosen for their variability and their reliability (*DIA**, *ESTD**, *GPI**, *MDH-1** and *PGM**); the sixth locus (*MDH-2**) was scored on the same gels as *MDH-1**. The MDH patterns immediately enabled any non-*K. scalarina* specimens to be eliminated from the stock-structure analysis.

Where there were multiple loci, the locus encoding the fastest migrating allozyme was designated '1'. In the between-species comparisons, alleles were lettered from 'a' for the fastest migrating allozyme. In the *Katelysia scalarina* stock-structure analysis, alleles were numbered according to the anodal mobility of their product relative to that of the most common allele observed in the Cockle Creek (TAS2) sample, which was designated '100'. Allele identities between the two studies, where known, are identified in Table 6. Sample sizes were substantially larger for the stock structure analysis (Table 5).

Statistical analysis

For the between-species comparisons, mean sample sizes, numbers of alleles, percent polymorphism (a locus was considered polymorphic if the most common allele had a frequency less than 0.95) and heterozygosities (both observed and unbiased Hardy Weinberg expected values) were estimated by the BIOSYS-1 package (Swofford and Selander 1981).

For the *K. scalarina* stock-structure analysis, individual sample and locus tests for goodness-of-fit to Hardy-Weinberg expectations used Fisher's exact test, after all but the most common allele had been pooled at each locus. Two genetic diversity parameters were estimated: F_{IS} (the correlation between two uniting alleles relative to the subpopulation, and defined as the ratio of the difference between the expected and observed heterozygosities to the expected heterozygosity) and F_{ST} (the correlation between two gametes drawn at random from each subpopulation, defined as the ratio of the difference between the expected total heterozygosity and the average expected subpopulation heterozygosity to the expected total heterozygosity). F_{IS} essentially estimates deviations from Hardy-Weinberg expectations, while F_{ST} estimates the extent of genetic differentiation of subpopulations. Values of Fisher's exact test, F_{IS}, and F_{ST} were estimated by BIOSYS-1 (Swofford and Selander 1981). Allele frequency homogeneity across samples was tested by the randomised Monte Carlo chi-square procedure of Roff and Bentzen (1989), which obviates the need to pool rare alleles. For each test, 1,000 randomisations of the data were carried out.

For both sets of comparisons, BIOSYS-1 was used to calculate unbiased genetic distances between samples (Nei 1978) and to cluster the resulting distance matrices with the UPGMA (unweighted pair-group method with averaging) algorithm.

When multiple tests of a single hypothesis were carried out, the standard Bonferroni procedure was applied. The *P* value for a specific test had to be less than, or equal to, $0.05/n$ (where n is the number of tests) to be deemed statistically significant (Miller 1980).

5.15.4 RESULTS

Between-species Comparison

Sixteen loci were examined in four samples, one each of *Katelysia rhytiphora* and *K. peronii*, and two of *K. scalarina* (Table 3). Levels of variation in each sample were high, despite quite small sample sizes, with an average of 2.5 to 2.8 alleles per locus and between 62.5% and 81.25% polymorphism. Observed and expected heterozygosities per locus ranged from 0.257 to 0.314 and from 0.283 to 0.377 respectively (Table 4). Sample sizes were too small to warrant Hardy-Weinberg tests.

All species pairs showed diagnostic loci, although only *SOD**, which was nearly monomorphic and different in each species, allowed all three species to be discriminated unambiguously. Other diagnostic loci were: *K. scalarina* - *K. rhytiphora*, *AK**, *APK**, *IDHP-2**, *MDH-2**,

*PGDH**, *SOD**; *K. scalarina* - *K. peronii*, *ADA**, *AK**, *MDH-2**, *PGM**; *K. rhytiphora* - *K. peronii*, *APK**.

Katelysia rhytiphora and *K. peronii* were more closely related to one another (*D* = 0.337) than either was to *K. scalarina* (mean *D* = 1.047, range 0.859 - 1.238) (Table 5, Fig. 3). The within-species genetic distance of *K. scalarina* (*D* = 0.068) was much less than any of the between-species genetic distances (mean *D* = 0.905, range 0.337 - 1.238) (Table 5, Fig. 4).

Katelysia scalarina *population structure*

Three samples of *Katelysia scalarina* from Tasmania and three from the mainland of Australia were examined to assess population structure, both on medium scales (comparisons of Tasmanian samples) and broad scales (comparisons among mainland samples, and of mainland with Tasmanian samples) (Table 6).

Thirty-six tests of Hardy-Weinberg equilibrium (6 loci x 6 subpopulations) were performed (data not shown). Two had *P* values less than 0.05 (*GPI** at TAS3, *P* = 0.030, and *DIA** at SA, *P* = 0.013), but neither was significant after Bonferroni corrections for 36 tests. Six tests (one per locus) of whether the average value of F_{IS} was significantly different from zero were performed (Table 7); one was significant after Bonferroni corrections. This was for *DIA**, and at 0.177 (*P* = 0.003) indicated an overall homozygote excess.

All loci showed significant heterogeneity in allele frequencies (*P* = 0.005) across the six subpopulations (Table 7). For five loci - *DIA**, *MDH-1**, *MDH-2**, *GPI** and *ESTD** - the extent of differentiation, although significant, was low, with F_{ST} values of about 0.05 or less. For one locus - *PGM** - the extent of differentiation was much more extensive (F_{ST} = 0.298). At this locus, nearly 30% of the allele frequency variation could be attributed to differentiation among subpopulations, and there was very little overlap in the allele frequencies of the Tasmanian and mainland subpopulations. The two common alleles in Tasmania (*PGM**120 and *PGM**100) were uncommon on the mainland, and the two common mainland alleles (*PGM**85 and *PGM**75) were uncommon in Tasmania (Table 6).

Comparing the three Tasmanian subpopulations with one another found only *DIA** to show evidence of spatial differentiation (*P* = 0.038), but this result became non-significant after Bonferroni corrections for 6 tests. Only one of the 18 pairwise subpopulation tests gave a *P* value less than 0.05 (*DIA**, *TAS2*-*TAS3*, *P* = 0.047), a result clearly non-significant after Bonferroni corrections. Thus no significant heterogeneity was observed among the Tasmanian subpopulations.

The three mainland populations showed more differentiation. Three loci - *DIA**, *MDH-2** and *PGM** - showed evidence of spatial differences in allele frequencies, and all remained significant after Bonferroni corrections (*P* = 0.001, 0.006 and 0.002, respectively). The three mainland populations were compared pairwise for these three loci; all comparisons with *P* < 0.05 included the Western Australia subpopulation (*DIA**, WA-VIC, *P* = 0.027, WA-SA, *P* = 0.014; *MDH-2**, WA-VIC, *P* = 0.003; *PGM**, WA-VIC, *P* = 0.010, WA-SA, *P* = 0.001). In none of these pairwise tests was the VIC-SA comparison significant.

Clustering the pairwise subpopulation genetic distances over all six loci (Fig. 4) confirmed these general findings. The three Tasmanian subpopulations clustered together and away from the mainland subpopulations. The SA and VIC subpopulations were genetically very similar, with the WA subpopulation more distinct.

5.15.5 DISCUSSION

All three species of *Katelysia* are genetically highly variable. Sixteen loci were examined. The average observed heterozygosities per locus ranged from 0.257 to 0.314, and percent polymorphism from 62.5 to 81.3 (Table 4). The average heterozygosity for molluscs, assessed from 105 species and an average of nearly 22 loci per species, is about 0.145 (Ward et al. 1992).

The genetic distance estimates between the different species pairs ranged from 0.337 to 1.238, with the corresponding identity values ranging from 0.714 to 0.290 (Table 5). These values fall within the typical invertebrate between-species identity range of about 0.80 to 0.20 (Thorpe 1983). *Katelysia rhytiphora* and *K. peronii* appear to be the most closely related species-pair, a conclusion reached earlier by Roberts (1984) from comparing general protein-stained isoelectric focusing gels of the three species. Diagnostic allozyme loci were identified to enable unambiguous species identification - knowledge that proved useful for distinguishing several *K. rhytiphora* in the putative *K. scalarina* samples.

The data were examined for the possible occurrence of between-species hybridization. This seemed possible, as the samples of *Katelysia rhytiphora*, *K. peronii*, and one of the *K. scalarina* samples were sympatric, and the spawning periods, at least of *K. rhytiphora* and *K. scalarina*, overlap (Nielsen 1963, Roberts 1984). However, in the between-species study, no heterozygotes of the apposite hybrid F1 genotypes were observed for the diagnostic *SOD** locus, although sample sizes were small and rare hybrids would not have been detected.

In the *Katelysia scalarina* stock-structure analysis, which used much larger sample sizes, there was evidence of possible F1 hybrids between *K. scalarina* and *K. rhytiphora*. Two of the three Tasmanian animals that were *MDH-2**b/c heterozygotes were also *MDH-1**b/c heterozygotes (one in TAS2 and one in TAS3) — an unlikely chance occurrence given the low frequency of *MDH-1**b/c heterozygotes. In Tasmania, the expected frequency of such a double heterozygote in *K. scalarina* would be about 0.2%, only about one-third the observed frequency of 0.7%. Possibly one or both these animals were F1 hybrids between *K. rhytiphora* and *K. scalarina*, despite the failure of laboratory experiments to produce such hybrids (Nielsen 1963). No such evidence for hybridization was recorded for the mainland subpopulations — none of the six *MDH-2**b/c heterozygotes observed in the VIC sample nor the single SA *MDH-2**b/c heterozygote was an *MDH-1**b/c heterozygote.

The stock-structure analysis of *Katelysia scalarina* revealed three distinct genetic groups: the three Tasmanian samples; the Victorian and South Australian samples; and the Western Australian sample. The lack of significant differentiation, at least at the scale of resolution afforded by this study, between the three well-separated Tasmanian subpopulations (and between the Victorian and South Australian subpopulations) suggests significant levels of coastal gene flow. On the other hand, the sizeable differences between the Tasmanian and mainland subpopulations, especially at the *PGM** locus, suggest minimal gene flow across Bass Strait. The differences at the *PGM** locus seemed so large that initially the hypothesis of two unrecognised and allopatric sibling species was entertained, but the genetic distance between the SA and TAS2 subpopulations, 0.068, suggests a level of differentiation more typical of within- than between-species differentiation (Thorpe 1983).

Coastal gene flow mediated by stepping-stone migration between adjacent subpopulations probably accounts for the similarities between subpopulations on the same land mass, whereas the short larval life of the species (only about 10 to 12 days; *Katelysia scalarina*, Kent et al.

1998; *K. rhytiphora*, Nell et al. 1994) is likely to severely restrict gene flow across the Bass Strait between Tasmania and the mainland. Gene flow between the WA and SA subpopulations might be hindered by the intervening presence of long sections of exposed coastline dominated by cliffs and without sheltered bays; these may have fragmented possible clam habitats between these two sampled localities.

What are the implications of these results for the clam industry? The lack of significant differentiation among the Tasmanian subpopulations suggests that these can be managed as a single stock, although the standard caveats must be made that it takes only a little gene flow to render subpopulations effectively panmictic, and that larger sample sizes and more loci might reveal heterogeneity. A second point is that the substantial genetic differences between Tasmanian and mainland subpopulations suggests that there could be local co-adaptation of genes and gene complexes, in which case any Tasmanian hatchery production should use Tasmanian rather than mainland animals as broodstock, and vice versa. The use of spat from Tasmanian broodstock to enhance mainland subpopulations might be counter-productive through the introduction of ill-adapted genotypes.

5.15.6 ACKNOWLEDGMENTS

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5.15.7 REFERENCES

Allendorf, F. W & R. S. Waples. 1996. Conservation and genetics of salmonid fishes. pp. 238-280. *In*: J. C. Avise and J. L. Hamrick (eds.). Conservation Genetics: Case Histories from Nature. Chapman and Hall, New York.

Anon. 1994. Policy Document and Fishery Development Plan for the Tasmanian Developmental Clam Fishery. Department of Primary Industry and Fisheries, Tasmania.

Bellchambers, L. M. & A.M.M. Richardson. 1995. The effect of substrate disturbance and burial depth on the venerid clam, *Katelysia scalarina* (Lamarck, 1818). *J. Shellfish Res.* 14: 41-44.

Coleman, N. 1982. Population density and biomass of the bivalves *Tellina mariae* and *Katelysia rhytiphora* from a seagrass bed in Western Port. *J. Malac. Soc. Aust.* 5:141-149.

FAO. 1995. Review of the state of world fishery resources: aquaculture. FAO Fisheries Circular 886, FAO, Rome.

FAO. 1996. Aquaculture production statistics. FAO Fisheries Circular 815, Revision 8, FAO, Rome.

Hebert, P. D. N. & M. J. Beaton. 1989. Methodologies for allozyme analysis using

cellulose acetate electrophoresis. Helena Laboratory, Beaumont, Texas.

Kent, G. N., Maguire, G. B., John, M., Cropp, M. and Frankish, K., 1998 Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina* (Lamarck 1818). *J. Shellfish Research*, 17(4): 1065-1070.

Lamprell, K. & T. Whitehead. 1992. Bivalves of Australia. Volume 1. Crawford House Press, Bathurst, New South Wales.

Manzi, J. J. 1991. Clams, cockles and arkshells. pp. 139-159. *In*: C.E. Nash (ed.). Production of Aquatic Animals. Elsevier, Amsterdam/Oxford.

Miller, R. G. 1980. Simultaneous Statistical Inference. Springer Verlag, New York.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89:583–590.

Nell, J. A., W. A. O'Connor, M. P. Heasman & L. J. Goard. 1994. Hatchery production for the venerid clam *Katelysia rhytiphora* (Lamy) and the Sydney cockle *Anadara trapezia* (Deshayes). *Aquaculture* 119:149-156.

Nielsen, B. J. 1963. Studies of the genus *Katelysia* Romer 1857 (Mollusca, Lamellibranchiata). *Mem. Nat. Mus. Vic.* 26:219-257.

Rhymer, J. M. & D. Simberloff. 1996. Extinction by hybridization and introgression. *Annu. Rev. Ecol. Syst.* 27:83-109.

Richardson, B. J., P. R. Baverstock & M. Adams. 1986. Allozyme Electrophoresis. A Handbook for Animal Systematics and Population Studies. Academic Press, Sydney.

Roberts, D. 1983. The effects of substrate and shell form on burrowing in the genus *Katelysia* (Bivalvia: Veneridae) in southern Australia. *J. Malac. Soc. Aust.* 5:33-40.

Roberts, D. 1984. The genus *Katelysia* (Bivalvia: Veneridae) in southern Australia. *J.*

Malac. Soc. Aust. 6:191-204.

Roff, D. A. & P. Bentzen. 1989. The statistical analysis of mitochondrial DNA

polymorphisms: chi-square and the problem of small sample. *Molec. Biol. Evolut.* 6:539-545.

Thorpe, J. P. 1983. Enzyme variation, genetic distance and evolutionary divergence in relation to levels of taxonomic separation. pp 131-152. *In*: G. S. Oxford & D. Rollinson (eds.). Protein Polymorphism: Adaptive and Taxonomic Significance. Academic Press, London.

Swofford D. L. & R. B. Selander. 1981. BIOSYS-1: A FORTRAN program for the

comprehensive analysis of electrophoretic data in population genetics and

systematics. *J. Heredity* 72:281-283.

Ward, R. D., D. O. F. Skibinski & M. Woodwark. 1992. Protein heterozygosity, protein structure and taxonomic differentiation. *Evolutionary Biology* 26:73–159.

5.15.8 TABLES AND FIGURES

Table 1. Collection details for *Katelysia* species. *n* = number of clams collected.

Site	State	Abbreviation	Location	Date collected	*n*
K. scalarina					
Cockle Creek	Tasmania	TAS1	43˚33'S, 146˚52'E	Aug 95	120
Ansons Bay	Tasmania	TAS2	41˚03'S, 148˚21'E	Sep 95	120
Smithton	Tasmania	TAS3	40˚50'S, 145˚00'E	Sep 95	120
Queenscliff	Victoria	VIC	38˚16'S, 144˚39'S	Nov 95	81
Ceduna	South Australia	SA	32˚07'S, 133˚46'E	Dec 95	58
Albany	Western Australia	WA	35˚01'S, 117˚58'E	Dec 95	110
K. rhytiphora					
Ceduna	South Australia	SA:RHY	32˚07'S, 133˚46'E	Dec 95	16
K. peronii					
Ceduna	South Australia	SA:PER	32˚07'S, 133˚46'E	Dec 95	16

Table 2. Enzymes used in this study. Tissue: d - digestive gland (visceral mass), m - adductor muscle. Gel: ca - cellulose acetate, s - starch (see text). Multiple loci encoding for the same enzyme are designated by consecutive numbers, with '1' denoting the fastest migrating system.

Enzyme	EC Number	Locus Abbreviation	Tissue	Gel
Adenosine deaminase	3.5.4.4	*ADA**	m	s
Adenylate kinase	2.7.4.3	*AK**	m	s
Arginine phosphokinase	2.7.3.3	*APK**	m	ca
Aspartate aminotransferase	2.6.1.1	*AAT**	m	s
Diaphorase	1.8.1.4	*DIA**	d	s
Esterase-D (UV, umb. acetate)	3.1.–.–	*ESTD**	m	s
Fumarate hydratase	4.2.1.2	*FH**	d	s
Glucose-6-phosphate isomerase	5.3.1.9	*GPI**	m	s
Isocitrate dehydrogenase	1.1.1.42	*IDHP-1**	d	ca
		*IDHP-2**	d	ca
Malate dehydrogenase	1.1.1.37	*MDH-1**	m	s
		*MDH-2**	m	s
Peptidase (val-leu)	3.4.–.–	*PEP**	m	ca
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	m	ca
Phosphoglucomutase	5.4.2.2	*PGM**	d	s
Superoxide dismutase	1.15.1.1	*SOD**	d/m	s

Table 3. Allele frequencies at 16 loci in *Katelysia rhytiphora*, *K. peronii*, and two populations of *K. scalarina*. *n* = number of individuals.

		K. rhytiphora	*K. peronii*	*K. scalarina*	
Locus	**Allele**	**SA:RHY**	**SA:PER**	**SA**	**TAS2**
*ADA**	a	0.031	0.219	-	-
	b	0.219	0.531	-	-
	c	0.063	0.250	0.031	-
	d	0.656	-	0.031	0.333
	e	0.031	-	0.938	0.667
	n	16	16	16	12
*AK**	a	0.958	1.000	-	-
	b	0.042	-	-	-
	c	-	-	0.969	1.000
	d	-	-	0.031	-
	n	12	15	16	10
*APK**	a	0.036	-	-	-
	b	0.179	-	-	-
	c	0.786	0.042	-	-
	d	-	0.125	0.906	1.000
	e	-	0.833	0.094	-
	n	14	12	16	12
*AAT**	a	-	-	0.031	-
	b	0.893	0.367	0.906	1.000
	c	0.107	0.633	0.063	-
	n	14	15	16	12
*DIA**	a	0.156	0.219	0.094	0.042
	b	0.594	0.250	0.531	0.333
	c	0.219	0.188	0.281	0.500
	d	-	0.094	-	-
	e	0.031	0.250	0.094	0.125
	n	16	16	16	12
*ESTD**	a	-	0.031	0.031	0.167
	b	0.969	0.844	0.969	0.708
	c	0.031	0.063	-	0.125
	d	-	0.063	-	-
	n	16	16	16	12
*FH**	a	0.844	0.938	0.594	0.583
	b	0.156	0.063	0.406	0.417
	n	16	16	16	12
*GPI**	a	-	0.469	-	-
	b	-	0.094	-	-
	c	0.625	0.438	0.188	0.208
	d	0.125	-	0.344	0.333
	e	0.094	-	0.063	0.333
	f	0.094	-	0.406	0.042
	g	0.063	-	-	0.083
	n	16	16	16	12
*IDHP-1**	a	-	-	0.063	-
	b	-	0.083	0.250	0.042

		K. rhytiphora	*K. peronii*	*K. scalarina*	
Locus	**Allele**	**SA:RHY**	**SA:PER**	**SA**	**TAS2**
	c	1.000	0.667	0.656	0.875
	d	-	0.250	0.031	0.083
	n	14	12	16	12
*IDHP-2**	a	0.036	-	0.063	-
	b	0.929	0.531	0.125	-
	c	0.036	0.031	0.813	1.000
	d	-	0.438	-	-
	n	14	16	16	12
*MDH-1**	a	-	0.031	0.031	0.045
	b	1.000	0.969	0.031	0.227
	c	-	-	0.938	0.727
	n	16	16	16	11
*MDH-2**	a	0.094	-	-	-
	b	0.906	1.000	0.063	0.042
	c	-	-	0.938	0.958
	n	16	16	16	12
*PEP**	a	-	-	-	0.208
	b	-	-	0.219	0.417
	c	0.179	0.031	0.281	0.292
	d	0.786	0.438	0.438	0.083
	e	0.036	0.469	0.063	-
	f	-	0.063	-	-
	n	14	16	16	12
*PGDH**	a	0.107	-	-	-
	b	0.250	0.292	-	-
	c	0.643	0.667	0.083	0.042
	d	-	0.042	0.917	0.792
	e	-	-	-	0.125
	f	-	-	-	0.042
	n	14	12	12	12
*PGM**	a	0.031	-	-	0.417
	b	0.219	-	0.156	0.458
	c	0.563	-	0.813	0.125
	d	0.188	0.438	0.031	-
	e	-	0.531	-	-
	f	-	0.031	-	-
	n	16	16	16	12
*SOD**	a	-	0.031	-	-
	b	-	0.969	-	-
	c	1.000	-	-	-
	d	-	-	0.969	1.000
	e	-	-	0.031	-
	n	15	16	16	12

Table 4. Summary of genetic variability values at 16 loci in *Katelysia rhytiphora*, *K. peronii*, and two subpopulations of *K. scalarina*.

	K. rhytiphora	*K. peronii*	*K. scalarina*

Locus	SA:RHY	SA:PER	SA	TAS2
Mean sample size per locus	14.9±0.3	15.1±0.4	15.8±0.3	11.3±0.7
Mean number of alleles per locus	2.7±0.3	2.8±0.3	2.8±0.2	2.5±0.3
Polymorphism % (0.95)	68.75	75.00	81.25	62.50
Observed heterozygosity per locus	0.291±0.059	0.314±0.063	0.276±0.059	0.257±0.070
Expected heterozygosity per locus*	0.283±0.058	0.377±0.065	0.299±0.059	0.333±0.071

*unbiased estimate (Nei, 1977)

Table 5. Unbiased genetic distance (*D*) (above diagonal) and identity (*I*) values (below diagonal) (Nei, 1978) between *Katelysia rhytiphora, K. peronii*, and two subpopulations of *K. scalarina*.

	K. rhytiphora	***K. peronii***	***K. scalarina***	
	SA:RHY	**SA:PER**	**SA**	**TAS2**
SA: RHY	-	0.337	0.859	0.919
SA:PER	0.714	-	1.173	1.238
SA	0.424	0.309	-	0.068
TAS2	0.399	0.290	0.934	-

Table 6. Allele frequencies at six loci in six populations of *K. scalarina*. *n* = number of individuals. Allele homologies with Table 3, where known, are in parentheses.

Locus	Allele	TAS1	TAS2	TAS3	VIC	SA	WA
*DIA**	125	-	-	0.004	0.025	-	-
	120 (a)	0.103	0.092	0.081	0.075	0.107	0.051
	110 (b)	0.310	0.298	0.275	0.342	0.286	0.280
	100 (c)	0.413	0.447	0.449	0.308	0.429	0.364
	90 (e)	0.136	0.158	0.140	0.217	0.161	0.178
	75	0.022	0.004	0.051	0.033	0.018	0.121
	60	0.016	-	-	-	-	0.005
	n	92	114	118	60	56	107
*ESTD**	150	-	-	0.008	0.019	0.009	0.005
	120 (a)	0.183	0.157	0.171	0.179	0.155	0.141
	100 (b)	0.731	0.712	0.729	0.790	0.836	0.836
	65 (c)	0.075	0.127	0.083	0.012	-	0.018
	50	0.011	0.004	0.008	-	-	-
	n	93	118	120	81	58	110
*GPI**	145	0.082	0.065	0.060	0.050	0.018	0.047
	125 (c)	0.212	0.178	0.282	0.094	0.140	0.142
	100 (d)	0.321	0.300	0.312	0.331	0.333	0.392
	80 (e)	0.228	0.252	0.192	0.206	0.167	0.151
	55 (f)	0.114	0.152	0.124	0.294	0.316	0.255
	35 (g)	0.043	0.048	0.030	0.025	0.026	0.014
	n	92	115	117	80	57	106
*MDH-1**	165	0.063	0.064	0.042	0.012	0.035	0.037
	135 (b)	0.195	0.182	0.229	0.056	0.053	0.023
	100 (c)	0.742	0.754	0.729	0.926	0.912	0.936
	65	-	-	-	0.006	-	0.005
	n	95	118	120	81	57	109
*MDH-2**	140	-	-	-	-	-	0.005
	120 (b)	-	0.008	0.004	0.044	0.009	-
	100 (c)	0.995	0.992	0.992	0.956	0.991	0.991
	70	0.005	-	0.004	-	-	0.005
	n	93	118	120	80	57	107
*PGM**	125	0.011	0.008	0.004	-	-	-
	120 (a)	0.317	0.314	0.383	0.006	0.035	-
	100 (b)	0.548	0.610	0.533	0.086	0.096	0.046
	85 (c)	0.091	0.059	0.075	0.790	0.728	0.713
	75 (d)	0.032	0.004	0.004	0.111	0.140	0.222
	55	-	0.004	-	0.006	-	0.019
	n	93	118	120	81	57	108

Table 7. Genetic diversity statistics for six loci and six populations of *Katelysia scalarina*

Locus	F_{IS}	*P*	F_{ST}	*P*
*DIA**	0.177	0.003	0.008	<0.001
*MDH-1**	0.030	0.575	0.053	<0.001
*MDH-2**	-0.027	0.090	0.015	0.005
*GPI**	0.009	0.692	0.017	<0.001
*ESTD**	-0.030	0.195	0.013	<0001
*PGM**	0.043	0.217	0.298	<0.001

F_{IS} data analysed by t-tests of individual subpopulations against expected value of 0. F_{ST} data analysed by Monte-Carlo chi-square tests of allele frequency homogeneity.

Figure 1. Venerid clams included in this study. From left to right: *Katelysia rhytiphora* (52.7 mm shell length, from Portland Harbour, Victoria; *K. scalarina* (41.2 mm, from Macrae, Port Phillip, Victoria; *K. peronii* (43.7 mm, from St. Kilda, Victoria). Specimens from the Western Australian Museum collection of venerids.

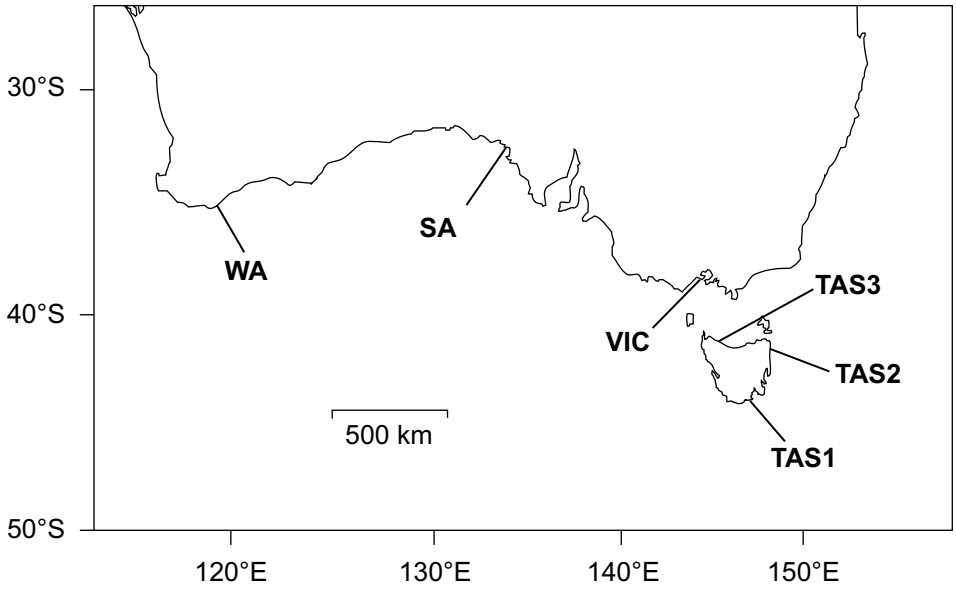

Figure 2. Southern Australia, showing approximate sample sites.

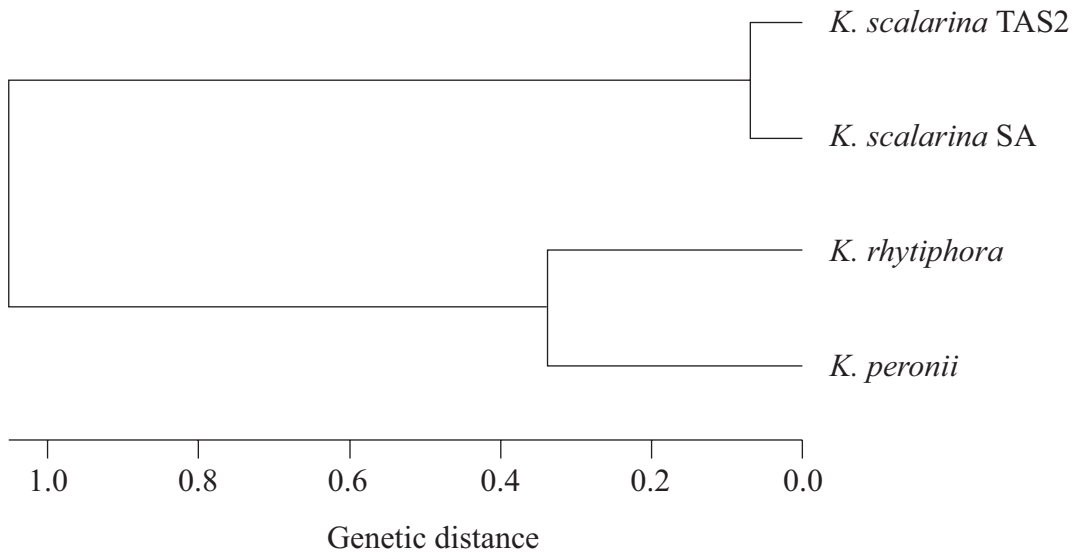

Figure 3. UPGMA cluster analysis of Nei's (1978) unbiased genetic distance (D) among three species of *Katelysia,* based on 16 allozyme loci.

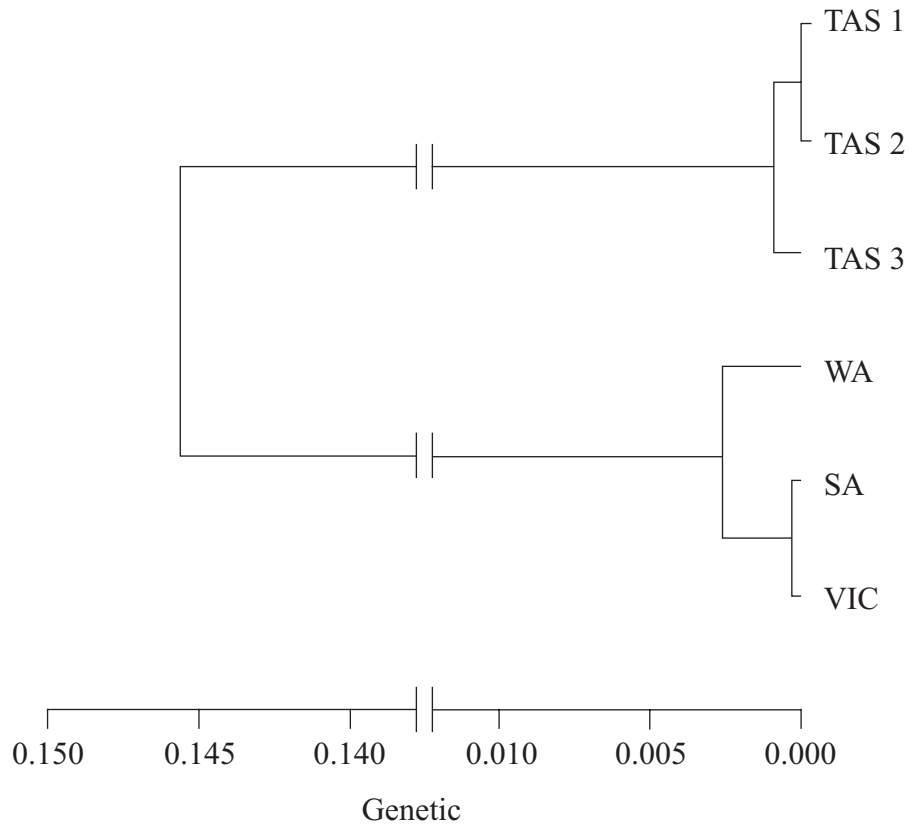

Figure 4. UPGMA cluster analysis of Nei's (1978) unbiased genetic distance (D) among six populations of *Katelysia scalarina,* based on six allozyme loci.

5.16 MANUSCRIPT 16

Genetic comparison of populations of a venerid clam *Ruditapes largillierti* (Philippi 1849) in Tasmania and New Zealand

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5.16.1 ABSTRACT

Three samples (20-22 clams per site) of *Ruditapes largillierti*, one from Tasmania, Australia (St Helens) and two from New Zealand (Whangateau Harbour, North Island and Croiselles Harbour, South Island) were compared for 12 allozyme loci. There were no diagnostic loci which could be used to discriminate unambiguously the Tasmanian population from the two New Zealand populations. Variability levels were high, with average expected heterozygosities per locus ranging from 0.268 to 0.278, and percent polymorphism from 58.33 to 66.67 based on a definition of polymorphism in which the most common allele has a frequency 0.95. These results are consistent with the view that *R. largillierti* is a New Zealand endemic which has been translocated to Tasmania (the only location from which it has been recorded in Australia). The two New Zealand populations were very similar (Nei unbiased genetic distance of <0.001). These results, although based on only two widely separated populations in New Zealand and with limited numbers of loci and small sample sizes, do not enable us to reject the null hypothesis of a single New Zealand stock.

5.16.2 INTRODUCTION

Ruditapes largillierti is considered to be endemic to New Zealand where it is distributed throughout that country including Stewart, The Chathams, Auckland and Campbell Islands (Cook in press). *R. largillierti* can grow to a length of 70 mm and a height of 50 mm and is found subtidally in both muddy and sandy substrates in shallow estuarine waters with high current flow (Cook in press). This species was previously known as *Venerupis largillierti*, a nomenclature still adopted by some Australian researchers (Paturusi, 1994). It occurs widely in Tasmania (Greenhill 1965) but has not been recorded from mainland Australia. Moreover, there is no evidence from the fossil record of its long term occurrence around mainland Australian although other venerid species including *Katelysia* spp. are well represented (G. Kendrick, Western Australian Museum, pers. comm. 1998). It was first observed in Tasmania in 1963 although Greenhill (1965) suggested that it could have been present earlier but misidentified as native *Katelysia* spp. Several other invertebrates in Tasmania, including a gastropod *Maoricolpus roseus* and a starfish *Patiriella regularis*, are considered to have originated from New Zealand in modern times (Greenhill 1965; Turner 1998). Translocation during the live oyster trade from New Zealand to Tasmania during 1880-1930 has been suggested as a likely opportunity for such translocations (Turner 1998).

Ruditapes largillierti is fished commercially in Tasmania in the St Helens inlet (Figure 1) and its aquaculture potential is being evaluated (Paturusi 1994; Kent *et al*. 1999; Manuscripts 2, 11). It is not commercially exploited in New Zealand although this could eventuate (R. Creese pers. comm. 1997). It is prudent to understand the population structure of venerid clams for fisheries management purposes or for managing translocations within a country in association with aquaculture (Soh et al. 1998). In a broader perspective, it has been asserted by Duda (1994),

who studied the genetic structure of the clam *Potamocorbula amurensis* in San Francisco Bay, that "high levels of variability could represent a universal characteristic of invading species". This has been the case with inadvertent and deliberate introductions respectively of Pacific oysters, *Crassostrea gigas*, into New Zealand (Smith et al. 1984) and Australia (English et al. 2000).

This paper reports the use of allozymes to assess the population structure of *Ruditapes largillierti* in Tasmania and New Zealand. This technique has proved useful for identifying genetically distinct, conspecific populations of venerid clams (Humphrey and Crenshaw, 1989). It complements an equivalent study (Soh et al. 1998) of *Katelysia scalarina,* the other major venerid clam species in Tasmania to be commercially exploited by fishermen (Manuscript 13) and which is also being evaluated for aquaculture or fisheries enhancement (Kent et al. 1998).

5.16.3 MATERIALS AND METHODS

Samples of *Ruditapes largillierti* were collected from three sites: one in Tasmania and one each from the North and South Islands of New Zealand (Figure 1; Table 1). Clams were transported live by road or air-freight in cool-boxes with ice-packs. On arrival at the laboratory, tissues were dissected and stored at -80°C in 2 ml microcentrifuge tubes. Adductor muscle and digestive gland tissue were kept separately.

Genetic analysis

Tissues were homogenised manually with 2 to 4 drops of distilled water and centrifuged for 5 min at 10,000 rpm; the supernatants were used for electrophoresis. Either starch or cellulose acetate gels were used. Starch gels used 9% Connaught starch with a discontinuous histidine-citrate buffer system run at 100 V for 4.5 h (gel buffer 0.005M histidine HCl pH 7.0; electrode buffer 0.41M trisodium citrate pH 7.0), the cellulose acetate gels were Helena Titan III plates run at 150 V for 1 h (gel and electrode buffer 0.075 M tris and 0.025 M citric acid, pH 7.0). Staining techniques were largely as in Richardson et al. (1986) and Hebert and Beaton (1989). Details of the enzymes used are given in Table 2. Heterozygote banding patterns were consistent with known quaternary structures (Ward et al. 1992).

Twelve loci were scored in this study. Where there were multiple loci, the locus encoding the fastest migrating allozyme is designated '1'. Alleles were lettered according to the anodal mobility, with 'a' going to the allele with the slowest mobility product.

Statistical analysis

Mean sample sizes, numbers of alleles, percent polymorphism (defining a locus as polymorphic if the most common allele had a frequency ≤ 0.95 or, as a separate estimate, ≤ 0.99), and heterozygosities (both observed and unbiased Hardy Weinberg expected values) were estimated by the BIOSYS-1 package (Swofford and Selander 1981). Allele frequency homogeneity across samples was tested by the randomised Monte Carlo chi-square procedure of Roff and Bentzen (1989), which obviates the need to pool rare alleles. For each test, 1000 randomisations of the data were carried out. BIOSYS-1 was used to calculate unbiased genetic distances between samples (Nei, 1978). When multiple tests of a single hypothesis were carried out, the standard Bonferroni procedure was applied. The P value for a specific test had to be less than, or equal to, $0.05/n$, where n is the number of tests (here equivalent to the number of variable loci), to be deemed statistically significant (for $n = 12$, adjusted critical P value is 0.004)

5.16.4 RESULTS

Electrophoretic patterns were similar across the three sites with no consistent evidence of divergence of the Tasmanian population from the New Zealand populations. Four 'private' alleles (those unique to a particular sample) were found in Tasmania, one in NZ 1 and three in NZ 2. These alleles were all rare (≤0.005) and probably represent sampling artefacts rather than true site differences (Table 3). All twelve loci were variable. Allele frequencies at all loci were homogeneous across sites after Bonferroni correction (Table 4).

There were no significant genotype deviations from Hardy-Weinberg expectations in any of the 12 valid tests (where a valid test is defined as one with an expected cell count ≤1, after pooling rare alleles as appropriate). The twelve valid tests were on the loci Aat, Gpi, Est-D and Dia, in each of three populations.

Levels of variation in each sample were high, despite quite small sample sizes, with an average of 2.6 to 2.8 alleles per locus, between 58.3% and 66.7% polymorphism (loci with the most common allele at a frequency ≤ 0.95) and observed heterozygosities per locus ranging from 0.260 to 0.290 (Table 5). The mean Hardy-Weinberg expected heterozygosity per locus was, for each population, very close to, and not significantly different from, the mean observed heterozygosity (P>0.05).Nei distance analyses indicated the three sites were genetically very similar (maximum Nei unbiased genetic distance was 0.005) (Table 6).

5.16.5 DISCUSSION

The overall pattern indicates that the Tasmanian population of *Ruditapes largillierti* is very similar to the two New Zealand populations which in turn were indistinguishable. Earlier, Greenhill (1965) had noted that the appearance of Tasmanian samples of this species was variable but was consistent with descriptions of New Zealand shells. Coastal gene flow mediated by stepping-stone migration between adjacent subpopulations probably accounts for the similarities between populations from North and South Islands. However, the short larval life of the species (only about 11-16 days; Kent *et al.* 1999) is likely to prevent gene flow across the Tasman Sea between Tasmania and New Zealand. Australian commercial penaeid shrimp species which have a similar larval duration to *R. largillierti* also do not occur in New Zealand. One of these species, *Melicertus plebejus*, has a broad Australian range extending from mainland coastal areas into Tasmanian inlets near Georges Bay. However, it should be noted that adult migration is a significant factor for this species (Kailola *et al*. 1993). The genetic similarities between the Tasmanian and New Zealand populations therefore are likely to reflect the genetic make-up of the original invading animals rather than gene flow, with the necessary corollary that that there has not been enough time or sufficient environmental differences for significant genetic drift or selection to have occurred in Tasmania. Similarly, Smith et al. (1986), Duda (1994) and English et al. (2000) found that exotic populations of bivalves in New Zealand, San Francisco Bay and Australia respectively, isolated this century from source populations presumed to be from Asia, could not be distinguished electrophoretically from samples drawn the putative source populations.

As several other Tasmanian venerid species also occur in abundance along the mainland Australian coast (Lamprell and Whitehead 1992), and as *Ruditapes largillierti* has a broad distribution in New Zealand, it is likely that this species would become established if introduced to mainland Australia. The Bass Strait thus appears to constitute a genetic barrier between Tasmania and mainland Australia. This also is the case with the endemic *Katelysia scalarina* which has genetically distinct populations either side of Bass Strait (Soh *et al*., 1998).

The assumption that *Ruditapes largillierti* was introduced into Tasmania from New Zealand is based on the absence of palaeontological (fossils and middens) evidence of its long-term occurrence in Tasmania (E. Turner, Tasmanian Museum, pers. comm., 1998) and on the lack of genetic differentiation of stocks from the two countries. Exactly when the introduction occurred is unclear. During the period 1880-1930 several marine species including the European lobster *Homarus gammarus* were deliberately introduced into Tasmania (Turner 1998), although no historical records indicate deliberate introduction of *R. largillierti.* It is possible that the introduction was inadvertent during the live oyster trade from New Zealand during that period. This has been postulated as the cause of introductions of other New Zealand invertebrates into Tasmania (Turner 1998). However, as the first records of *R. largillierti* in Tasmania are from 1963 (Greenhill 1965), it is not possible to confirm this.

All three populations of *R. largillierti* are genetically highly variable, with average observed heterozygosities per locus ranging from 0.260 to 0.290. The average heterozygosity for molluscs, assessed from 105 species, is around 0.145 (Ward *et al*. 1992). The high heterozygosity value for the New Zealand and Tasmanian populations is consistent with the theory postulated by Duda (1994) of invading populations being characterised by high levels of variability.

The lack of differentiation between the New Zealand populations of *R. largillierti*, as indicated by the very small Nei distance, might suggest that these can be managed as a single stock. However, the standard caveats must be made that it takes only a little gene flow to render subpopulations effectively panmictic and that the analysis of further allozymes or further animals might reveal significant differences. Moreover, while allozyme electrophoresis has proved very useful in delineating the population structure of venerid clams (Humphrey and Crenshaw 1989), the population structure of *R. largillierti* in New Zealand may warrant re-examination involving additional sites and more powerful DNA techniques as recently applied to other clam species (Caporale *et al.* 1997).

5.16.6 ACKNOWLEDGMENTS

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5.16.7 REFERENCES

Cook, S. *ed*. In press: New Zealand Coastal Invertebrates. Canterbury University Press, Christchurch, New Zealand.

Caporale, D. A.; Beal, B. F.; Roxby, R.; Van Beneden, R. J. 1997: Population structure of *Mya arenaria* along the New England coastline. *Molecular Marine Biology and Biotechnology 6:* 33-39.

Duda, T. F. Jr. 1994: Genetic population structure of the recently introduced Asian clam, *Potamocorbula amurensis*, in San Francisco Bay. *Marine Biology 119*: 235-241.

English, L. J.; Maguire, G. B.; Ward, R. D. 2000: Genetic variation of wild and hatchery populations of Pacific oyster, *Crassostrea gigas* (Thunberg), in Australia. *Aquaculture* 187(3-4): 283-298.

Greenhill, J. F.: New records of marine Mollusca from Tasmania. *Papers and Proceedings of the Royal Society of Tasmania* 99: 67-69.

Hebert, P. D. N.; Beaton M. J. 1989: Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratory, Beaumont, Texas.

Humphrey, C. M.; Crenshaw, J. W. Jr. 1989: Clam genetics. *In*. Manzi, J. J.; Castagna, M. *ed*. Clam mariculture in North America. Developments in Aquaculture and Fisheries Science, vol 19. Elsevier, Amsterdam. 323-356.

Kailola P. J.; Williams, M. J.; Stewart, P. C.; Reichelt, R. E.; McNee, A.; Grieve, C. 1993: Australian Fisheries Resources. Bureau of Resource Sciences and the Fisheries Research and Development Corporation, Canberra, Australia.

Kent, G. N.; Maguire, G. B.; Duthie, I. 1999: Spawning, settlement and growth of the New Zealand Venerid *Ruditapes largillierti* (Philippi 1849) in culture. *New Zealand Journal of Marine and Freshwater Research* 33: 55-62.

Kent, G. N.; Maguire, G. B.; John, M.; Cropp, M.; Frankish, K. 1998: Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina* (Lamark 1818). *Journal of Shellfish Research, 17(4):* 1065-1070.

Lamprell, K.; T. Whitehead. 1992: Bivalves of Australia. Volume 1. Crawford House Press, Bathurst, NSW, Australia.

Nei, M. 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics 89*: 583–590.

Paturusi, A. 1994: Effects of stocking density and initial size on the performance of the clam *Venerupis largillierti* (Phillipi, 1849) in experimental cages in Georges Bay, Tasmania. Unpublished Thesis, University of Tasmania, Launceston.

Richardson, B. J., Baverstock, P. R.; Adams, M. 1986: Allozyme electrophoresis. A handbook for animal systematics and population studies. Academic Press, New York, NY.

Roff, D. A.; Bentzen, P. 1989: The statistical analysis of mitochondrial DNA

polymorphisms: chi-square and the problem of small sample. *Molecular Biology. Evolution 6:*539-545.

Smith, P. J.; Ozaki, H.; Fujio, Y. 1986: No evidence for reduced genetic variation in the accidentally introduced oyster *Crassostrea gigas*. in New Zealand. *New Zealand Journal Marine of Freshwater Research 20:* 569-574.

Soh, S. W. L.; Maguire, G. B.; Ward, R. D.1998: Genetic studies of the venerid clam genus *Katelysia. Journal of Shellfish Research, 17(4):* 1057-1064.

Turner, E. 1998: A brief history of the oyster, scallop and rock lobster industries in Tasmania, and their relationships to the Port of Hobart to 1985. Unpublished manuscript, Tasmanian Museum, Hobart, Australia.

Swofford, D. L.; Selander, R. B. 1981: BIOSYS-1: A FORTRAN program for the

comprehensive analysis of electrophoretic data in population genetics and

systematics. *Journal of Heredity 72:*281-283.

Ward, R. D.; Skibinski, D. O. F.; Woodwark, M. 1992: Protein heterozygosity, protein structure and taxonomic differentiation. *Evolutionary Biology 26:*73–159.

5.16.8 TABLES AND FIGURES

Table 1. Sample sites for *Ruditapes largillierti*.

Site	Region	Abbreviation	Date collected	n
St Helens	Tasmania (North -East), Australia	Tas	1995	22
Whangateau Harbour	Warkworth, North Is, New Zealand	NZ1	1995	20
Croiselles Harbour	Nelson, South Is, New Zealand	NZ2	1995	20

Tas St Helens (Inlet) 148°, 20' E; 41°, 15' S
nearest town = St Helens (to the west)

NZ1 Whangateau Harbour 174°, 45' E; 36°, 19.5' S
nearest town = Warkworth (to the west).

NZ2 Croiselles Harbour 173°, 39.5' E; 41°, 06.5' S
nearest town = Nelson (to the South).

Table 2. Details of enzymes used in this study. Tissue: D - digestive gland, A - adductor muscle. Gel: ca - cellulose acetate, s - starch (see text). Multiple loci encoding for the same enzyme are designated by consecutive numbers, with '1' denoting the fastest migrating system.

Enzyme	EC number	Locus	Tissue	Gel System	Subunit number
Aspartate aminotransferase	2.6.1.1	Aat	A	starch	2
Esterase D	3.1.1.-	EstD	A	starch	2
Isocitrate dehydrogenase	1.1.1.42	Idh-1, Idh-2	D	c.a.	2
Malate dehydrogenase	1.1.1.37	Mdh-1, Mdh-2	A	starch	2
Peptidase (val-leu)	3.4.11.-	Pep-vl	D	c.a.	2
Peptidase (leucine-proline)	3.4.11.-	Pep-lp	D	c.a.	2
6-Phosphogluconate dehydrogenase	1.1.1.44	6Pgdh	D	c.a.	2
Glucosephosphate isomerase	5.3.1.9	Gpi	A	starch	2
Phosphoglucomutase	5.4.2.2	Pgm	A	c.a.	1
Diaphorase	1.8.1.4	Dia	D	starch	1

Table 3. Allele frequencies, sample sizes (*n* = number of individuals sampled), and locus heterozygosities (het. = Hardy-Weinberg expected heterozygosity) for three *Ruditapes largillierti* populations based on 12 loci.

Locus	allele	Tas	NZ1	NZ2
Idh-1	a	0.023	-	0.025
	b	0.977	1.000	0.975
	N	22	20	20
	het.	0.044	0	0.049
Idh-2	a	0.955	0.950	0.925
	b	0.045	0.050	0.075
	N	22	20	20
	het.	0.087	0.095	0.139
6Pgdh	a	0.909	0.850	0.875
	b	0.091	0.125	0.075
	c	-	0.025	0.050
	N	22	20	20
	het.	0.165	0.261	0.226
Pgm	a	-	-	0.050
	b	0.818	0.825	0.775
	c	0.023	0.125	0.175
	d	0.136	0.050	-
	e	0.023	-	-
	N	22	20	20
	het.	0.311	0.301	0.366
Aat	a	0.295	0.130	0.167
	b	0.386	0.565	0.667
	c	0.318	0.304	0.167
	N	22	23	24
	het.	0.662	0.571	0.500
Pep-lp	a	0.023	-	0.100
	b	0.909	0.825	0.850
	c	0.023	0.150	-
	d	0.045	0.025	0.050
	N	22	20	20
	het.	0.170	0.296	0.265
Pep-vl	a	-	0.025	-
	b	1.000	0.975	1.000
	N	22	20	20
	het.	0	0.049	0
Gpi	a	0.045	0.063	0.021
	b	0.318	0.354	0.292
	c	0.591	0.542	0.667
	d	0.045	0.042	0.021
	N	22	24	24
	het.	0.545	0.576	0.470
Est-D	a	0.023	-	-
	b	0.568	0.565	0.563
	c	0.409	0.435	0.417
	d	-	-	0.021

Locus	allele	Tas	NZ1	NZ2
	N	22	23	24
	het.	0.509	0.491	0.510
Mdh-1	a	0.955	1.000	1.000
	b	0.045	-	-
	N	22	24	24
	het.	0.087	0	0
Mdh-2	a	-	0.021	-
	b	0.977	0.979	0.958
	c	0.023	-	0.042
	N	22	24	24
	het.	0.044	0.041	0.080
Dia	a	0.023	-	-
	b	0.136	0.021	0.083
	c	0.318	0.125	0.229
	d	0.386	0.646	0.500
	e	0.136	0.188	0.146
	f	-	0.021	0.042
	N	22	24	24
	het.	0.712	0.531	0.668

Table 4. Probabilities of homogeneity among the three populations of *Ruditapes largillierti* for 12 loci. Bonferroni corrected *P* value is 0.004.

Locus	chi-square	P
Aat	9.345	0.053
Dia	15.328	0.095
Est-D	4.050	0.970
Gpi	2.372	0.886
Idh-1	0.975	1.000
Idh-2	0.390	0.900
Pep-vl	2.117	0.642
Mdh1	4.427	0.099
Mdh2	3.879	0.603
Pgdh	2.792	0.639
Pgm	17.187	0.010
Pep-lp	15.367	0.011

Table 5. Summary of genetic variability in the three collections (± standard errors) for 12 loci in *Ruditapes largillierti.* Two definitions of polymorphism used, one where the most common allele has a frequency ≤0.95, and one where the most common allele has a frequency ≤0.99.

Sample	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic 0.95	Percentage of loci polymorphic 0.99	Mean Hardy Weinberg expected heterozygosity per locus (Observed)	Mean Hardy Weinberg expected heterozygosity per locus (Expected)
Tas	22.0	2.83±0.34	58.33	91.67	0.277±0.072	0.278±0.075
NZ1	21.8	2.58±0.34	66.67	83.33	0.290±0.073	0.268±0.066
NZ2	22.0	2.67±0.33	66.67	83.33	0.260±0.063	0.273±0.066

Table 6. Matrices of genetic distance (12 loci) among three collections of *Ruditapes largillierti*. Above diagonal, Nei (1978) unbiased genetic distance, below diagonal, Nei (1972) genetic distance.

	Tas	NZ 1	NZ 2
Tas	-	0.005	0.004
NZ1	0.014	-	<0.001
NZ2	0.013	0.008	-

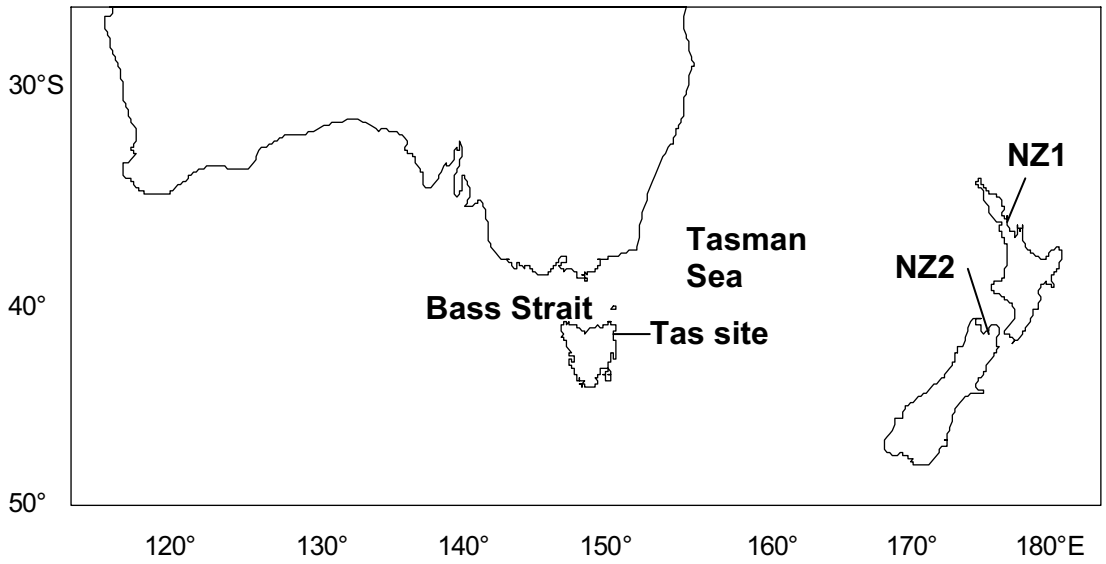

Figure 1. Map of southern Australia and New Zealand (NZ) showing approximate sample locations. (Tas = Tasmania; NZ = New Zealand)

5.17 MANUSCRIPT 17

Summary of health studies related to FRDC Clam Project, and relationship to CRC for Aquaculture Project A.2.1. (Early Mollusc Health)

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5.17.1 BACKGROUND

Sections of *Katelysia scalarina* clams from gonad development samples, processed as part of the FRDC project Enhancing Tasmanian Clam Resources, and hatchery records of several experimental *K. scalarina* hatchery spawnings from the project and commercial hatchery spawnings were examined as part of a CRC for Aquaculture project on early mollusc health. Herpes virus infection was diagnosed as a cause of sudden deaths in 7 to 8 day old *K. scalarina* larvae. As well as a high level of intra-nuclear inclusions in all larvae from the affected spawning by day 8, and herpes virus confirmation by electron microscopy, the CRC studies subsequently demonstrated low numbers of similar inclusions in testes of some broodstock from that spawning. No inclusions were recognised in other tissues, suggesting that testes was a likely reservoir of infection.

The availability of stained whole body sections for the FRDC project from several years of monthly gonad and condition monitoring of this species allowed retrospective assessment of the incidence, duration and seasonal pattern of testicular inclusions at two sites, including the site of origin of the broodstock for the confirmed herpes virus infection. These findings, together with findings from the larvae and analysis of clinical observations from hatchery records, will be reported in a paper in preparation entitled *Larval mortality associated with Herpes virus infection in the Australian clam* Katelysia scalarina *from Tasmania*, to be submitted to Diseases of Aquatic Organisms.

The incidence and distribution of other pathogens and parasites in the adult clams was recorded concurrently. These will be compared with previous single point samples from this species, and reported separately in a paper in preparation entitled *Pathogens and parasites of the clam* Katelysia scalarina *in Tasmania*, (to be submitted to Molluscan Research).

A brief summary of both studies is given here.

5.18 MANUSCRIPT 18

Pathogens and parasites of the clam *Katelysia scalarina* in Tasmania

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5.18.1 METHODS

The availability of histological sections from over 700 *K. scalarina* from this FRDC project, for retrospective assessment of herpes virus like intra-nuclear inclusions, allowed concurrent assessment of the incidence of other pathogens of potential significance in clam health in these populations. Sections available included two years of monitoring of 20 adult clams per month from Anson's Bay, and four years of monitoring of 10 adult clams per month at Little Swanport (Manuscript 1)**.** All sections were a single cross section of sea water formalin fixed shucked whole clams, routinely processed and stained by haematoxylin and eosin, which included gonad and foot, usually gills, and sometimes gut. All sections were scored for herpes virus like inclusions in testes and other tissues, and other pathogens. Apparent differences in incidence between sexes and between sites were tested using Fisher's exact test.

5.18.2 RESULTS

A total of 719 clams, from two sites, were examined, of which 320 were adult males, 399 adult females.

As well as occasional herpes type inclusions (to be reported elsewhere), the following pathogens and parasites were detected.

Flukes

Three types of parasite believed to be intermediate stages of digenean flukes were found, these being by far the most common parasites detected.

Small sporocyts stages and their daughter products cercaria, representing first host stages of digenean flukes, were identified in gill and or gonad of 19 of 362 clams (5.2%) from Anson's Bay, and 29 of 357 clams (8.1%) from Little Swanport. These conform to immature fluke stages usually referred to as *Bucephalus*-like, though the actual fluke type has not yet been identified. These were more common in the gill than gonad, and dual site infections occurred, with 4 of the 10 clams with gonad infection showing flukes in both gills and gonad. The parasites in gills and gonad appeared identical suggesting that the same species was invading both tissues. In infected clams, large numbers were usually present, resulting in effective complete castration from gonad infections to the extent that only occasional gonad cells were present to indicate the host sex, and presumably compromised circulatory function from heavily parasitised gills. Haemocyte reactions in infected gills were variable, with eosinophilic granular cells dominating effuse reactions. Many other gills showed similar heavy reactions, suggesting possible prior fluke infection of these gills.

Two types of apparent metacercaria or second host stages of digenean flukes were seen. These were larger parasites than the above, and differed from each other in both appearance and tissue location.

Apparent metacercarial infection was found in tissues furthest from the foot section, in an area adjacent to that of the shell hinge. These were identified in 27 clams, but may have been present in a larger number of clams as this area was not retained in all sections. These usually appeared to embedded just under mantle tissue, which is not uncommon for trematode metacercaria (Lauckner, 1983). However in some clams it was unclear if parasites were also present within gut lumen near this site, which is suggestive of turbellarian infection.

The third presumed trematode was of a similar size but different appearance, and was encysted, usually within the foot muscle, in 136 of the Ansons Bay clams (37.6%), and 230 from Little Swanport (64.4%). Several such flukes were present in the longitudinal section of foot muscle in most infected clams. Host reaction was variable but often significant and dead flukes, surrounded by intense host reaction, were not uncommon. In one clam the reaction round dead flukes constituted a large abscess.

Metacercaria occasionally extended into the peri-gut, gonad or kidney region.

All flukes are still to be identified. Identification could at best be to family level as identification of fluke species from intermediate hosts alone is uncertain.

Statistical analysis of fluke data:

This analysis of the presence of the parasites in foot muscle in clams at the two sites indicates that there is a probability of 0.6002×10^{-12} of achieving the observed result by random chance. The relationship between site and the foot muscle parasites worms is highly significant. Analysis of foot muscle parasites infestation and sex indicates that there is no significant relationship between sex and the presence of these parasites (P=0.599).

Analysis of the interaction of sex and site indicated that the apparent differences in sex ratio between sites was not significant, with a 9% chance of achieving this result by random chance, and no significant relationship between sex and foot muscle parasites.

Data for the other flukes (i.e. no flukes, gill/gonad infection, infection near the shell hinge), when analysed with data for site (AB, CD), sex (M, F) and foot muscle parasites (Y, N) indicate no significant relationship between the occurrence of these flukes and site or sex, and no association between foot infestation and the other flukes.

Other pathogens and parasites

Other much larger unidentified helminth parasites were seen as single examples in the gut or gonad interstitial tissue of four clams, three from Little Swanport and one from Ansons Bay. Most appeared live. All elicited a host reaction but this was more marked to dead helminths, with the reaction to one dead helminth apparently including syncitial giant cells.

In general, the interstitial tissue of many of these clams, especially those with parasite infections, showed a uniform mixed diffuse haemocytosis, with eosinophilic granular cells dominating and a high level of brown cells and other evidence of a high cell turnover. Increased levels of this type of reaction were common round dead and sometimes live parasites, and focal abscessation was also seen occasionally without the cause being obvious in section. In one occasion crustacean fragments were present in a gill abscess.

Fine intracellular bodies typical of *Rickettsia* or *Chlamydia* were seen twice in gut cells of clams from Ansons Bay, and twice in gill epithelia and once in kidney ducts in Little Swanport clams. One similar body was seen in the testes, though the identity is uncertain. However, this

may have been an underestimation of the incidence in gut, as this organ was not present in all sections. The *Rickettsia*-like organisms appear similar to those previously reported in gut cells in Tasmanian oysters. Rickettsia in clams has been reported elsewhere, with infection of gills more common than in oysters (reviewed by Bower et al., 1994).

Coccidial parasites resembling *Pseudoklossia* species were seen in at least three kidneys from this study.

Occasional larger bodies with some resemblance to *Rickettsia* infected cells were seen in gill and interstitial tissue, but re-examination suggests they are unlikely to be related. Neither their identity or significance is known at this stage. Similar bodies seen in one coccidia infected kidney suggests they could be a coccidian stage, but there is no other evidence to support this at this stage.

Small numbers of an unidentified protozoan parasite were seen in the epithelium of gut tubules of one clam. There were intra-cellular and similar to the intracellular ciliates described from mussels (See Bower et al., 1994)

Small refractile spore like bodies resembling haplosporidia necrotic debris and a marked host reaction were seen in occasional tubules of two testes. At this stage it is not known if these are parasites of the clam, or hyperparasites of flukes, as has been reported occasionally of a number of fluke species (reviewed by Lauckner, 1983). One of the clams showed possible fragments of dead flukes in the testes, and the other gonad clearly showed fluke sections plus an extensive reaction, as well as a focus of spores.

5.18.3 SUMMARY AND DISCUSSION

In common with other clam species, digenean flukes appear to be the most common parasites of *K. scalarina* in Tasmania, with clams acting as both a first stage and second stage host. However, while shortened life cycles with both sporocysts / cercaria and metacercaria occurring in the same species, and even the same host, are not unknown, data from other species suggests this is likely to represent three separate species (reviewed by Lauckner, 1983). High levels of infection of either stage may be highly deleterious, depleting energy reserves and possibly altering behaviour to increase exposure to predators. Low levels of infection without significant compromisation of the host are very common world-wide. The likely effect of these parasites on the potential of *K. scalarina* as an aquaculture species, is dependent on the levels found. Differences between sites, as found for parasite embedded in foot muscle, may be significant factors in stock or site selection. As the samples from the two sites were collected over different time spans, annual variations may also have influenced these results.

The other parasites reported herein are either of unknown potential in an aquaculture environment, but at low levels, or are recognised low grade pathogens such as *Rickettsia* and which are similar to those present in other local aquaculture species, without ill effect at the levels seen.

The herpes virus detected in testes of adult clams and epithelial cells of larvae (reported elsewhere) has the potential to prevent hatchery rearing of this species, unless hatchery conditions to avoid infection and or early mortality are developed. The effect on adult clams appears minimal.

5.18.3 REFERENCES

Bower, S.M., McGladdery, S.E., and Price, I.M., 1994. Synopsis of infectious diseases and parasites of commercially exploited shellfish. Annual Review of Fish Diseases 4:1-199.

Lauckner, G. 1983. Disease of Mollusca: Bivalva. Diseases caused by Metazoan agents Trematoda. In *Diseases of Marine Animals, Volume II*. (O Kinne ed) pp 632-750. Biologische Anstalt Helgoland, Hamberg.

5.19 MANUSCRIPT 19

Notes on Herpes virus associated larval mortality in the Australian clam *Katelysia scalarina* in Tasmania

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5.19.1 ABSTRACT

The histological and electron microscopic appearance of affected larvae and testes will be reported in full elsewhere.

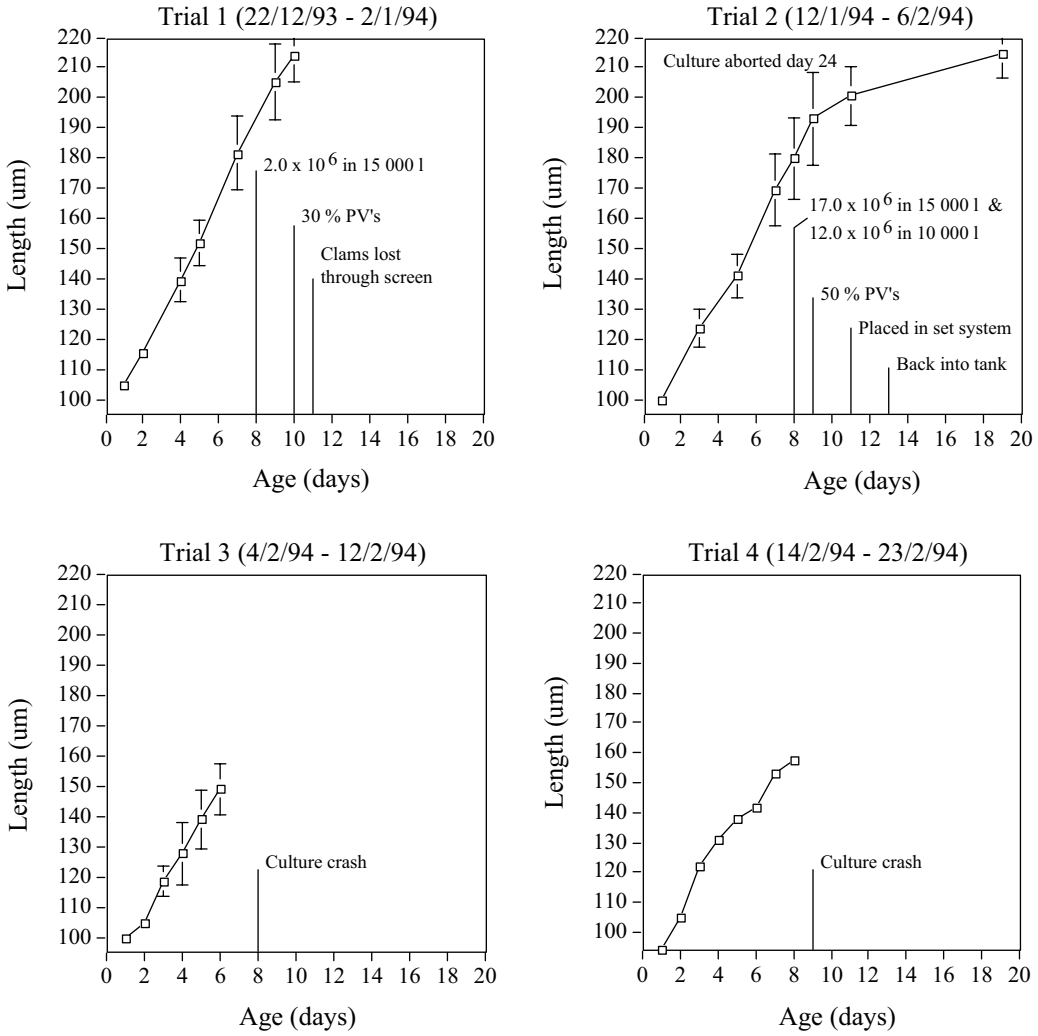
Records from three hatcheries showed that from 11 artificial spawnings, 6 showed catastrophic mortality at day 7 to 9, while in four batches a significant proportion of larvae survived to settlement but did not settle well. One spawning showed heavy mortality at day 4, which was diagnosed as related to bacterial overload.

The dates, outcomes and growth curves of five project related spawnings are shown in Figure 1. Larval rearing temperatures for Trials 1-4 fluctuated slightly over the range 21-25.5°C, with little overall difference between batches and no apparent relationship of temperature to larval success. Broodstock were drawn from sites not included in the monthly monitoring program (Cockle Creek (Trials 2 and 3), or Swanwick and Mussel Roe Bay (Trial 4).

Five spawnings at another hatchery included two from March 1995 and two from February 1996, which crashed at day 7 to 8, and one from October 1995 which survived through to settlement but with some mortalities on day 14 and poor settlement. Spawning of another clam species in this hatchery was unaffected. All broodstock were from the Ansons Bay monitoring site, but held in the hatchery for up to one month pre-spawning. Temperatures varied from 13.5-19°C, with the more successful batch run at ambient 16-17°C.

All of the batches which crashed at day 7 to 8, and the batch which crashed at day 4 were spawned in early autumn (February to March - while those surviving to were spawned earlier, from October to Mid-January.

Hatchery notes were compared from Trials 1 and 2 which survived to settlement, and Trials 3 and 4, which crashed at day 7-9.



Mean growth expressed as length of *Katelysia scalarina* larvae over 4 trials conducted in 1994
Note:
1. PV = Pediveliger larvae
3. Values are mean ± standard deviation (n=20 to 38)

Figure 1. Summary of four larval rearing trials with *K. scalarina* (see Kent et al., 1998).

5.19.2 REFERENCE

Kent, G.N., Maguire, G.B., John, M., Cropp, M., and Frankish, K., 1998. Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina* (Lamark 1818). Journal of Shellfish Research, 17(4): 1065-1070.

6.0 BENEFITS

K. scalarina

The research on growth and age of clams at three significant locations has assisted greatly with the sustainable management of this fishery as has the research on natural variables that affect mortality (eg predators, parasites and low salinity) and growth (eg density, beach position and natural diet). Genetic analyses are helpful in dealing with translocation and population management issues.

Commercial production of hatchery-produced *K. scalarina* spat was not successful and will require more trials by industry if demand for spat occurs. The broodstock, larval and disease research covered in this report will assist greatly in these efforts. (Please note that the disease research arose largely out of CRC for Aquaculture funding but relied significantly on samples and histology produced by this FRDC research grant.)

Large scale growout trials with industry could not be carried out because of the lack of hatchery-produced spat. However, the growout trials with wild spat were very useful in assessing limiting factors and optimising management variables. Clearly, this species has not proved to be suitable for farming at a range of Tasmanian Pacific oyster leases.

Ruditapes* (=*Venerupis*) *largillierti

The more limited range of research that could be sustained within this grant on this inadvertently introduced New Zealand clam shows that it has promise for enhancement and aquaculture. The results from this project have created interest among NZ researchers. However, further development of technology for subtidal clam technology would be needed (use of protective mesh over enhanced scallop beds, as pioneered more recently by NSW Fisheries researchers may be a useful approach). During the project a massive spatfall occurred within the key fishery (St Helens, Tasmania) and such occurrences may negate the need for enhancement with this species.

Scientific benefits

The project provided biochemical tools for clarifying misidentifications of *K. scalarina*, *K. rhytiphora* and *K. peronii* as well as establishing genetic relationships among southern Australian populations of *K. scalarina* and Tasmanian and New Zealand populations of *Ruditapes largillierti*. (Note that the "accepted" scientific name, within Australia for this last species, changed during the course of this project.) The project also yielded or fostered well received Honours, Masters qualifying, Masters and a PhD projects and refereed publications have/are appearing in the scientific literature.

Original proposed benefits

The project has contributed to the sustainability of the *K. scalarina* fishery but not to its expansion. The risk of adverse effects on consumers has been greatly reduced by facilitation of an extension of the Pacific oyster quality assurance program to Tasmanian clam fisheries. Beach clam fisheries have proved to be very difficult to manage worldwide because of erratic recruitment and mass mortality, commercial/recreational fisher conflict and biotoxin problems (McLachlan et al., 1996).

Hatchery operators, fishers and oyster farmers are now far more knowledgable about *K. scalarina.* Environmental managers are now better informed about the potential impact of clam fishing on bird life such as oystercatchers.

The researchers within the grant were successful in fostering awareness of clams as a novel menu item for restaurants.

To date the project has not been successful in fostering diversification of the Australian molluscan aquaculture industry. However, close collaboration occurred with a parallel project on farming clams in New South Wales and now significant pilot scale commercial initiatives are being undertaken by oyster farmers there using *Tapes dorsatus* (see Nell et al., 1995, Nell and Paterson, 1997, Paterson and Nell, 1997). The fact that different clam species show relatively different degrees of promise for aquaculture is not a novel result.

7.0 FURTHER DEVELOPMENT

Until progress is made with avoiding herpes - virus induced mortality with *K. scalarina* larvae or better methods emerge for farming subtidal clams, there are not likely to be major commercial initiatives occurring with enhancement of *K. scalarina* fisheries or aquaculture of *Ruditapes* (=*Venerupis*) *largillierti* respectively.

8.0 CONCLUSION

As indicated in sections 1 and 7, there proved to be major biological constraints on achieving the commercial goals. In general, this is an inherent risk with new species projects. Specifically, this project sought to evaluate the potential of these two clam species. The conclusion that the potential is relatively limited biologically or constrained by current technology is an answer to a research question rather than constituting project failure.

The original grant application had a wide range of specific objectives (section 4) and very substantial progress was made. The outcomes in relation to these objectives are listed below and given in greater detail in Appendix 3.

1. **Spawning seasons** *(objective achieved)*

2. **Meat condition** *(objective achieved)*

3. **Spat production** *(objective partly achieved)*

This was the major "failure" of the project - small numbers of *K. scalarina* spat could be produced in research trials but not by commercial operators on a scale that would allow cooperating fishers to undertake the large scale reseeding trials they proposed.

4. **Larval and nursery experiments** *(objective partly achieved)*

A range of trials were undertaken and led to success with broodstock conditioning, thermal spawning stimuli, hormonal induction of spawning of males (strip spawning was not successful), fecundity estimates, larval rearing, settlement strategies and (for *Ruditapes largillierti*) land and sea-based nursery phases. *K. scalarina* remains a challenging hatchery species although considerable progress has been made. (See Manuscripts 3-4.)

5 and 6. **Growout trials and variables** *(objectives largely achieved)*

Key variables were addressed although the failure of commercial larval batches prevented large scale growout trials with commercial fishers.

7. **Importance of sediment characteristics** *(objective partly achieved)*

Because of the distance from staff locations to appropriate *K. scalarina* locations and local sedimentation rates in potential sites, the logistics of addressing this question with plot trials were not favourable. However other techniques adopted were useful (covariance analyses, with sediment characteristics within growout trails).

A major trial was established with wild caught juvenile *R. largillierti* in replicate fish boxes, containing different sediment types, linked via a recirculating system to a 5 m diameter pool at the University of Tasmania's Launceston campus. A second 5 m diameter pool was used to produce algal blooms which were regularly pumped into the recirculating system. Unfortunately, despite considerable effort and expense, low survival and growth rates led to the abandonment of this trial.

In summary, this was one of the less successfully addressed objectives within the grant.

8. **Gut contents** *(objective achieved for K. scalarina*).

9. **Age determination** *(objective achieved)*

10. **Clam genetics** *(objective achieved)*

11. **Salinity tolerance** *(objective achieved for K. scalarina)*

12. **Public awareness of clams** *(objective partly achieved)*

In addition to writing popular articles for fisheries and aquaculture publications (see Appendix 3), press interviews were also given on a popular fishing program on Sydney radio. As well, grant staff contributed material for a major food column on clams (Appendix 3). However, as the grant did not lead to increased production of clams, a more intensive effort aimed at increased demand to match increased production was not attempted.

13. **Foster quality assurance program** *(objective achieved)*

14**. Help develop an optimum management regime** *(objective achieved)*

9.0 REFERENCES

McLachlan, A., Dugan, J.E., Defeo, O., Ansell, A., Hubbard, D.M., Jaramillo, E. and Penchaszadeh, P.E., 1996. Beach clam fisheries. Annual Review of Oceanography and Marine Biology, **34**: 163-232.

Nell, J.A., O'Connor, W.A., Hand, R.E. and McAdam, S.P., 1995. Hatchery production of diploid and triploid clams, *Tapes dorsatus* (Lamarck 1818): a potential new species for aquaculture. Aquaculture, **130**: 389-394.

Nell, J. A. and Patterson, K. J. 1997. Salinity studies on the clams *Katelysia rhytiphora* (Lamy) and *Tapes dorsatus* (Lamarck). Aquaculture Research, **28**: 115-119.

Paterson, K. J. and Nell, J. A., 1997. Effects of different growing techniques and substrate types on the growth and survival of the clams *Tapes dorsatus* (Lamarck) and *Katelysia rhytiphora* (Lamy). Aquaculture Research, **28**: 707-715.

Spencer, B.E., Edwards, D.B. and Millican, P.F., 1992. Protecting Manila clam (*Tapes philippinarum*) beds with plastic netting. Aquaculture, **105**: 251-268.

10.0 APPENDICES

APPENDIX 1: INTELLECTUAL PROPERTY

The program has not produced any confidential information and all data have been made freely available to the industry and researchers. During the project very detailed 6 monthly reports were provided to Tasmanian government fisheries research collaborators in this project, for distribution to clam fishers. A clam R&D workshop was also held. Researchers often visited fishers or hatchery operators (mostly UTAS staff) or had meetings with the fishers (mostly Tas. DPIF staff). There has been close interaction and information transfer between researchers and commercial bivalve hatchery operators.

APPENDIX 2: STAFF

Name	Position	Institution	Time (%)	Qualifications
Dr Greg Maguire	Senior Lecturer	UTAS	20	B Sc (Hon), PhD
Dr Lynda Bellchambers	(PhD student)	UTAS	100	B Sc (Hon), PhD
Dr A. Richardson	Assoc. Professor	UTAS	5	B Sc (Hon), PhD
As'ad Paturusi	Masters student M Appl Sc (Aqua)	UTAS	100 (1 year)	B Fish Tech,
Shirlena Soh	Honours student	UTAS	100 (1 year)	B Appl Sc (Hon)
Greg Kent	Masters Qual student	UTAS	10	Ass. Dip, B App Sc
Various	Casual Technical officers	UTAS	10	Dip Appl Sc,
Will Zacharin/ Sean Riley	Research Scientists	Tas DPIF	20	B Sc, M. Sc/ B Sc Hon
Robert Green	Technical Officer	Tas DPIF	20	

Smaller but valuable time contributions were made by Dr Judith Handlinger Tas DPIF, Dr Stephen Edwards, Colleen O'Meley, Ian Duthie and Elizabeth Cox (UTAS), commercial fishers (particularly Alan Flintoff and Dale Ridges), and commercial hatchery staff/managers including Martin John (Shellfish Culture P/L), Miles Cropp and Ken Frankish (Marine Shellfish Hatcheries) and Richard Pugh (Geordy River Aquaculture).

Please note that the Schools of Aquaculture and Zoology, within the University of Tasmania and the Tasmanian Department of Primary Industries and Fisheries have a joint research identity as the Tasmanian Aquaculture and Fisheries Institute, TAFI (see logo on cover).

TAS DPIF is now part of a larger agency, the Tasmanian Department of Primary Industries, Water and Environment Tas DPIWE.

APPENDIX 3: DETAILED PROGRESS IN RELATION TO OBJECTIVES

1. **Spawning seasons** *(objective achieved)*

These were delineated for both species based on lengthy series of monthly samples. The species had quite different reproductive characteristics both in terms of seasonal patterns and variability in gamete development within an individual gonad and within a population. However, no hermaphrodites were detected for either species. (See Manuscripts 1, 2 and 11.)

Katelysia scalarina commences a phase of gonad maturation at Little Swanport in autumn and becomes mature in late winter-spring and regresses in summer - early autumn. These results suggest that peak spawning periods are more likely to be in summer - early autumn whereas other published studies indicate peak spawning periods in September-October in Victoria and April-June in southern Western Australia.

Ruditapes largillierti gonads are relatively difficult to stage because of the tendency towards co-occurrence of several gamete development stages within the one gonad section. Mature individuals were found in most months but in only September (spring) samples were most individuals mature. However, the incidence of mature individuals differed greatly between the summers of 1993/94 (rare) and 1994/95 (still common in December).

2. **Meat condition** *(objective achieved)*

Seasonal trends were again delineated for both species based on lengthy series of monthly samples. In general, meat condition and hence marketability of clams varied much less than for Pacific oysters *Crassostrea gigas* in Tasmania. The condition index of *R. largillierti* was more stable than for *K. scalarina*. (See Manuscripts 1, 2 and 11.)

3. **Spat production** *(objective partly achieved)*

This was the major "failure" of the project - small numbers of *K. scalarina* spat could be produced in research trials but not by commercial operators on a scale that would allow cooperating fishers to undertake the large scale reseeding trials they proposed. As indicated in the original risk analysis, use of wild spat did at least allow research objectives to be progressed.

The original risk analysis also foreshadowed the need for disease testing of aquaculture product and adherence to translocation protocols (disease testing of juveniles translocated between waterways was required). Thus this Disease/parasite sampling was undertaken both through the grant and through a complementary CRC for Aquaculture project (by Dr J. Handlinger) using FRDC generated samples and other samples.

This work revealed critically important information. (See Manuscripts 17-19.) Larval mortality around day 8 has been a common problem with *K. scalarina* in both experimental and commercial hatchery trials (see Manuscripts 3). Herpes virus infection was diagnosed as a cause of sudden deaths in 7 to 8 day old *K. scalarina* larvae. A high level of intra-nuclear inclusions was detected in all larvae from the affected spawning by day 8, and herpes virus confirmation was achieved by electron microscopy. In larval rearing trials with *K. scalarina* in the experimental hatchery at the University of Tasmania, many larvae were invaded by a marine ciliate (resembling *Uronema nigricans*) probably in response to increased bacterial numbers.

A total of 719 adult clams, from two sites, were also examined. In addition to occasional herpes type inclusions, three types of digenean flukes were by far the most common parasites detected. Fine intracellular bodies typical of *Rickettsia* or *Chlamydia* were also seen.

4. **Larval and nursery experiments** *(objective partly achieved)*

A range of trials were undertaken and led to success with broodstock conditioning, thermal spawning stimuli, hormonal induction of spawning of males (strip spawning was not successful), fecundity estimates, larval rearing, settlement strategies and (*for Ruditapes largillierti*) land and sea-based nursery phases. *K. scalarina* remains a challenging hatchery species although considerable progress has been made. (See Manuscripts 3-4.)

Mean fecundity for *K. scalarina* in mass spawning trials ranged between 0.7 and 2.4x10^6 eggs. $female^{-1}$ while fecundity of *R. largillierti* induced to spawn by thermal stimulus ranged from 0.5 - 0.9 x 10^6 $eggs.female^{-1}$. For *K. scalarina,* eggs (69 ± 2 µm) developed to D veliger larvae (110 ± 1.3 µm) within 48 h at 20°C. Metamorphosis to spat (210.9 ± 2.1 µm) was observed from day 20 following treatment with 10^{-4} M norepinephrine for 60 min on day 19. For *R. largillierti,* fertilised eggs developed into trochophore larvae by 24 h at 20°C and D veligers with a mean shell length of 85.3 ± 4.7 µm within 48 h. Early larvae were frequently deformed and their mortality rates were very high. Development to pediveliger stage (mean shell length 200.3 ± 7.3 µm) took between 11 and 16 days at 20°C, and metamorphosis to spat (mean shell length 240 µm) occurred between Days 16 and 19.

5 and 6. **Growout trials and variables** *(objectives largely achieved)*

Farming trial results for *Ruditapes largillierti* were quite promising although biofouling of predator exclusion mesh is a major problem for subtidal farming (see Spencer et al., 1992). Density/size relationships were established. (See Manuscript 11.)

Growth results for wild caught *K. scalarina* were less promising, in mesh baskets, than for *R. largillierti* however excellent data were obtained on effects of beach position, stocking density and type of growout site (see Manuscripts 8,9 and 10). Growth rates in baskets for this species at Moulting Lagoon (Manuscript 8) were about ten times better than equivalent trials by other authors in Western Australia, however, conservation issues (see below) will probably preclude commercial activities at this site despite its attractive attributes (large areas of stable, almost flat, intertidal seabed not dominated by anoxic sediment. Long term growout trials were conducted with tagged *K. scalarina* in defined but not enclosed 20 m^2 plots (no predator protection) at three sites. These growth data indicate low growth rates (4-6 years to commercial size, ≥32 mm, depending on site). These trials were used to estimate natural mortality rates M, without predator protection, which were relatively high (0.24-0.35 $year^{-1}$) whereas annual mortality is mesh baskets at favourable locations was usually negligible except at elevated locations in the intertidal range. (See Manuscripts 8, 9 and 13.)

Both species exhibit an attractive characteristic in that, across a wide range of densities, growth is largely independent of stocking density (see Manuscripts 8, 9 and 11). However, density effects on sediment condition, for example pH, and interaction of density and occasional extreme environmental stress need to be considered (see Manuscript 13). Performance of *K. scalarina* is adversely affected at the most elevated beach positions assessed but otherwise beach position was not an influential factor. (See Manuscripts 10 and 11).

Predation is considered to be a major issue in clam farming (Spencer et al., 1992). Involvement of grant staff facilitated identification of European shore crabs (*Carcinus maenas*) and drilling gastropods as significant predators (see Appendix 5 - Additional publications 2-3). Financial contributions by commercial clam fishers to the grant allowed assessment of predation by wading birds both from a predator perspective and from an impact of fishing perspective (importance

of clams to bird diets and disturbance of bird behaviour or non-target benthic species by clam fishers (see Manuscript 14). *K. scalarina* (20-40 mm) formed the major part of the diet of Pied oystercatchers (*Haematopus longirostris*) at Ansons Bay, the major area for commercial *K. scalarina* fishing. Populations of this bird are characterised by extremely low productivity and high annual survival rates and are hence susceptible to disruptions in food supply. Such conservation issues are likely to reduce opportunities for expansion of clam fishing areas. This is particularly the case if fixed quota harvesting levels have been pursued regardless of variable recruitment rates. If protective mesh, that hinders predation by birds, was used in aquaculture or enhancement activities, as is likely, there may be conflict between such activities and bird conservation policies. In Manuscript 14, parallels are drawn with the conflict between clam harvesting in Europe and the needs of oystercatchers.

7. **Importance of sediment characteristics** *(objective partly achieved)*

Because of the distance from staff locations to appropriate *K. scalarina* locations and local sedimentation rates in potential sites, the logistics of addressing this question with plot trials were not favourable. [Paterson and Nell (1997) regularly removed surface sediment from the *Tapes dorsatus* plots.] Instead, *K. scalarina* were grown at a range of locations, with differing natural sediment conditions, so that sediment effects could be inferred. Unfortunately, survival at most of the sites was usually poor probably, in part, because of unfavourable sediment characteristics (see Manuscript 10). As an alternative approach, sediment characteristics were examined as covariates in a major stocking density trial at Moulting Lagoon (see Manuscript 8). In that analysis, sediment particle size was not a significant covariate but sediment pH within experimental cages (but not nearby outside the cages) did affect growth rates.

Research initiated prior to the grant commencing was finalised and published with assistance from this grant yielded an estimate of the maximum depth that *K. scalarina* can survive (10 - 30 cm, depending on size and site (see Appendix 5 - Publication 1).

A major trial was established with wild caught juvenile *R. largillierti* in replicate fish boxes, containing different sediment types, linked via a recirculating system to a 5 m diameter pool at the University of Tasmania's Launceston campus. A second 5 m diameter pool was used to produce algal blooms which were regularly pumped into the recirculating system. Unfortunately, despite considerable effort and expense, low survival and growth rates led to the abandonment of this trial (no data presented). However, the system was used subsequently to condition successfully triploid Pacific oyster broodstock for producing tetraploid offspring.

In summary, this was one of the less successfully addressed objectives within the grant.

8. **Gut contents** *(objective achieved for K. scalarina*).

K. scalarina were shown to selectively consume benthic diatoms from an array of potential microalgal feed items within the water column in Dick Bay, Smithton, Tasmania. Supplementary feeding/sacrifice trials in aquaria confirmed that if flagellates were consumed they could still be recognised within the time span that occurred between field collections and gut preservation; this suggested that the much greater occurrence of diatoms in the clam guts reflected active food consumption rather than differential digestion rates.

While the techniques applied were not as successful with gut contents of Pacific oysters on the adjacent oyster lease, it was clear that substantial dietary overlap exists between *K. scalarina* and Pacific oysters. This result, combined with unfavourable sediment characteristics, indicates that the original vision of farming *K. scalarina* under and around oyster racks to

reduce environmental impact from bioturbation is not feasible both in terms of poor survival and growth rates and the high risk of competition for food between clams and oysters. Pacific oyster farmers are concerned over bivalve carrying capacity of commercial growing areas.

9. **Age determination** *(objective achieved)*

This required much greater time input from Tasmania DPIF staff (now TAFI and Tas DPIWE-see above) than originally envisaged. Cellulose acetate peels were attempted for 900 *K. scalarina.* Growth ring analyses indicated that this species lives for up to 29 years. Both the tagging (see objective 5-6 above) and ageing studies indicate that this is a slow growing species (see Manuscript 13) and that growth rate is site dependent.

10. **Clam genetics** *(objective achieved)*

Allozyme techniques were used for both species (Manuscripts 15-16). Six samples of *K. scalarina* (three groups from Tasmania and one group each from Victoria, South Australia and Western Australia), examined for six variable loci, revealed three distinct groups: the three Tasmanian samples; the Victorian and South Australian samples; and the Western Australian sample. Differentiation between the Tasmanian and mainland subpopulations was striking while the three Tasmanian samples very similar.

Additional sampling allowed an examination of the relationships between the three major *Katelysia* spp. found in southern Australia and indicated that *Katelysia rhytiphora* and *K. peronii* appear to be the most closely related species-pair. Diagnostic allozyme loci were identified to enable unambiguous species identification - knowledge that proved useful for distinguishing several *K. rhytiphora* in the putative *K. scalarina* samples. There was also evidence suggesting F1 hybrids between *K. scalarina* and *K. rhytiphora* in Tasmanian samples collected from the wild. Confusion between broodstock of these two species had occurred in at least one commercial hatchery.

Three samples of *Ruditapes largillierti*, one from St Helens, Tasmania and two from New Zealand (Whangateau Harbour, North Island and Croiselles Harbour, South Island) were compared for 12 allozyme loci. There were no diagnostic loci which could be used to discriminate unambiguously the Tasmanian population from the two New Zealand populations. These results are consistent with the view that *R. largillierti* is a New Zealand endemic which has been translocated to Tasmania (the only location from which it has been recorded in Australia).

11. **Salinity tolerance** *(objective achieved for K. scalarina)*

Adult *K. scalarina* exposed to salinities in the range 5-55 ppt displayed a salinity tolerance range of 25-50 while juvenile *K. scalarina* displayed wider salinity tolerance with substantial mortality occurring only in the 50-55 treatments). Even outside of this 21 day tolerance range, adult *K. scalarina* exposed to low salinities (5-15) survived for approximately 10 days. (See manuscript 5.) Adults subject to a near lethal exposure to a low salinity and then returned to 35 ppt survived well. This suggests that mass mortalities observed with wild populations of *K. scalarina* are not due to low salinity unless rainfall induced freshes are very persistent. (See Manuscript 13.)

K. scalarina is essentially an osmo- and ionic conformer, particularly at salinities >25, that relies on the mechanism of shell valve closure to isolate the body tissues from unfavourable salinities. Calcium concentration and the incidence of valve closure displayed inverse relationships with salinity except at 55. Ca^{2+} is probably mobilised from $CaCO_3$ in the shell during shell closure to

buffer the production of anaerobic by - products. K^+ was constantly hyperionic to the external medium indicating some degree of regulation. (See manuscript 5.)

However, prolonged shell valve closure is not utilised as much by *K. scalarina* over the tolerated salinity range. Cell volume regulation over the tolerated salinity range appeared to be controlled by fluctuations in the free amino acid (FAA) pool with Taurine + arginine comprising about 25% of the pool. (See manuscript 6.)

Respiration and algal clearance trials were conducted. Irregular valve closure patterns limit oxygen consumption and algal clearance which may in turn limit growth potential. Oxygen consumption by adult *K. scalarina* was depressed in salinities outside 35 even for salinities within the tolerance range. Both juveniles and adults display a substantial decrease in algal clearance outside the salinity tolerance range and both groups displayed optimal algal clearance rates between 25 and 45. (See manuscript 7.) Nell and Paterson (1997) found that algal clearance rate correlated well with growth data for other clam species.

12. **Public awareness of clams** *(objective partly achieved)*

In addition to writing popular articles for fisheries and aquaculture publications, press interviews were also given on a popular fishing program on Sydney radio. As well grant staff contributed material for a major food column on clams. However, as the grant did not lead to increased production of clams, a more intensive effort aimed at increased demand to match increased production was not attempted.

1. Constance, M. Shell shock: eating bait! The Australian Financial Review Magazine.
2. Maguire, G. B., 1994. Clam project attracts research funding. Fishing Today, 6(6): 34.
3. Zacharin, W., 1993. Interim management plan for the clam fishery released. Fishing Today, 6(4): 32-33.

13. **Foster quality assurance program** *(objective achieved)*

See manuscript 14. This was a significant achievement as uncontrolled harvesting of clams from undesirable locations had the potential to adversely impact on Tasmanian bivalve industries including the valuable Pacific oyster industry.

14**. Help develop an optimum management regime** *(objective achieved)*

This is an ongoing process but significant progress has been made. See Manuscript 13 and the following Tasmanian Government publications.

1. Zacharin, W. F., Stuart, R. D., Griffiths, H. and Maguire, G. B., 1994. *Sheltered Waters Clam Fishery.* New Fisheries Development Assessment Document, Tasmanian Dept. Primary Industries and Fisheries, Hobart, 24 pp.
2. DPIF Tasmanian Marine Resources Division, 1994. Policy and fishery development plan for the Tasmanian development clam fishery. Department of Primary Industry and Fishery Tasmania, 19 p.

APPENDIX 4: MANUSCRIPTS (FROM SECTION 5)

The aim has been to make each manuscript internally consistent in style rather than making all manuscripts conform to the one style. They largely follow the style of the journal or monograph in which they have been or should be published.

Reproductive biology

1. Maguire, G. B. and Kent, G. N. Gametogenesis and condition index of the stepped venerid, *Katelysia scalarina* (Lamarch 1818), at two sites in Tasmania, Australia.

2. Maguire, G. B. and Kent, G. N. Gametogenesis and condition index of the New Zealand venerid *Ruditapes largillierti* (Philippi 1849) from St Helens, Tasmania, Australia.

Hatchery phase

3. Kent, G. N., Maguire, G. B., John, M., Cropp, M. and Frankish, K., 1998 Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina* (Lamarck 1818). J. Shellfish Research, 17(4): 1065-1070.

4. Kent, G. N., Maguire, G. B., and Duthie, I., 1999. Spawning, settlement and growth of the New Zealand venerid *Ruditapes largillierti* (Philippi 1849). NZ J. Marine and Freshwater Research, 33: 55-62.

Environmental requirements

5. Bellchambers, L. M., Maguire, G. B. and Richardson, A. M. M. Effects of salinity on an estuarine clam; *Katelysia scalarina* (Lamarck, 1818) (Bivalvia: Veneridae). I. Osmo- and ionic regulation.

6. Bellchambers, L. M., Edwards, S. and Maguire, G. B. Effects of salinity on an estuarine clam; *Katelysia scalarina* (Lamarck, 1818) (Bivalvia: Veneridae). II. Regulation of free amino acids.

7. Bellchambers, L. M., Edwards, S., Maguire, G. B. and Richardson, A. M. M. Effects of salinity on an estuarine clam; *Katelysia scalarina* (Lamarck, 1818) (Bivalvia: Veneridae). III. Respiration and algal clearance.

Growout

8. Bellchambers, L. M., Maguire, G. B. and Richardson, A. M. M. Absence of effects of intra-specific competition on the growth and survival of *Katelysia scalarina* (Lamarck, 1818) (Bivalvia: Veneridae).

9. Bellchambers, L. M., Maguire, G. B. and Richardson, A. M. M. Effect of tidal position and density on *Katelysia scalarina* (Lamarck 1818) (Bivalvia: Veneridae).

10. Bellchambers, L. M. and Maguire, G. B. The effect of growout site on the mortality of *Katelysia scalarina* (Lamarck, 1818) (Bivalvia: Veneridae).

11. Maguire, G. B. and Paturusi, A. Performance of clams, *Ruditapes largillierti* (Phillipi, 1849), stocked at different densities and sizes in experimental cages in Georges Bay, Tasmania

Natural diet

12. Bellchambers, L. M. and Maguire, G. B. Observations of the gut contents of *Katelysia scalarina* under and away from oyster racks at Duck Bay, Smithton.

Fisheries biology and environmental issues

13. Riley, S. Green, R., Zacharin W. and Maguire, G. B. Growth models and age determination for the intertidal venerid clam *Katelysia scalarina* (Lamarck 1818) from three sites in Tasmania, Australia.

14. Taylor, I. R., 1995. Clam harvesting and the conservation of wading birds in Tasmania. Johnstone Centre of Parks, Recreation and Heritage Report no. 42, 90 p. (Research funded via direct fishermen contributions to support this grant).

Genetics

15. Soh, S. W. L., Maguire, G. B. and Ward, R. D., 1998. Genetic studies of the venerid clam genus *Katelysia.* J. Shellfish Research, 17(4): 1057-1064.

16. Maguire, G. B. and Ward, R. D. Genetic comparison of populations of a venerid clam *Ruditapes largillierti* (Philippi 1849) in Tasmania and New Zealand.

Diseases and parasites

17. Handlinger, J. H. Summary of health studies related to FRDC Clam Project, and relationship to CRC for Aquaculture Project A.2.1. (Early Mollusc Health).

18. Handlinger, J. H. Pathogens and parasites of the clam *Katelysia scalarina* in Tasmania.

19. Handlinger, J. H. Notes on Herpes virus associated with larval mortality in the Australian clam *Katelysia scalarina* in Tasmania.

APPENDIX 5: ADDITIONAL PUBLICATIONS

1. Bellchambers, L. M. and Richardson, A. M. M., 1995. The effect of substrate disturbance and burial depth on the venerid clam, *Katelysia scalarina* (Lamarck, 1818). J. Shellfish Research, 14(1): 41-44.

2. Chilcott, N. C., 1996. Predation by Drilling Gastropods on Tasmanian Intertidal Bivalves. Unpublished Honours thesis, University of Tasmanian, Hobart.

3. Mackinnon, C. J., 1997. Impact of the Introduced European Green Crab, *Carcinus* maenas on Tasmanian Bivalve Populations. Unpublished Honours thesis, University of Tasmanian, Hobart.

4. Maguire, G. B., Bellchambers, L. M. and Zacharin, W., 1994. Papers from FRDC Clam Research Workshop, University of Tasmania, Launceston, 19 pp.