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LABORATORY AND FIELD STUDIES OF THE LARVAL DISTRIBUTION AND DURATION OF THE INTRODUCED SEASTAR *ASTERIAS AMURENSIS* WITH UPDATED AND IMPROVED PREDICTION OF THE SPECIES SPREAD BASED ON A LARVAL DISPERSAL MODEL.

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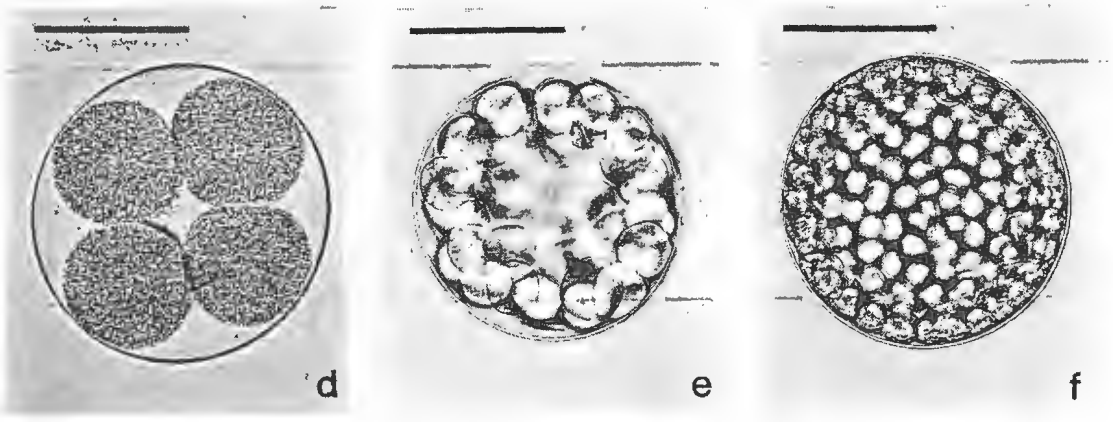

CSIRO
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1. NON - TECHNICAL SUMMARY

The northern Pacific seastar, *Asterias amurensis*, occurs naturally on the coasts of Japan, eastern Russia, the Korean Peninsula and is also found in Alaska. It has recently been introduced to Australian (specifically, south-east Tasmanian) waters probably via larvae in ballast water of ships. A significant breeding population now occurs in the Derwent River estuary.

A. amurensis produces pelagic, feeding (planktotrophic) larvae that are well adapted to long distance dispersal. This project was established to investigate aspects of the species' early life history in southern Tasmania and provide basic information to enable modelling the spread of *A. amurensis* by larval dispersal.

There were three main components to the study. First, larvae of *A. amurensis* and the two other dominant seastars found in the Derwent River that also have planktotrophic larvae (*Patiriella regularis* and *Coscinasterias calamaria*), were reared in the laboratory. This was in order to document development, provide an estimate of larval duration and to provide reference material for identification of field collected larvae. Second, *A. amurensis* larvae were sampled in the Derwent River estuary and at Spring Bay (Triabunna) on the Tasmanian east coast to document their vertical and horizontal distribution. Third, these data were used to refine a model of larval advection in southern Tasmanian and southern Australian waters.

1.1 LABORATORY STUDIES

A. amurensis has indirect development typical of starfish with planktotrophic larvae. Development proceeds through bipinnaria to brachiolaria larvae.

Larval duration is both variable and protracted. Reported larval durations range from 50-60 to 115-120 days and are dependent on temperature and feeding regime. Larvae were reared to 46 days (at 15^{o}C) by which time they had developed to bipinnaria but were not yet competent to settle. Minimum larval duration was highly significantly correlated with temperature amongst reported rearings of starfish larvae. Based on this relationship and our observations of reared material, we estimate larval duration in *A. amurensis* to be at least 66-91 days under local conditions.

Linear discriminant function analysis (LDA) was used to examine morphological differences between reared *A. amurensis*, *P. regularis*, *C. calamaria* and wild caught starfish larvae. Wild caught larvae were not significantly different to reared *A. amurensis* but both groups were significantly different to *P. regularis* and *C. calamaria*. This suggested that wild caught larvae were *A. amurensis*.

Three other factors support the view that wild caught seastar larvae were *A. amurensis*. First, the concentration of larvae was extraordinarily high in the Derwent, particularly in areas where both adult and juvenile *A. amurensis* were most abundant. Second, the development of larvae at the time of sampling was consistent with that expected from spawning within the peak (August) period recorded for *A. amurensis*. Third, spawning in other Tasmanian starfish (where known) occurs during late spring and summer rather than winter, as is the case in *A. amurensis*, and thus capturing large numbers of larvae of other starfish species at this time was less likely.

1.2 FIELD STUDIES

A. amurensis larvae were extremely abundant in the Derwent River estuary during the September and October sampling periods. Sampling failed to locate larvae at Spring Bay (Triabunna) on the east Tasmanian coast.

The distribution of *A. amurensis* larvae varied with depth. The concentration of larvae was not significantly different between the surface and 5 m, but larval concentration at both these depths was significantly higher than at 10 m. The concentration of *A. amurensis* larvae within the upper 5 m of the water column suggests that wind driven circulation is likely to play a major role in larval dispersal and consequently, that modelling advection based on surface circulation patterns is appropriate.

A. amurensis larvae appear to be ideally suited to uptake and translocation in ballast water, thus further supporting this as the most likely vector for introduction to Tasmanian waters. *A. amurensis* is highly fecund, has long lived pelagic larvae which, at least in the current study, larvae are abundant within the upper 5 m of the water column from where ballast water is most commonly drawn.

Concentrations within the Derwent represent some of the highest reported for starfish larvae world-wide. Some of the highest concentrations were recorded near port facilities. This raises the possibility that the species may be further translocated by coastal shipping taking on ballast water in Hobart. In this respect, the Derwent may act as a source for further ballast mediated introductions to mainland Australia and international destinations. The length of time that *A. amurensis* larvae are present within the Derwent is unknown, although both the protracted spawning and larval periods suggests that larvae may be available for considerable periods and, at least, from July to December.

The distribution of larvae, juveniles and adults suggests that at least some *A. amurensis* larvae are retained and complete their development within the Derwent. This is also supported by modelling results. Predominant wind patterns during the spawning/larval period tend to retain larvae within the

Derwent and this may partially explain the slower than expected rate of spread of the species.

The initial point of introduction into Tasmanian waters cannot be ascertained with certainty. Introduction to either the Triabunna area or Derwent estuary is possible. However, despite the higher frequency of ballast water discharge at Triabunna from likely source localities, observed patterns of larval distribution and dispersal support the Derwent as the initial introduction port. Aperiodic reversals in wind direction and interannual variability in their strength and duration may result in export of larvae from the Derwent system and account for recent sightings of juveniles and adults in other localities.

1.3 MODELLING

Modelling supports the hypothesis of a dominant pattern of larval retention within the Derwent estuary, particularly for those larvae emanating from spawning near the port of Hobart. Furthermore, simulations suggest that the initial spread of *A. amurensis* southwards towards the mouth of the Derwent would have been slow and may have taken several years.

The model supports that with the establishment of adults in the lower reaches of the estuary, colonies in Frederick Henry Bay could have been derived from the Derwent and thus from an original colony established in the vicinity of Hobart.

Simulations demonstrate that the Triabunna juveniles could have emanated from colonies established near Dunalley. The model also predicts that in certain years, the Dunalley larvae may be advected into Great Oyster Bay and beyond.

Based on these observations, the model predicts that (provided settlement requirements are met) colonies of *A. amurensis* may become established in the vicinity of the port of Triabunna and the northern reaches of Great Oyster Bay. Simulations undertaken by Lyne (1993) also suggest that if a spawning population does become established in Great Oyster Bay, then the dominant wind patterns during the spawning season would facilitate retention of larvae within that area and may subsequently lead to enhanced recruitment.

2. BACKGROUND

Asterias amurensis is a boreal seastar, inhabiting cold-temperate, sub-littoral and shallow waters of the north and north-east areas of the Pacific Ocean. It is native to the coasts of Japan, Korea and eastern Russia (D'Yakanov 1968, Onguru and Okutani 1991) and although also found through the Bering Sea to Alaska, it is uncertain whether the Alaskan populations are native or introduced (Fukuyama 1994).

In its native environment, *A. amurensis* is the dominant inshore predator and sometimes (McLoughlin and Bax 1993). In Japan, it sometimes undergoes massive, sporadic, population outbreaks which cause significant damage to both natural and cultured shellfish beds (Nojima et al 1986).

In 1986, a specimen of *A. amurensis* was collected in the Derwent River, southern Tasmania, and lodged with the Tasmanian Museum (Turner 1992). At that time its identity was confused with a native, morphologically similar species, *Uniophora granifera* . The specimen was correctly identified in March 1992. *A. amurensis* has proliferated in the Derwent River and is now a dominant part of the shallow water fauna of the lower estuary, adjoining bays and channels with concentrations of up to 9.44 per m^2 (Buttermore et al. 1994). A much smaller population has also been discovered on the east coast near the wood chip export terminal of Triabunna (Figure 1).

The most likely vector for the introduction of *A. amurensis* to southern Tasmania is considered to be via ballast water (Davenport and McLoughlin 1993). This hypothesis is reasonable considering the reported protracted pelagic larval duration of the species (> 50 d) and thus its presumed ability to survive the 15-20 d voyage from north Pacific source localities. The transport of pelagic larvae in ballast water and the potential for ballast water mediated introductions were first suggested by Ostenfeld (1908) and are now well documented. Carlton and Geller (1993) recorded 367 taxa in plankton samples taken from ballast water in ships originating from Japan. These authors further document the established invasion of 49 species world-wide after ballast water mediated introductions. Similar ballast water introductions have already resulted in the establishment in Tasmanian waters of the Japanese seaweed *Undaria pinnatifida* (Jones 1991) and the toxic dynoflagellate, *Gymnodinium catenatum* (Bolch and Hallegraff 1990).

The discovery of *A. amurensis* in southern Tasmania has raised considerable concern amongst biologists and the fishing industry regarding the potentially serious impact such a species may have on native ecosystems and the aquaculture industry.

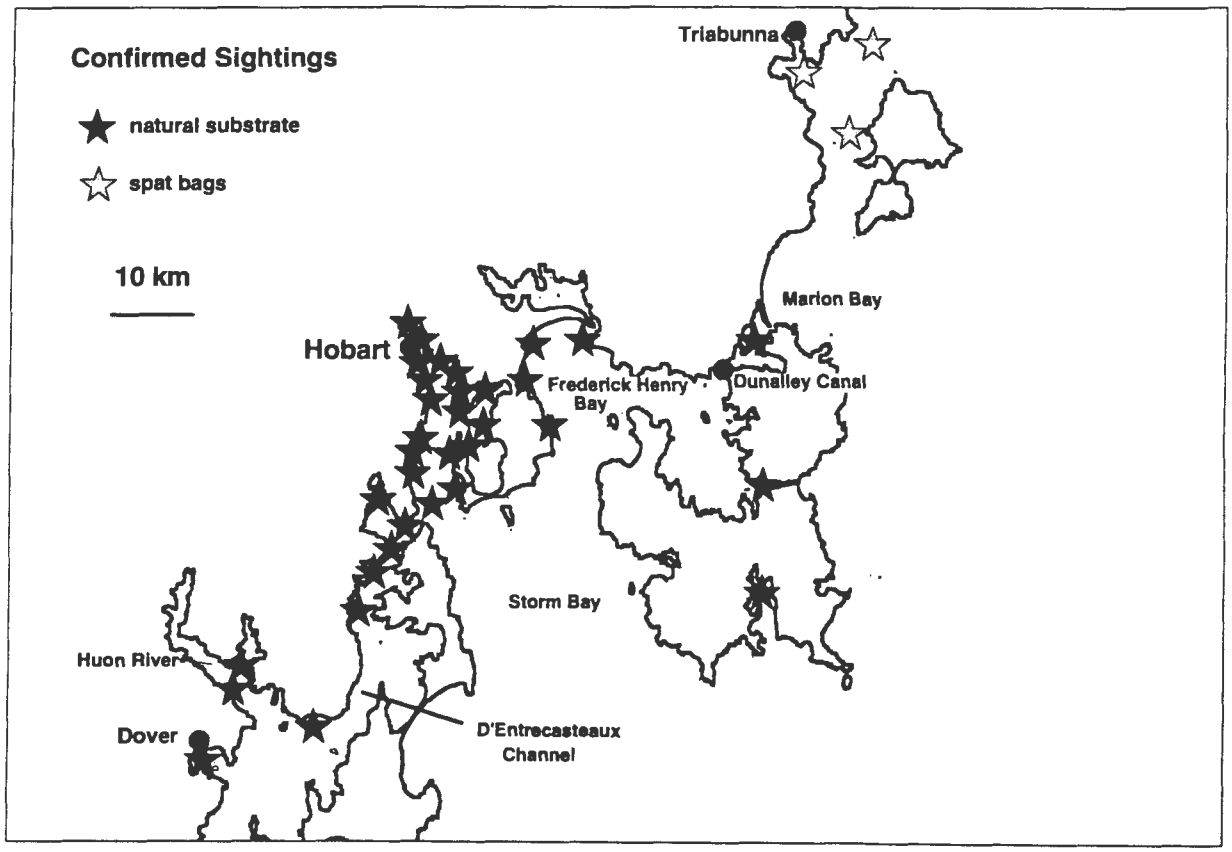

Figure 1 *Current distribution of* A. amurensis *in southern Tasmania (after Buttermore et al 1994).*

The proliferation of *A. amurensis* in southern Tasmania has also raised concerns regarding the potential of the species to spread throughout remaining Tasmanian waters and northwards to mainland Australia. The dynamics of dispersal, the mechanisms responsible and time scales involved are thus critical to the formulation of an action plan for the species.

3. NEED

In May 1993, the CSIRO Division of Fisheries was commissioned by the Fisheries Research and Development Corporation (FRDC) to review known information on *A. amurensis* and predict its potential impact on Australian fisheries and marine ecosystems. The conclusions, detailed in a status report produced in June (Davenport and McLoughlin, 1993), were that:

- *A. amurensis* colonised southern Tasmanian waters probably due to the introduction of larvae from ballast water.

- *A. amurensis* is an opportunistic feeder and will consume almost any animal tissue it can capture, but prefers mussels, scallops and clams.

- In its native environment, it has no significant natural predators, may undergo massive, cyclic, changes in population size and, at high densities, has caused millions of dollars worth of damage to shellfish industries (Nojima et al 1986).

- Although the impact of *A. amurensis* in southern Australian waters cannot yet be stated with certainty, the ecological plasticity of the species and the problems it presents in its natural environment are cause for grave concern. It is possible that *A. amurensis* could have a devastating impact on coastal wild fisheries (especially shellfish and crustacean fisheries) mariculture and the coastal marine ecosystem. *A. amurensis* could well impact the southern Australian marine environment to an extent similar to the effects of rabbits on Australia's terrestrial environment.

- *A. amurensis* larvae could spread from Tasmanian waters north into Bass Strait, and possibly, to mainland Australia. The rate of spread was based on the assumptions that larvae are present in surface waters and that they drift as passive particles. It was emphasised, in the review, that the predicted dispersal rate and pattern was very preliminary and that much of the information required to fully assess the dispersion of *A. amurensis* was sketchy or unavailable.

Hence a major recommendation of the report was:

"to fill in the gaps with reliable data on the reproductive and larval biology and ecology of the starfish - time of spawning, duration of spawning, fecundity, larval

duration, where eggs and larvae are dispersed in the water column (and) habitat requirements of larvae and adults ...".

The potential of pelagic dispersal of *A. amurensis* cannot be underestimated. A similar species (*Asterias rubens*) has a larval stage in excess of 140 days and was considered by Barker and Nichols (1983) to have the capabilities to cross the Atlantic during this period. Existing laboratory data on the larval duration of *A. amurensis* (Sagara and Ino 1954, Nojima *et al* 1986) are ambiguous and, given the broad ecological tolerance of the species, it is not possible to accurately predict either its potential dispersal, ecological impact or the time frames involved without real information on the species under local conditions.

4. OBJECTIVES

The objective of the current study was :

- To obtain locally applicable information on the larval biology of the introduced seastar, *A. amurensis* , through field and laboratory studies, in order to refine models of the species dispersal and produce more accurate forecasts of its spread in southern Australian waters.

There were three main components to the study.

(i) *A. amurensis* and the two other dominant seastars found in the Derwent River that have planktotrophic larvae (*Patiriella regularis* and *Coscinasterias calamaria*), were reared in the laboratory. Larval rearing was undertaken to document development, provide an estimate of larval duration and to provide reference material for identification of field collected larvae.

(ii) Field sampling was undertaken in the Derwent River estuary and at Spring Bay (Triabunna) to document the vertical and horizontal distribution of *A. amurensis* larvae under local conditions.

(iii) These data were used to refine a model of larval advection in southern Tasmanian and southern Australian waters.

Each component will be discussed in turn.

5. LABORATORY STUDIES

5.1 REARING AND DEVELOPMENT

5.1.1 METHODS

(A) *A. AMURENSIS*

Mature *A. amurensis* were collected between mid April and July from the Derwent River. Ovaries were removed in the laboratory and bathed in a sea water solution of $1x10^{-5}$ M 1-methyladenine for 60 to 90 minutes. Eggs were washed by sieving off water through a 60 µm filter and replacing with fresh seawater. Washed eggs were transferred to 500 ml beakers. A sperm solution was obtained from small pieces of mature, excised, testes diluted in seawater. Eggs were fertilised by adding a couple of drops of sperm solution into each beaker. Percentage fertilisation was checked and, if necessary, another drop of sperm solution was added. Fertilised eggs were again washed (as above) and transferred to fresh 500 ml beakers. Concentration of eggs in incubation beakers was 15-20 eggs/ml. Cultures were kept in a constant temperature cabinet at 15°C and stirred by rotating paddles. Cultures were washed and placed in fresh seawater after 3 days when larvae had developed to early bipinnaria. Feeding of larvae also commenced at this time.

Larvae were fed *Dunaliella tertiolecta* at a concentrations of 20 000 cells per ml. Initially, larvae were fed and the water changed, every two days. However, this proved to be disruptive and resulted in the loss of too many larvae. Feeding larvae every two days and changing water once per week proved to be the best compromise.

D. tertiolecta was grown in F/2 media (O'Mealy and Daintith 1992) in a constant temperature room at 15°C. The concentration of cells in the algal cultures was calculated using a haemocytometer. The appropriate volume of algae (for feeding) was transferred into centrifuge tubes in a laminae flow cabinet and then centrifuged for 10 minutes at 5 rpm. The supernatant was decanted off and the algae resuspended in seawater prior to feeding the larvae.

(B) *P. REGULARIS* AND *C. CALAMARIA*

Both *P. regularis* and *C. calamaria* were reared by G. Moreno at Sydney University. Adults were collected in the Derwent River during late December 1993 and January 1994 respectively, and sent, via air freight, to Sydney. Fertilisation and rearing techniques followed the same methodology as for *A. amurensis*. Larvae were fed on a diet of *Rhodomonas sp.* (strain CS-215 CSIRO) and reared at 19°C.

Definitions of developmental stages follow Byrne and Barker (1991), Table 1.

Table 1. *Definitions of developmental stages*

DEVELOPMENTAL STAGE	DESCRIPTION
fertilised egg	the appearance of a clear membrane around the egg
early coeloblastula	cell mass > 64 cells and has started to take on the appearance of a hollow ball
wrinkled blastula	folding of the blastoderm into blastocoel
coeloblastula/blastula	hollow ball of cells
early gastrula	invagination commenced, archenteron extending into the blastocoel
gastrula	elongation of archenteron and gastrula
advanced gastrula	mesenchyme cells budding off archenteron into the blastocoel
late gastrula	enterocoels on either side of the archenteron, presence of stomodaeum
early bipinnaria	fusion of the stomodaeum and the archenteron
bipinnaria	formation of complete pre and post oral ciliated tracks
late bipinnaria	thickening and elongation of bipinnarial processes
brachiolaria	budding of coelomic lumina on either side of the anterior lumen (axohydrocoel) which will form the lumen of the future posterior brachia
late brachiolaria	development of median and posterior brachials and the adhesive disc

5.1.2 RESULTS

A. amurensis larvae were reared in two separate trials. Single rearings of *P. regularis* and *C. calamaria* were undertaken (Table 2).

Table 2. *Summary of various rearing trials*

TRIAL	COMMENCED	FINISHED	DAYS REARED	TEMP (^{0}C)	STAGE ACHIEVED
A. amurensis 1	13.5.94	27.6.94	46	15	advanced bipinnaria
A. amurensis 2	28.6.94	2.8.94	36	15	advanced bipinnaria
P. regularis	31.12.93	28.2.94	60	19	juveniles
C. calamaria	31.1.94	3.3.94	32	19	late brachiolaria

5.1.3 DEVELOPMENT OF *A. AMURENSIS* (TABLE 3, FIGURE 2)

Very ripe ovaries release eggs readily and are fertilisable within 60 minutes of bathing in 1-methyladenine. Less ripe ovaries release eggs within 30 minutes and eggs are fertilisable within 90 minutes. The mature ova are spherical, 150 µm in diameter, light peach to orange in colour and have a granular texture (Figure 2a).

The fertilisation membrane (Figure 2b) appears within 30 to 60 seconds after introduction of sperm into rearing beakers. Polar bodies are extruded within 30 minutes (Figure 2c). Cleavage is radial and holoblastic. First cell division occurs 2.5 to 3 h post fertilisation and eggs develop into early coeloblastula (Figure 2d-f) by 5 to 8 hours. Blastular wrinkling (Figure 2g) occurs 12 to 13 h post fertilisation. This stage proceeds rapidly and is not as complex as in other species (eg. Komatsu 1975). Some embryos were observed to wrinkle and smooth out again within 15 minutes. Embryos resume smoothness by 13 to 14 h post fertilisation and ciliated coeloblastulae commence rotating within the fertilisation membrane by 18 to 21 h. Some individuals were observed to rotate in the late wrinkled stage before they had completely resume smoothness, although this was unusual.

Larvae hatch as coeloblastula 22 to 25 h after fertilisation (Figure 2i). Shortly after hatching, larvae begin to elongate and invagination commences. By 24 to 26 h embryos develop into early gastrula, each with a shallow blastopore (Figure 2j). The larvae continue to elongate as the archenteron extends into the blastocoel. By 45 h post fertilisation, the blind end of the archenteron expands. Mesenchyme cells bud from its tip (Figure 2k) and move into the blastocoel. Late gastrula develop by 52 h post fertilisation, when rudimentary left and right enterocoels form on either side of the archenteron tip (Figure 2l). Shortly

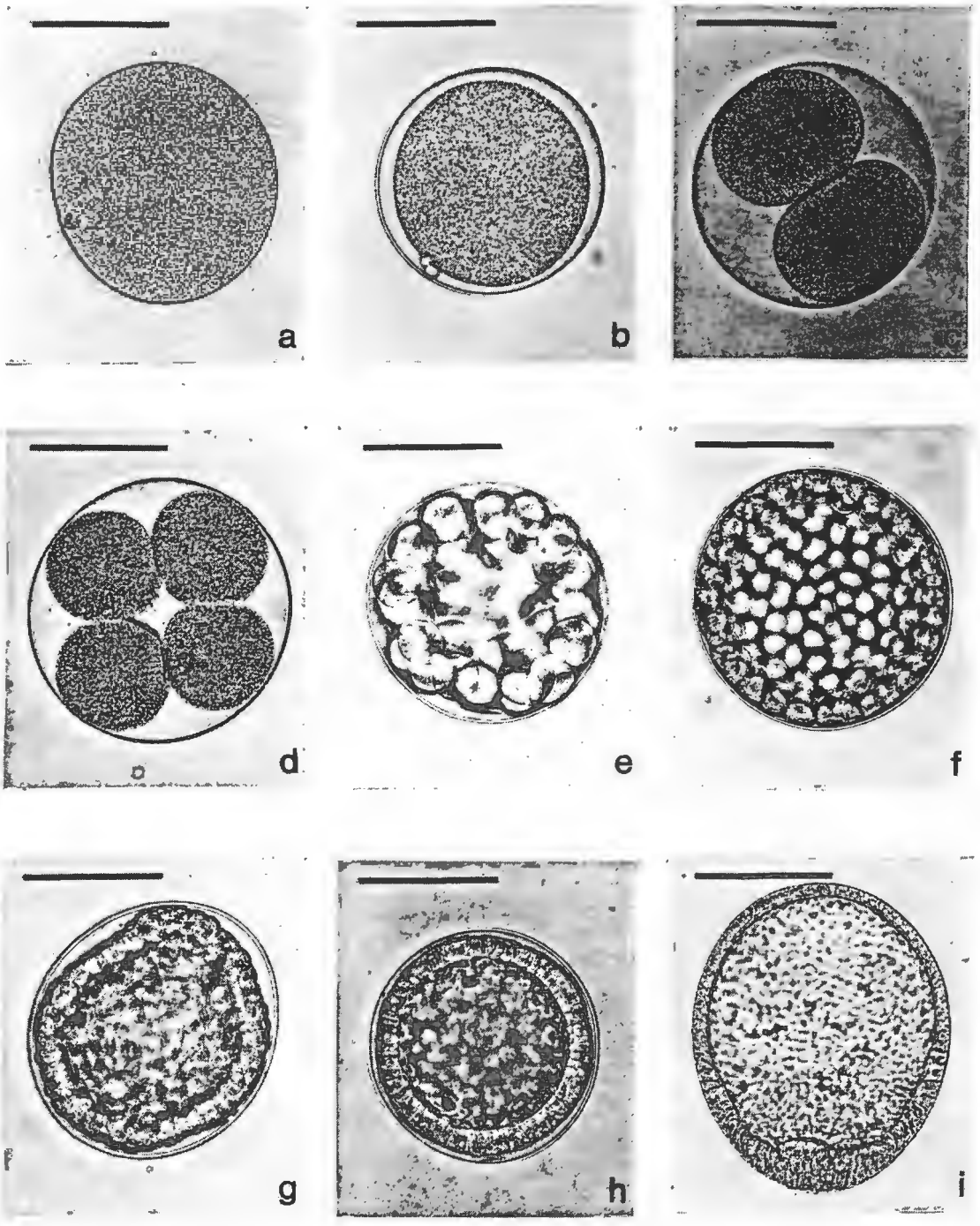

Figure 2 *Development of reared* A. amurensis: *(a) unfertilised egg; (b) fertilised egg with two polar bodies; (c) first cleavage; (d) second cleavage; (e) early coeloblastula; (f) blastula; (g) wrinkled coeloblastula; (h) coeloblastula - after resuming smoothness and prior to rotating within the fertilisation membrane; (i) hatched coeloblastula.Scale Bar = 100µm.*

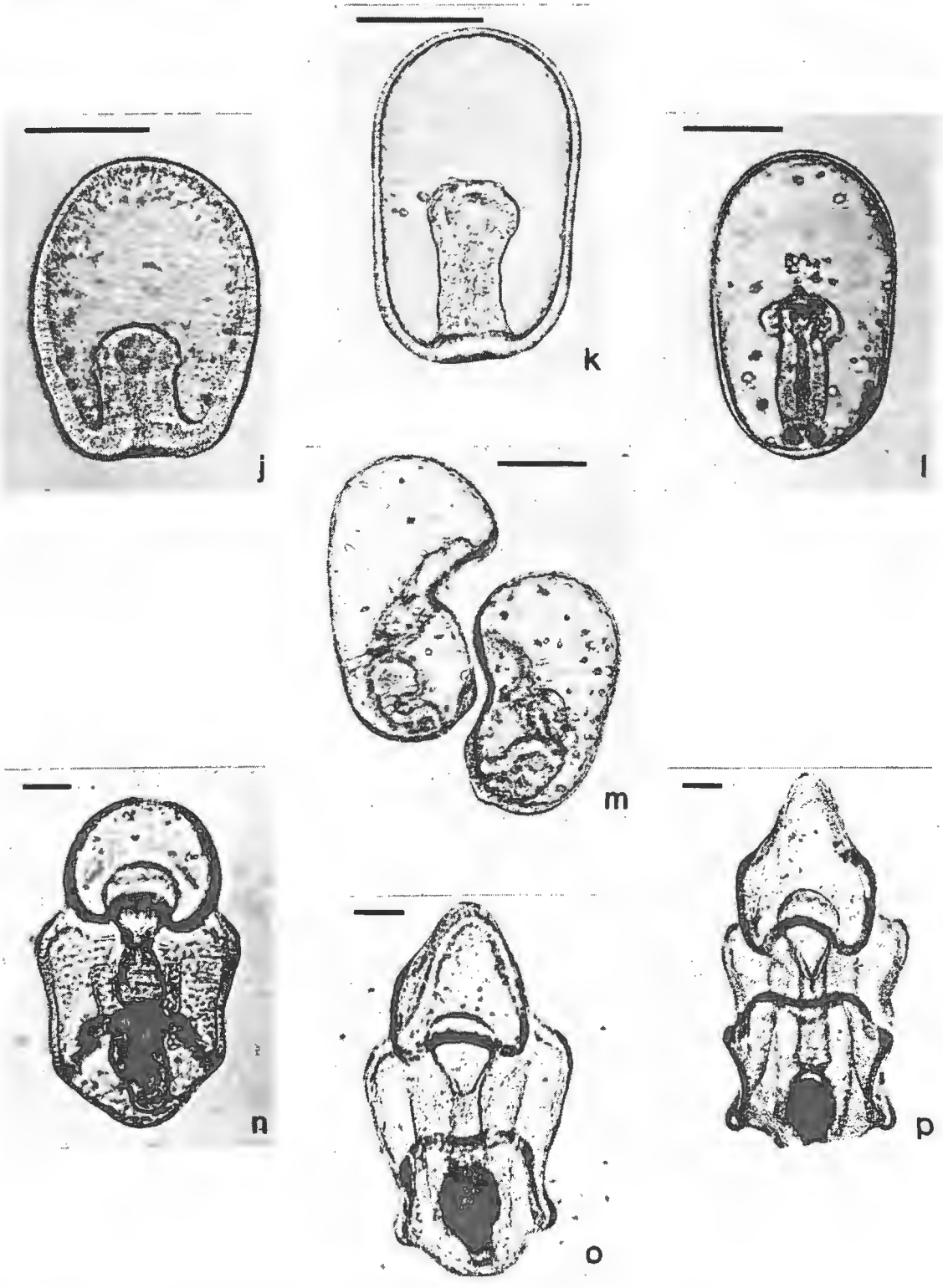

Figure 2 *(cont.) (j) early gastrula; (k) advanced gastrula; (l) late gastrula; (m) early bipinnaria - showing gastric tract and hydropore; (n) bipinnaria; (o) bipinnaria - enterocoels have extended posteriorly; (p) late bipinnaria - bipinnarial processes have begun to thicken and curl. Scale bar = 100 µm.*

thereafter, the anterior end of the archenteron bends ventrally toward the shallow stomodaeum. The enterocoels develop into small bunch like pouches and an obvious stomodaeum is present 67 h after fertilisation. By 75 h the archenteron fuses with the stomodaeum, whereby the stomodaeum becomes the mouth, the blastopore the anus and the archenteron develops into the larval gut. The larvae are now early bipinnaria.

The cardiac sphincter is present by 93 to 110 h post fertilisation and separates the developing oesophagus and stomach. The hydropore is present and extends dorsally out of the left enterocoel (Figure 2m). The mouth widens and takes on a gaping appearance. Pre and post-oral ciliated tracts begin to develop and the enterocoels are present in the form of small pouches on either side of the archenteron at the level of the cardiac sphincter.

Table 3. *Chronology of development of* A. amurensis

DEVELOPMENTAL STAGE	TIME
fertilisation membrane	30-60 seconds
first cleavage	2.5-3 h
second cleavage	4 h
third cleavage	5 h
fourth cleavage	6 h
early coeloblastula	8-9.5 h
wrinkled blastula	12 -13 h
coeloblastula	13-14 h
rotating coeloblastula	18- 21h
swimming coeloblastula	22-25 h
early gastrula	26 h
gastrula	40 h
advanced gastrula	42 h
advanced gastrula/early bipinnaria	52 h
early bipinnaria	75 h
bipinnaria	5 d
advanced bipinnaria	30-36 d

In 5 d larvae, the pre and post oral ciliary tracts form complete circuits and the bipinnarial processes start to develop. Enterocoels elongate anteriorly and extend approximately 1/4 the way up the oesophagus (Figure 2n). When the larvae are 6 d old, the oral hood curves over the mouth and both anterior and lateral bipinnarial processes become more pronounced. Larvae exhibit posterior flexure and contraction of the cardiac sphincter. By 9 d, the oral hood becomes pointed. The enterocoels extend both anteriorly to the mouth and commence extending posteriorly (Figure 2o). By 14 d, the 'tips' of bipinnarial processes, along the ciliated tracts, begin to thicken and curl, and the ciliated tracts become

more prominent and granular in appearance (Figure 2p). Larvae at this stage are 950-975 µm long. In 17 to 20 d larvae, the enterocoels extend half way along each side of the gut. Anterior and dorsal lateral processes develop as the ciliated tracts became more complex and convoluted. Enterocoels extend into the oral hood by 30 to 36 d post fertilisation and fuse to form the axohydrocoel (Figure 2q)

5.1.4 DEVELOPMENT OF *P. REGULARIS* AND *C. CALAMARIA*

Table 4 shows the chronology of development of *C. calamaria* and *P. regularis* reared during the present study.

Table 4. *Chronology of development of* P. regularis *and* C. calamaria

P. REGULARIS DEVELOPMENTAL STAGE	DAYS	*C. CALAMARIA* DEVELOPMENTAL STAGE	DAYS
fertilised	1	fertilised	1
elongate gastrula	2	elongate gastrula	3
early bipinnaria	4	early bipinnaria	5
bipinnaria	6	bipinnaria	7
late bipinnaria	11		
early brachiolaria	14	early brachiolaria	29
brachiolaria	21	brachiolaria	32
late brachiolaria	38	late brachiolaria	60
juveniles	41		

5.1.5 DEFORMED LARVAE

Deformities in laboratory reared larvae are not uncommon (M. Byrne pers comm.) and deformed larvae were observed in all rearing trials. The development of bent archenterons was the most common abnormality observed.

On one occasion adults were collected from shallow water after a particularly heavy period of rainfall. Fertilisation rate was poor (50%) and development abnormal. By the second day, the majority of the fertilised eggs had not developed beyond second cleavage and only 1% had developed to early coeloblastula. We also found that eggs from adults collected late in the spawning season (ie. late August - September) fertilised successfully but did not develop normally. Larvae developed to gastrula, however, most (90%) were abnormal and had bent archenterons. Similar observations have been reported in laboratory rearings by other researchers (J. Keesing pers. comm., M. Byrne pers. comm., Gemmill 1914).

5.1.6 DISCUSSION

(A) *A. AMURENSIS*

The development of *A. amurensis* differed from previously reported rearings in three areas. First, the rate of development was intermediate between rearings by Kas'yanov (unpublished report) and Komatsu (pers. comm.), Table 5. This difference was probably temperature related. Kas'yanov reared *A. amurensis* larvae at 19 ^{o}C and Komatsu at 10 ^{o}C. The most striking difference was the rate of enterocel development. Fusion of the axohydrocoel occurred at 10, 36 and 78 days respectively for Kas'yanov's, our study and Komatsu's rearings. Previous studies on echinoderm larvae have documented the temperature dependence of larval development (see also section on larval duration below). Second, previous work of Kas'yanov (unpublished report) and Kume and Dan (1968) do not record a wrinkled blastula stage. However, it is clear from the present study (Figure 2) and that of Komatsu's (pers comm) recent work, that the blastocoel of *A. amurensis* does fold and wrinkle, albeit over a short period (approximately 30 minutes). The short duration of this process may have contributed to it being overlooked in previous rearings. Third, Komatsu (pers comm) observed the development and subsequent re absorption of a posterior left enterocoel, although this structure was not observed during this study.

Table 5. *Developmental times for three rearings of* A. amurensis.

	KOMATSU	THIS STUDY	KAS'YANOV
TEMPERATURE	10 ^{0}C	15 ^{0}C	19 ^{0}C
STAGE		TIME	
Blastula	15-16 h	12-14 h	14-16 h
early bipinnaria	3 d	3 d	3 d
pre and post ciliated band		5 d	5 d
enterocoels fused-axohydrocoel	78 d	30-36 d	10-11 d
brachial development	102 d		40-45 d
brachiolaria- adhesive disk	115-120 d		50-60 d

(B) *P. REGULARIS* AND *C. CALAMARIA*

The development of *P. regularis* and *C. calamaria* was similar to that reported by Byrne and Barker (1991) and Barker (1978), with one exception. The post oral ciliated band of *C. calamaria* was much more elaborate than that reported by Barker (1978). This may be due to a difference in diet. Barker (1978) fed his larvae a mixture of *Isochrysis galbana* and *Dunaliella primolecta* but found they grew best on *D. primolecta*. A more rapid rate of development was achieved in

C. calamaria larvae fed on *Rhodomonas sp.* compared to those reared on *D. tertiolecta* in the present study, and thus all experimental rearing was undertaken using *Rhodomonas sp.* as a food source. Food quality is known to affect development of echinoderm larvae (Allison 1994, Paulay *et. al.* 1985) and has been shown to effect the growth of the ciliated band in echinoids (Hart and Schilbling 1988).

5.1.7 FURTHER OBSERVATIONS ON *A. AMURENSIS*

A. amurensis larvae were reared to bipinnaria and provided us with sufficient specimens to compare with local sea star larvae as well as adequate information to estimate larval duration. We were unsuccessful in rearing larvae to settlement and metamorphosis. The following observations may assist future rearing attempts.

- The protracted larval duration and very high mortality suggests that batch cultures, larger than the 500 ml beakers used during this study, are necessary to ensure survival of adequate numbers of larvae. M. Komatsu (pers comm.) recently reported successful rearing of *A. amurensis* to metamorphosis in 10L batch cultures.

- Bacterial contamination may have increased mortality. We observed several instances of unaccountable mortality of larvae in rearing beakers. Treatment with antibiotics during rearing has been reported to increase survival in larval seastar cultures (C. Johnson pers. comm.) and may similarly assist in rearing *A. amurensis.*

- During the second week of trial 2 we had trouble with clumping in *D. tertiolecta* cultures. Upon closer inspection, the individual algal cells appeared normal, although the clumps were too large for larvae to ingest. While seastar larvae are able to go without food for a number of days (Allison 1994, G. Moreno pers. comm.), this may have unduly stressed the larvae. The clumped algae also interfered with water changes as they would not pass through the 60 µm filter. In order to remove clumps from the culture, they were allowed to settle and the remainder of the culture decanted off and then sieved normally. The clumped algae was then discarded. This undoubtedly resulted in the loss of more larvae than usual. The reason for the observed clumping was not ascertained, although it has been previously observed in "old" algal cultures (C. Johnston pers. comm.).

- Mixed algal cultures have been reported to result in more rapid growth rates and enhanced survival in seastar larvae (C. Johnson pers. comm.). *D. tertiolecta* alone, may not be an adequate nutritional source for *A. amurensis.* Mixed algal cultures should be trialed in future rearings.

5.2 LARVAL DURATION

Estimates of larval duration in seastars are based largely on laboratory reared material. In most cases, some degree of abnormality and considerable variation in both developmental rate and time to settlement, has been recorded (Byrne and Barker 1991, Barker 1978, Strathman 1978, Gemmill 1914). Several factors can influence developmental rate and settlement in seastars including temperature, feeding regime, size of rearing containers and provision of appropriate settlement substrate (Barker and Nichols 1983, Barker 1977).

Very few estimates are available for seastar larval duration in the field and thus it is difficult to assess whether the observed variability in development reflects natural variability or represents a laboratory artefact. Barker and Nichols (1983) estimated the minimum larval duration of *Asterias rubens* in the field to be 77-84 d based on monitoring spawning events, larval distribution and subsequent settlement. This figure was close to their minimum laboratory duration of 87 d. Concurrently monitoring spawning, larval distribution and settlement offers probably the only validation of larval duration in the field.

Larval duration of *A. amurensis* appears to be both protracted and variable. Kas'yanov (unpublished report) recorded a larval duration of 50 - 60 d. Rearing temperature was not specified, however initial fertilisation was undertaken at 19°C and we therefore assume this to be the rearing temperature. A three to four week larval period inferred by Hawkes and Day (1993) for *A. amurensis* from Nojima et al (1986) is erroneous and refers to a separate species. Most recently, M. Komatsu (pers. communication) reported a larval duration of 115-120 d at 10-12°C. During the current study, *A. amurensis* was reared for 49 days (15°C) by which time larvae had developed to bipinnaria and were not yet competent to settle.

The relationship between minimum larval duration and temperature was investigated for 11 reported rearings of seastar larvae (Figure 3). The relationship was log-linear and highly significant (r^2=0.611, p=0.0027). This was surprising given the variety of rearing conditions reported. From this relationship, larval durations of 66 d, 74 d, 82 d, and 91 d are predicted for seastar larvae developing at 14, 13, 12, and 11°C respectively. Based on previous rearings of *A. amurensis* and our observations of development, these estimates are probably appropriate for this species. Ambient temperatures range from 11 - 14 °C in the Derwent during the spawning/larval period and thus we predict a larval duration of 66 - 91 d under local conditions. This is somewhat longer than the previous assumed duration of 50 d used in modelling the dispersal of *A. amurensis* larvae in southern Tasmania by Lyne (1993).

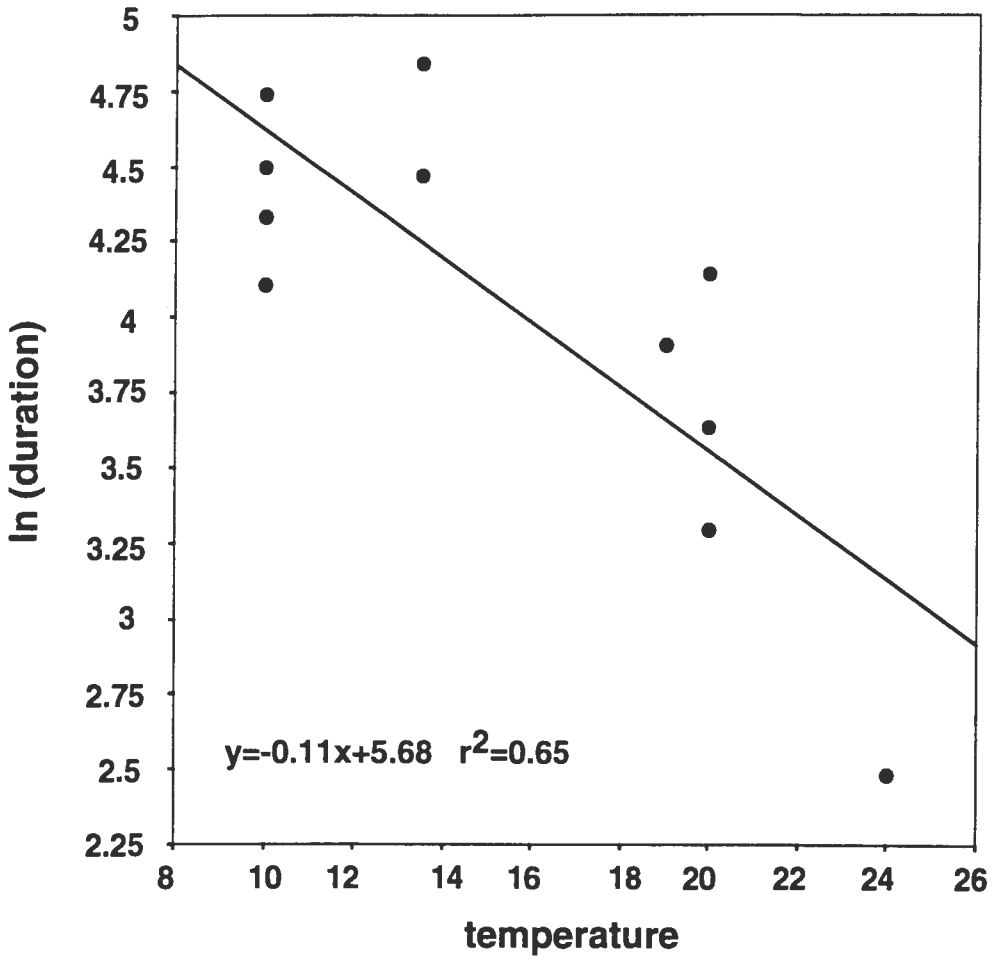

Figure 3 *Relationship between larval duration of seastars and temperature.*

5.3 IDENTIFICATION OF LARVAE

The inability to identify larvae from plankton samples has generally limited studies of larval ecology and recruitment processes in seastars. Slight morphological differences have been observed between planktotrophic larvae of different seastar orders (eg length of bipinnarial processes, M. Barker, M. Byrne pers. communication), however, species identification has previously been established for only a small number of wild caught larvae on the basis of morphological characters (eg. Barker and Nichols 1983).

Morphological comparisons were made between wild caught larvae from plankton samples, collected in southern Tasmania, with larvae of reared *A. amurensis* as well as two other seastars (*P. regularis* and *C. calamaria*). *P. regularis* and *C. calamaria* were selected because they are both abundant in the Derwent and also have planktotrophic larvae.

5.3.1 METHODS

Two methods were used to examine morphological differences. First, due to the large morphological variation within larvae of the same species and the overlap of characters between species, numerical analyses (one way multivariate analysis of variance (MANOVA) and linear discriminant function analysis) were applied to the four larval groups. Similar analyses have facilitated the identification of morphologically similar prawn larvae (Rothlisberg et al 1983) although they have not previously been used to identify starfish larvae. Second, larvae were examined microscopicaly to identify characters that could otherwise not be measured (eg complexity of ciliated bands) but which may be diagnostic for the species.

(A) NUMERICAL ANALYSES

Seven morphological measurements were taken from 18-20 larvae from each group (Figure 4). Larvae of the same developmental stage (bipinnaria) were selected to reduce the confounding effects of otogenetic variability in morphology. Characters that could be easily and reliably measured and that best described the shape of the larvae were selected. Four morphological characters (dependent variables) were used in the analysis, the oral hood (OH), posterior lateral process (PL), post oral process (POP) and the pre oral hood (PRO). Two characters, the dorsal process (D) and the posterior dorsal process (PD), had unacceptably high squared multiple correlations and were omitted in order to reduce the possibility of multicolinearity (Tabechnik and Fidell 1989). Morphological characters were standardised between specimens by dividing by total length (TL).

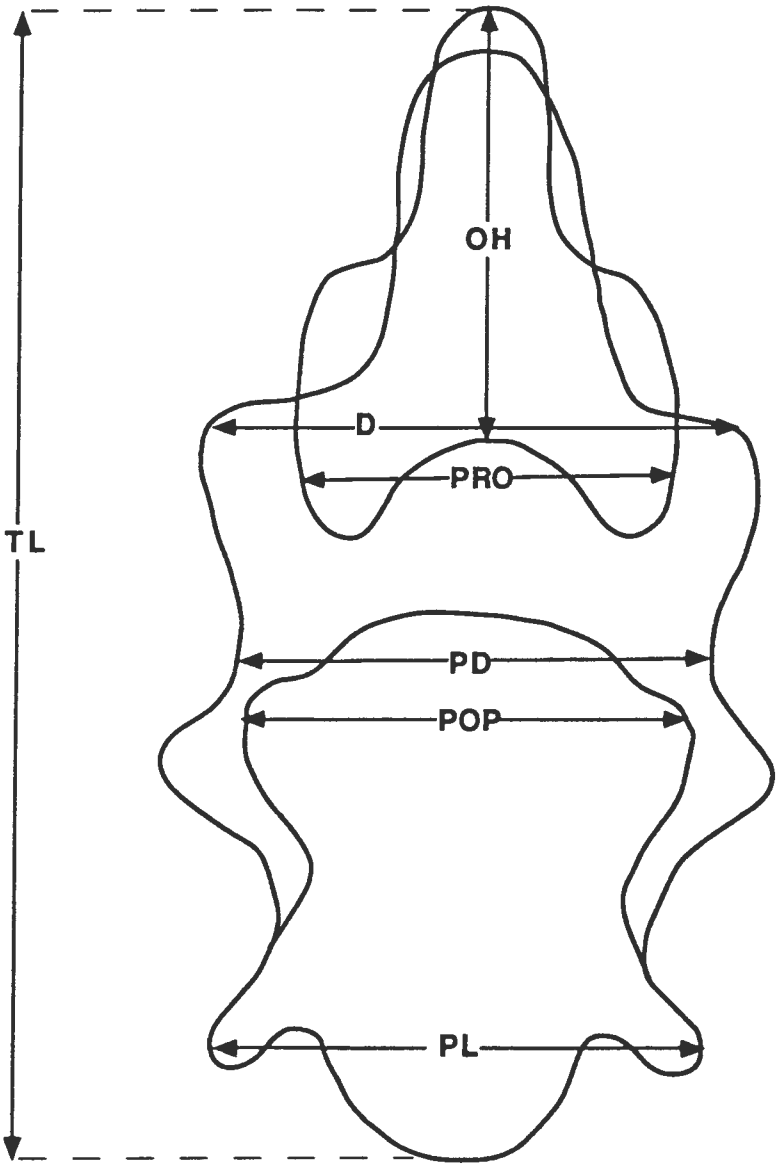

Figure 4 *Morphological measurements used in numerical analyses of seastar larvae.*

Data were tested for multivariate outliers, multivariate normality and homogeneity of variance-covariance matrices (Tabechnik and Fidell 1989). Data were transformed using log(x+1) to standardise the variance. All analyses were performed using SYSTAT.

Multivariate outliers were determined using Mahalanobis' distances evaluated as $\chi 2$ with degrees of freedom equal to the number of dependent variables (ie. the number of morphological characters) and $\alpha = 0.05$.

MANOVA was performed using Roy's greatest characteristic root (gcr) as a simultaneous test statistic to compare contrasts between the four groups. Contrasts were considered significant if the statistic $\theta_1/(1-\theta_1)$ exceeded the critical value of $R_{0.05}/(1-R_{0.05})$, where θ_1 is Roy's gcr for the contrast and $R_{0.05}$ is the critical gcr for the over-all test (Bird and Hadzi-Pavlovic 1983). The critical values of Roy's gcr were obtained by interpolation from the tables of Harris (1985).

Linear discriminant function analysis was performed using DISCRIM to further examine the group differences. DISCRIM maximises the separation between pre-defined groups while minimising the within group variation (Ter Braak and Prentice, 1988). Canonical correlations were used to assess the relative contribution of each character in distinguishing between the four groups. Canonical correlations represent the amount of variance each character shares with the discriminant function. Canonical coefficients represent the unique contribution of each character to the discriminant function but are less stable as they are influenced by intercorrelation between dependent variables (Tabechnik and Fidell 1989).

5.3.2 RESULTS

No outliers were detected for any of the four larval groups. Multivariate normality was assumed with 18-20 cases per group. Homogeneity of variance-co variance was assumed as the ratio of the largest to smallest sample size did not exceed 1:2. There was a suggestion of multicolinearity as the dependent variables POP and PRO had correlations of -0.829 and -0.862 respectively. However, the squared multiple correlations were at acceptably low levels (< 0.407) and therefore they were included in the analysis (Tabechnik and Fidell 1989).

Significant differences were detected between larval groups based on the four selected characters (PL. POP, PRO and OH; θ=0.482, s=4, M=.00, N=34.5). No significant difference was detected between Ast. wild and *A. amurensis* nor between *C. calamaria* and *P. regularis*, however both groups were significantly different to each other (Table 6).

Table 6. *Pairwise comparisons between the four larval groups using Roy's gcr simultaneous test procedure.* θ*=Roy's gcr. Critical gcr:* $R_{0.05}/(1-:R_{0.05})=.233$

COMPARISON		θ_1	$\theta_1/(1-\theta_1)$	SIGNIFICANCE
Ast. wild	*A. amurensis*	0.184	0.225	ns
Ast. wild	*C. calamaria*	0.295	0.418	*
Ast. wild	*P. regularis*	0.321	0.473	*
A. amurensis	*C. calamaria*	0.311	0.451	*
A. amurensis	*P. regularis*	0.355	0.550	*
C. calamaria	*P. regularis*	0.025	0.026	ns

*= P<0.05, ns = not significant

The discriminant function plot (Figure 5) displays 95% confidence limits around the centroids of the four larval starfish groups. Ast wild and *A. amurensis* separated from *C. calamaria* and *P. regularis* by the first discriminant function. Ast wild and *A. amurensis* were weakly, but not significantly, separated by the second discriminant function. A test of residual roots shows a reliable relationship between groups and predictors $\chi 2(12)=60.003$, $p<0.001$ which remained reliable after the removal of the first discriminant function $\chi 2(6)=16.122$, $p<0.01$ but was not significant with the removal of the second discriminant function. Therefore the first and second discriminant functions are true tests of variability between the four groups but not the third. The first and second functions explained 66.4% and 30.4% of the variance respectively.

Table 7 displays the canonical coefficients and canonical correlations of the first two discriminant functions. The correlations between characters are considered important if they exceed 0.3 (Tabachnik and Fidell, 1989). PL and POP were both negatively correlated with the first discriminant function. PRO was negatively correlated with the second discriminant function.

Table 7. *Canonical coefficients and canonical correlations of the first two discriminant functions using the four morphological characters as dependent variables.*

MORPHOLOGICAL CHARACTER	CANONICAL COEFFICIENTS		CANONICAL CORRELATIONS	
	1	2	1	2
oral hood (OH)	0.087	0.118	-0.126	-0.153
posterior lateral process (PL)	-0.435	0.829	-0.595	0.18
post oral process (POP)	-0.78	-0.729	-0.879	-0.461
pre oral process (PRO)	0.432	-0.862	0.153	-0.619

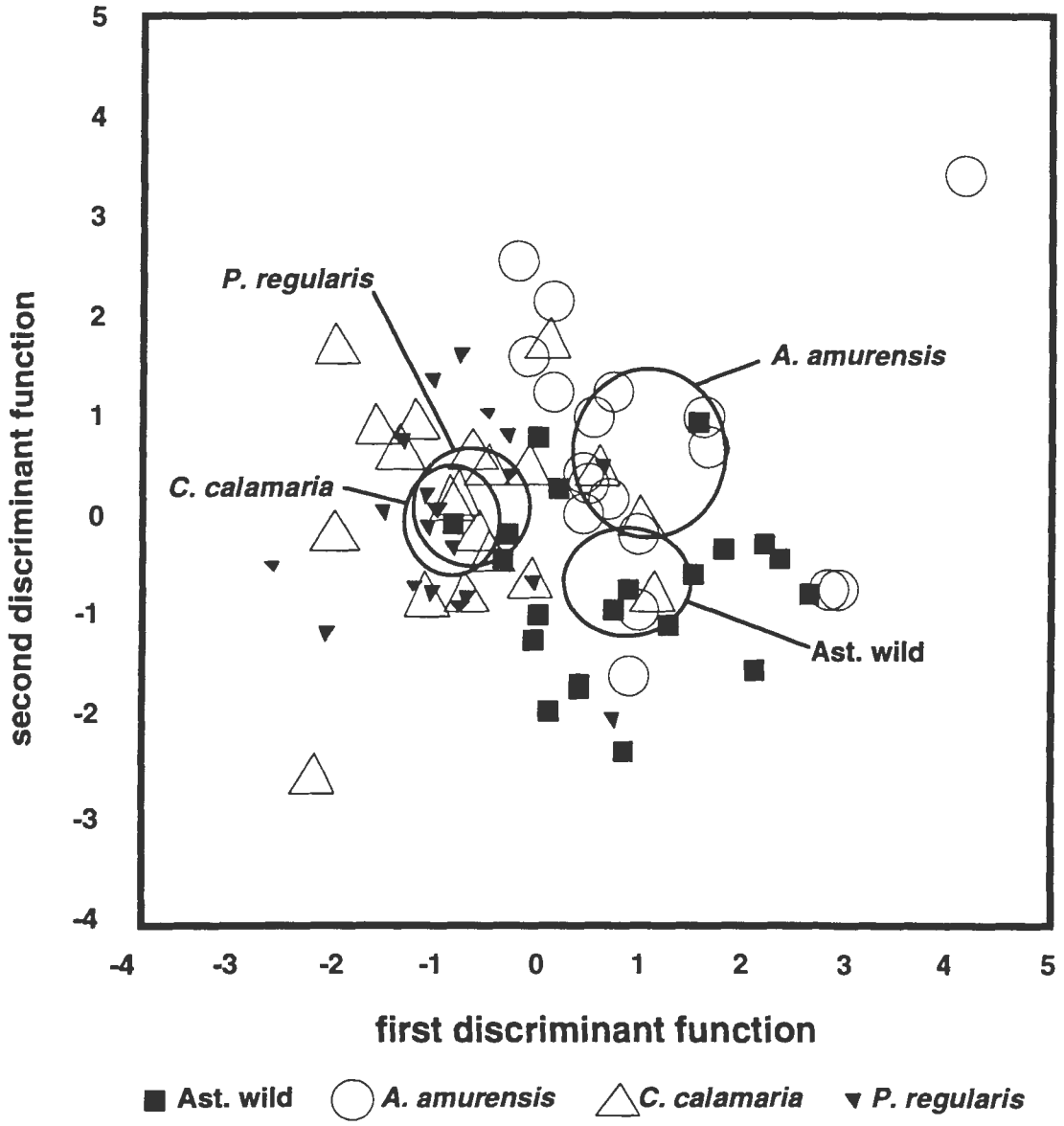

Figure 5 *Discriminant function plot for larval seastar groups. 95% confidence limits are displayed around centroids for each group.*

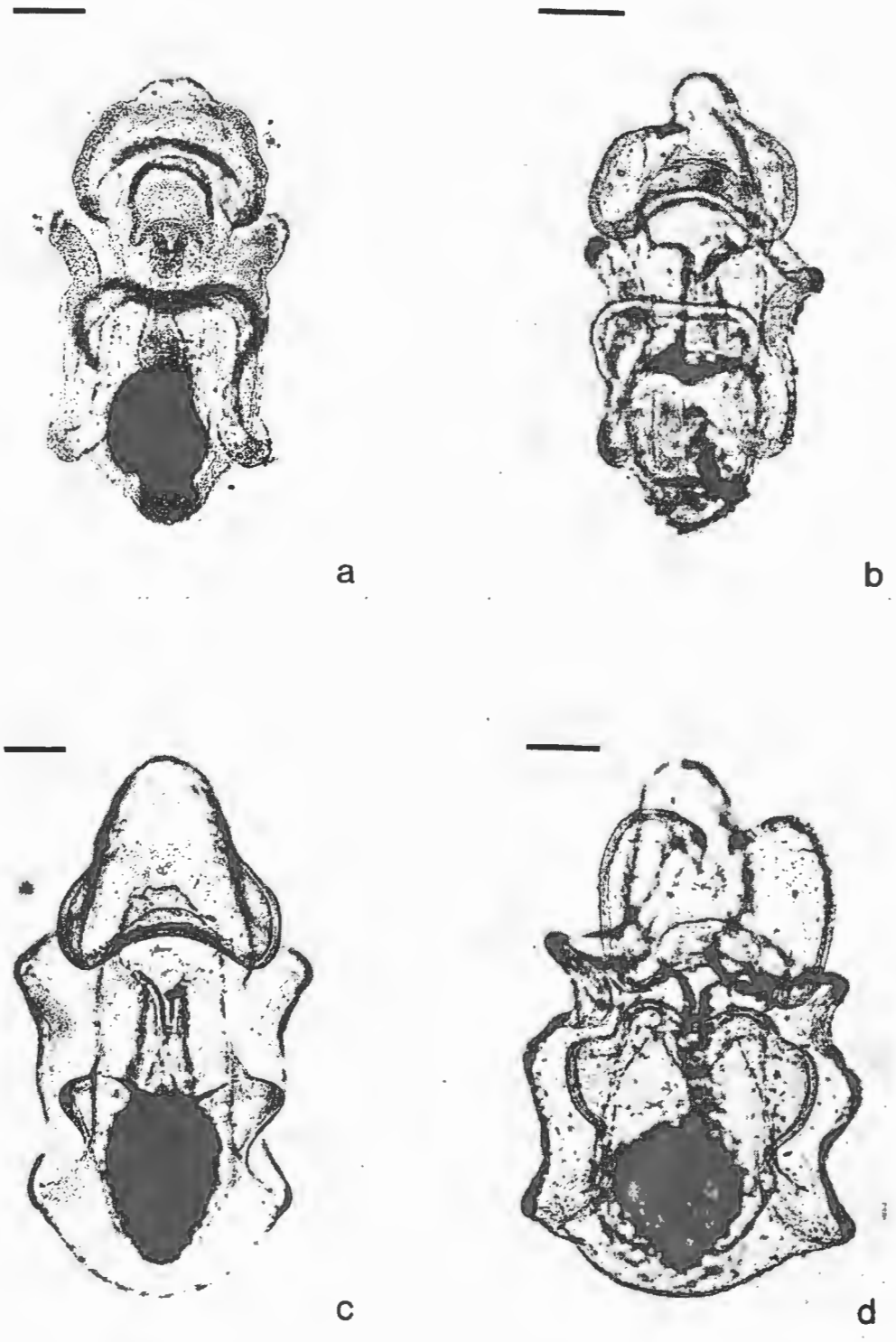

Figure 6 *Bipinnaria larvae of reared and field collected seastars: (a)* A. amurensis*; (b) Ast. wild; (c)* P. regularis*; (d)* C. calamaria*. Scale bar = 100 µm.*

PL and POP were larger in *C. calamaria* and *P. regularis* than Ast. wild and *A. amurensis*. PRO was largest in the Ast. wild and *P. regularis* and thus explains the significant but weaker association between Ast. wild and *A. amurensis* compared to the strong association between *C. calamaria* and *P. regularis* (Table 8).

Table 8. Least squares mean of each morphological character in each larval group

MORPHOLOGICAL CHARACTER	AST. WILD	A. AMURENSIS	C. CALAMARIA	P. REGULARIS
	n=20	n=18	n=20	n=20
oral hood (OH)	0.139	0.134	0.143	0.140
posterior lateral process (PL)	0.156	0.158	0.173	0.179
post oral process (POO)	0.124	0.105	0.148	0.152
pre oral process (PRO)	0.170	0.156	0.155	0.160

5.3.3 MICROSCOPIC EXAMINATION

Microscopic examination revealed several characters that separated the larval groups. Useful characters included the shape and complexity of the post oral ciliated band, the post oral field and the shape of the oral hood (Table 9). *P. regularis* has a simple post oral ciliated band, where as the other three groups had more complex bands. *C. calamaria* had the most complicated and tortuous band (Figure 6). The complexity of the post oral ciliated band was similar in *A. amurensis* and Ast. wild. *C. calamaria* had an elaborate post oral field, where as the others have relatively plain post oral fields (Figure 6). The shape of the oral hood is also very different among the groups, *P. regularis* have a bell shaped hood. *C. calamaria* have a pointed hood as well as prominent and very rounded anterior dorsal process. *A. amurensis* and Ast. wild have a pointed hood and a prominent anterior dorsal process, but, they are not as rounded as those of *C. calamaria* (Figure 6).

Table 9. *Summary of characters used for microscopic identification*

CHARACTER	A. AMURENSIS	C. CALAMARIA	P. REGULARIS	AST. WILD
post oral ciliated band	complex	very complex and tortuous	simple	complex
oral hood	pointed hood, anterior dorsal process not as rounded as *C. calamaria*	pointed hood, prominent and rounded anterior dorsal processes	bell shaped, no anterior dorsal processes	pointed, anterior dorsal process not as rounded as *C. calamaria*
post oral field	simple	very complex and elaborate	simple	simple

5.3.4 DISCUSSION

Both LDA results and microscopic examination suggest that wild caught larvae were *A. amurensis*. In general, larvae of *C. calamaria* and *P. regularis*, were relatively wider than larvae of Ast. wild or reared *A. amurensis* and differed in the complexity of the post oral ciliated band. In LDA analyses, Ast. wild and *A. amurensis* were separated from *C. calamaria* and *P. regularis* by the first discriminant function which explained 66.4% of the total variance. The second discriminant function, which only explained 30.4% of the total variance, grouped *C. calamaria* and *P. regularis*. The inability of LDA to separate *C. calamaria* and *P. regularis* does, however, suggest caution in establishing species identity from such analyses alone.

One difference was noted between the reared *A. amurensis* and Ast. wild larvae. Ast. wild larvae had less developed enterocoels, relative to total length, compared to those of *A. amurensis*. Enterocoel development of the Ast. wild was consistent with that recorded by Komatsu (pers. comm.) for *A. amurensis* reared at 10-12°C. Ambient temperatures in the Derwent River range from 11-14 °C, when larvae are present in the water column. These lower temperatures may thus account for the observed differences between wild caught larvae and larvae reared at 15°C. Different feeding regimes between reared and wild caught larvae may also contribute to variations in enterocel development. Decreased growth rate in echinoderm larvae due to food limitation is well documented (Hart and Strathman 1994, Paulay et. al. 1985, Boidron-Metairon 1988).

Three other, albeit circumstantial, results also suggest that our wild caught seastar larvae were *A. amurensis*. First, the concentration of larvae was extraordinarily high in the Derwent, particularly, in areas where both adult and juvenile *A. amurensis* were most abundant. Second, the development of larvae was consistent with that expected from spawning within the peak (August) period recorded for *A. amurensis* by Buttermore et al. (1994). Third, spawning in other Tasmanian seastars (where known) occurs during late spring and summer rather than winter, as is the case in *A. amurensis*, and thus capturing large numbers of larvae other seastar species was unlikely.

Despite the accumulated evidence suggesting that wild caught larvae were *A. amurensis*, the above criteria cannot provide unequivocal identification. This would be particularly apparent if identification of larvae in ballast water, or from samples taken at other times of the year (eg early Summer) was required. Unequivocal identification of larvae is probably only possible through the use of genetic technology (see section on Future Development).

6. FIELD STUDIES

Sampling were taken within the Derwent River and at Spring Bay (Figure 7) to determine the vertical and broad scale (horizontal) distribution of *A. amurensis* larvae. These sites were selected for three reasons. First, both included ports (Hobart and Triabunna) where discharge of ballast water occurs. Second, *A. amurensis* had been recorded at both localities. Third, the two sites differed in their physical environment. The Derwent River is a typical estuary influenced by fresh water input whereas Spring Bay is coastal/oceanic in physical attributes. The two sites were compared to assess variations in larval distribution between estuarine and coastal/oceanic environments.

6.1 METHODS

6.1.1 VERTICAL DISTRIBUTION (SAMPLING).

Two sites were sampled Spring Bay, two sites within the Derwent River estuary and one site within the D'Entrecasteaux Channel during September and October 1993 (Figures 8+9). Sample sites, times and depths are shown in Table 5.

Table 5. *Sampling sites and details (vertical distribution).*

SITE	DATE	STRATA SAMPLED (NO. REPS)
Derwent River		
Sullivans Cove	22/9/93	0, 5, 10 m (3)
Sullivans Cove	29/9/93	0, 5, 10 m (3)
Sandy Bay	29/9/93	0, 5, 10 m (1)
Sandy Bay	30/9/93	0, 5, 10 m (3)
D'Entrecasteaux Channel		
Bligh Pt	30/9/93	0, 5, 10 m (3)
Spring Bay		
Paddy's Pt	8/10/93	0, 5, 10 m (3)
Paddy's Pt	28/10/93	0, 5, 10 m (3)
Quarry Pt	28/10/93	0, 5, 10 m (3)

Sampling was undertaken from the Division's 5.4 m shark cat "Ophelia". At each station the vessel was anchored and larvae were sampled with a diaphragm pump. A 'Platypus' submersible data logger was attached to the hose intake and recorded sample depth, salinity and temperature at 5 second intervals. Larvae were collected by pumping water from 0, 5 and 10 m depth strata for a 10 minute period. Bottom depth ranged from 12-14 m between stations. Pumping rate was held constant and averaged 57.2 litres per min., thus giving an estimated average of 572 litres per sample per depth stratum. Three replicates were taken at each strata. Due to time constraints, the order in which strata

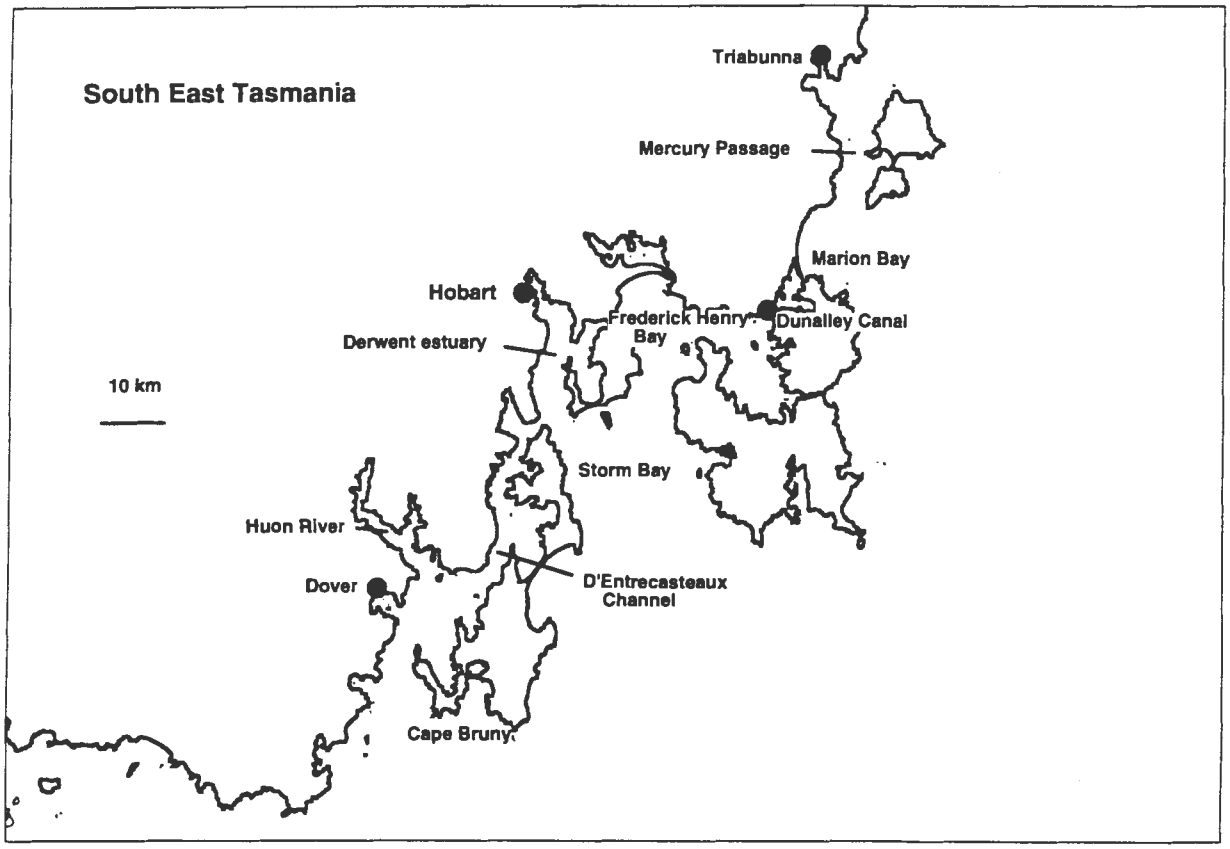

Figure 7 *The study area.*

were sampled was fixed. Sampling order was 10,5,0,0,5,10,10,5,0 m. Each vertical series thus comprised nine samples and took 2.5-3.0 hours to complete. Strata were fixed as depth from the surface and no allowance was made for tidal variation during the period of sampling.

Filtration was achieved by directing the outflow of the pump into a 100 µm mesh plankton net suspended on a frame beside the vessel. The pump was operated for two minutes at each new sample stratum, prior to filtering, in order to clear the hose of water from the previous stratum. One hundred µm mesh was selected based on the reported size of *A. amurensis* eggs (110 µm, Kas'yanov 1988). However, during rearing trials, some live eggs and newly hatched larvae passed through 100 µm mesh during water changes. Thus field samples were likely to underestimate the numbers of these stages. Samples were preserved in a 10% un-buffered formaldehyde/seawater solution and returned to the laboratory for sorting.

A full vertical series was completed at each site except Sandy Bay on 29 September, when weather and time constraints limited sampling to only one sample per stratum (Table 10). This series was omitted from subsequent analyses.

6.1.2 HORIZONTAL DISTRIBUTION (SAMPLING)

Sampling was undertaken at 8 stations within Spring Bay and 30 stations within the Derwent River estuary during October 1993. Spring Bay was sampled on two separate occasions (8/10/93 and 28/10/93), Figure 8. A single survey (14/10/93) was undertaken in the Derwent (Figure 9).

At each station, larvae were sampled using a weighted, free fall, plankton net (50 cm diameter, 100 µm mesh). The depth of sampling was recorded by a Tekna maximum depth indicating gauge attached to the frame of the net. Calibration trials estimated the rate of fall of the net at approximately 0.75 m per second and this value was used to estimate the required duration of each fall. Fall times were calculated to allow the net to sample from the surface to as close to the bottom as possible. A collar choke on the net prevented sampling during retrieval of the gear. Samples were fixed in 10% unbuffered formaldehyde/seawater solution and returned to the laboratory for sorting.

Vertical profiles of temperature and salinity were taken using a 'Platypus' submersible data logger (Spring Bay) and a Sea Bird CTD (Derwent River). Calibration trials between the two units revealed no significant difference between readings.

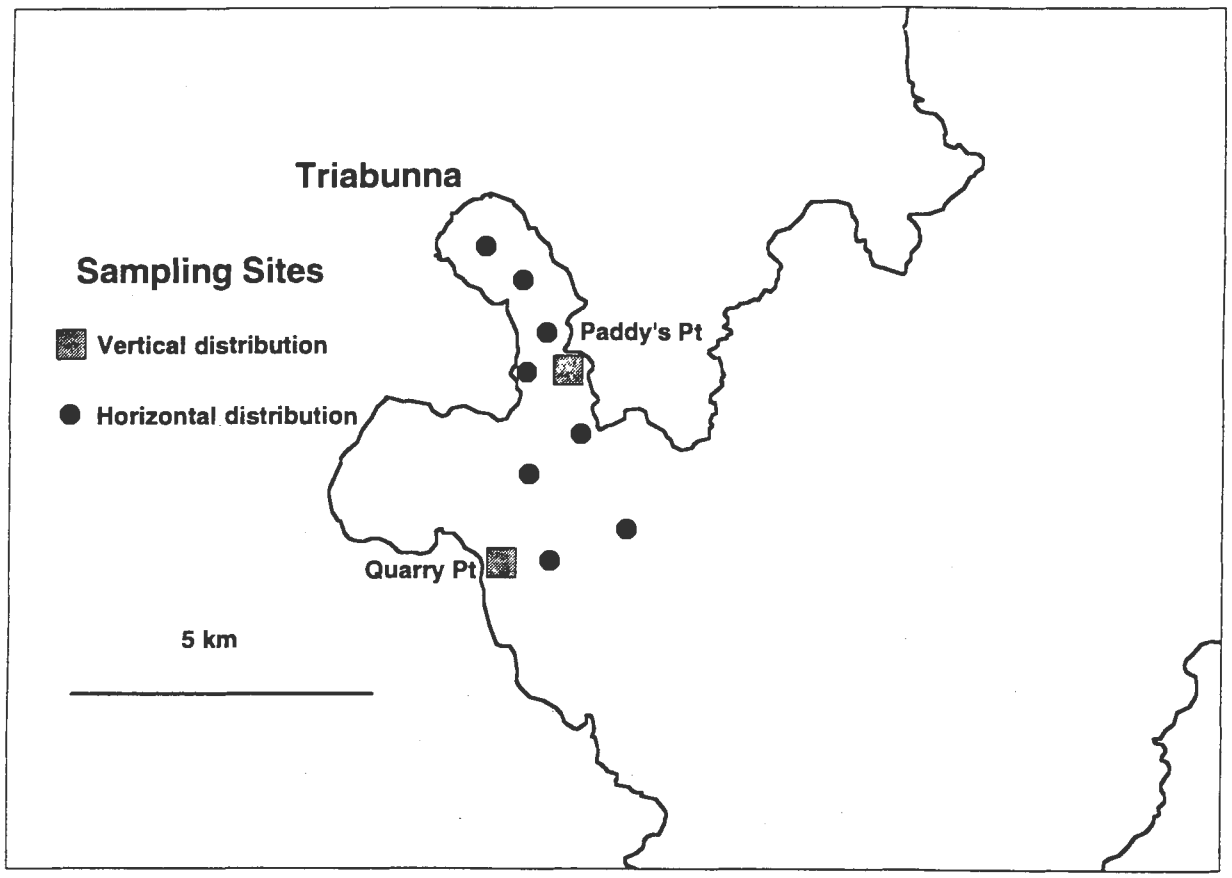

Figure 8 *Station locations - Spring Bay.*

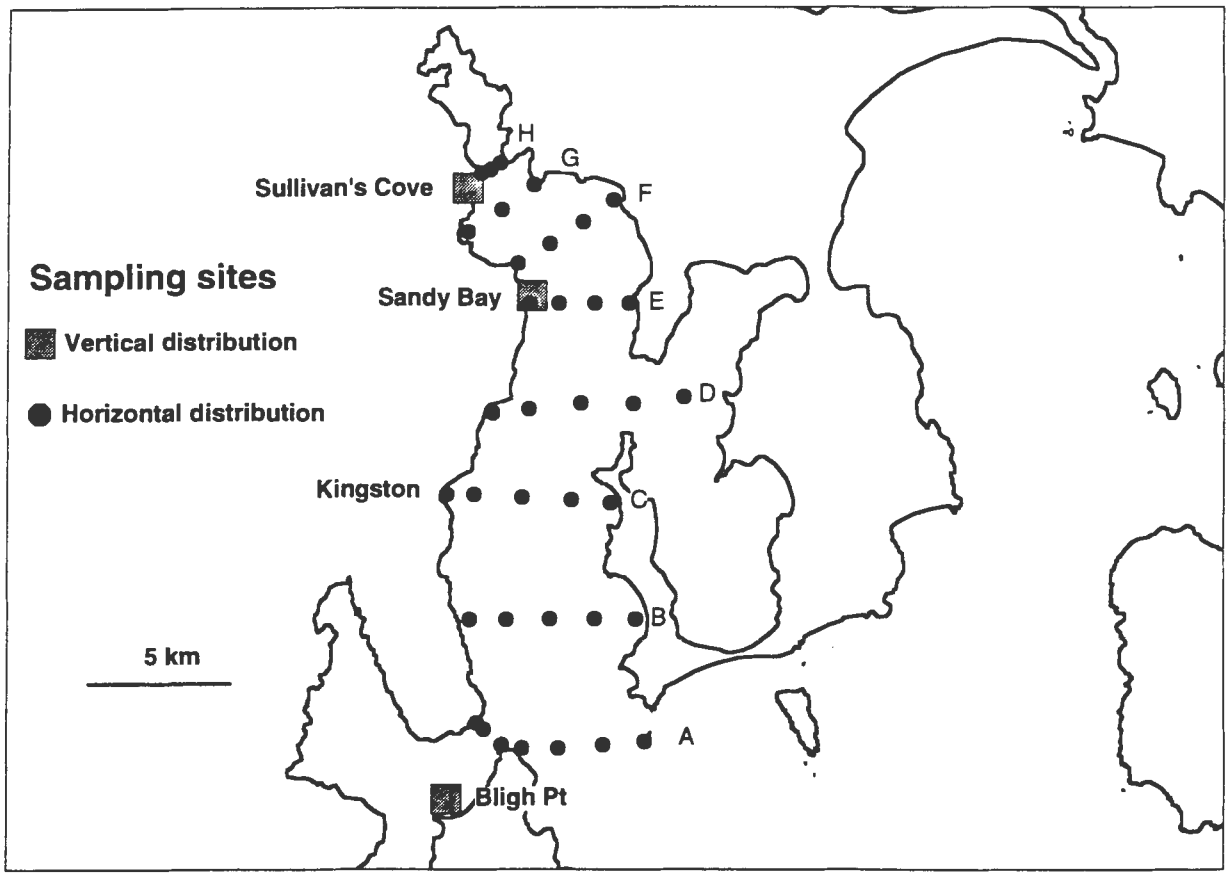

Figure 9 *Station locations - Derwent estuary.*

6.1.3 LABORATORY PROCEDURES

Vertical and broad scale samples were treated in the same way. Samples were rinsed in fresh water to remove formaldehyde and then sorted under a binocular microscope. Where possible, complete samples were sorted. For most samples, however, sub-sampling was undertaken using a Folsom splitter. Seastar larvae were removed, identified, assigned to a developmental stage and enumerated. The total number of larvae per sample was estimated by the formula: count x 2^n, where n is the number of splits (Omori and Ikeda 1984). Larvae were assigned to one of five developmental stages, gastrula, bipinnaria, advanced bipinnaria, brachiolaria or advanced brachiolaria following the criteria established in Table 1.

(A) DATA ANALYSES

All statistical analyses were performed using SYSTAT 5.2.1.

(B) VERTICAL DISTRIBUTION

Numbers of larvae were standardised to numbers collected per ten minutes of sampling for each depth strata (ie number per 0.572 m^3). Comparisons of standardised numbers of larvae between depths and sites, and (where appropriate) between depths, sites and sampling days, were made using a multiway ANOVA. Standardised numbers of larvae were transformed to $\log_e(N+1)$ as this transformation gave the most even distribution of residuals versus fitted values. A Tukey post hoc test was used to distinguish between groups when the effects were significant at $P \leq 0.05$.

(C) HORIZONTAL DISTRIBUTION

Numbers of larvae were standardised to the number per cubic metre, integrated over the depth sampled using the formula:

$$N = n \times (3.14159 \times (0.25)^2 / d)$$

where n was the total number of larvae in the sample and d was the maximum depth to which the net sampled.

6.2 RESULTS

6.2.1 PHYSICAL DATA

(A) SPRING BAY

Surface temperatures and salinities were relatively uniform at Spring Bay and reflected the overall coastal/oceanic influence at the locality. The water column at Paddy's Pt was well mixed on each sampling date (Figure 10). Temperatures were higher and salinities lower on 28 October (13.0-13.2^{o}C, 34.81-34.85 ppt) compared to 8 October (12.98-13.0^{o}C, 35.01-35.05 ppt).

A thermocline was present at Quarry Pt on 28 October, separating the surface 5 m (14.5^{o}C) from the cooler bottom layer (13.8^{o}C). Very little vertical structure was apparent in the salinity profile (Figure 11).

(B) DERWENT RIVER AND D'ENTRECASTEAUX CHANNEL

Very little vertical structure was apparent in temperature profiles at Sullivans Cove on 22 September with temperatures ranging from 11.3-11.5^{o}C. Salinity increased steadily with depth from 30.25 ppt at the surface to 33.75 at the bottom (Figure 12).

A marked, relatively warm and less saline, surface (0-1 m; 13.5^{o}C, 32.15 ppt) layer was present at Sullivans Cove on 29 September (Figure 12). A weaker thermo-halocline was present at 8 m (11.4-11.7^{o}C. 34.05-34.35 ppt).

Temperature and salinity at Sandy Bay, on 30 September, ranged from 11.5-14.02^{o}C and 31.01-34.35 ppt. (Figure 13). Temperature decreased steadily with depth. Salinity increased rapidly from 31.01-33.65 at 4 m and then steadily to 34.35 at the bottom.

Vertical profiles at Bligh Pt were dominated by a marked, relatively warm and less saline surface layer extending to 4.5-5.0 m (Figure 13). Temperatures and salinities were relatively stable from 5 m to the bottom (12.1-12.2^{o}C, 33.95-34.05 ppt).

Surface temperature and salinity, on 14 October, ranged from <12.5^{o}C and 34.00 ppt at the mouth of the Derwent to >14.0^{o}C and <25.00 ppt at the Tasman Bridge (Hobart) respectively (Figure 14). Contours indicated a flow of relatively warm, low salinity, Derwent River water southwards over the central and eastern regions of the estuary. This was particularly evident in vertical profiles (Figure 15). An inflow of high salinity water from the D'Entrecasteaux Channel and Storm Bay was evident on the western boundary of the estuary. Considerable vertical stratification was evident on all transects, particularly on

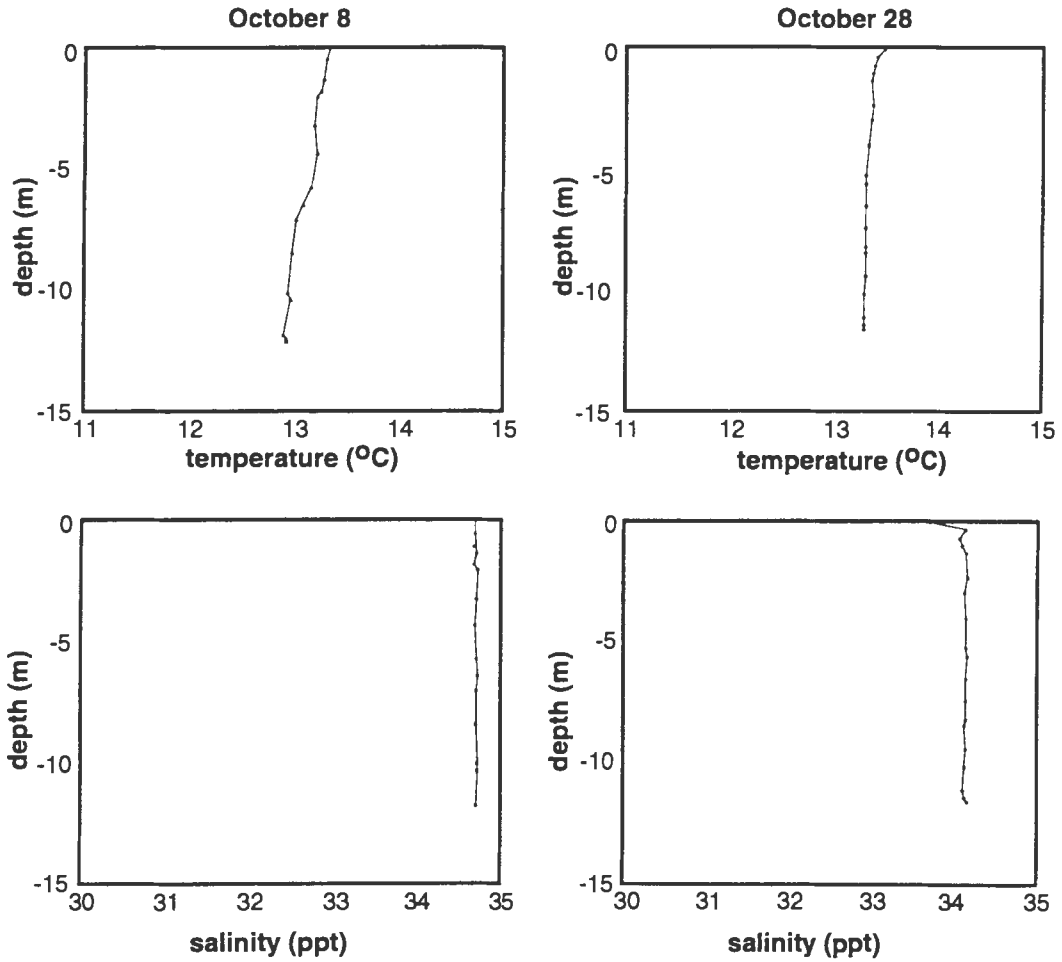

Figure 10 *Temperature and salinity profiles - Paddy's Point.*

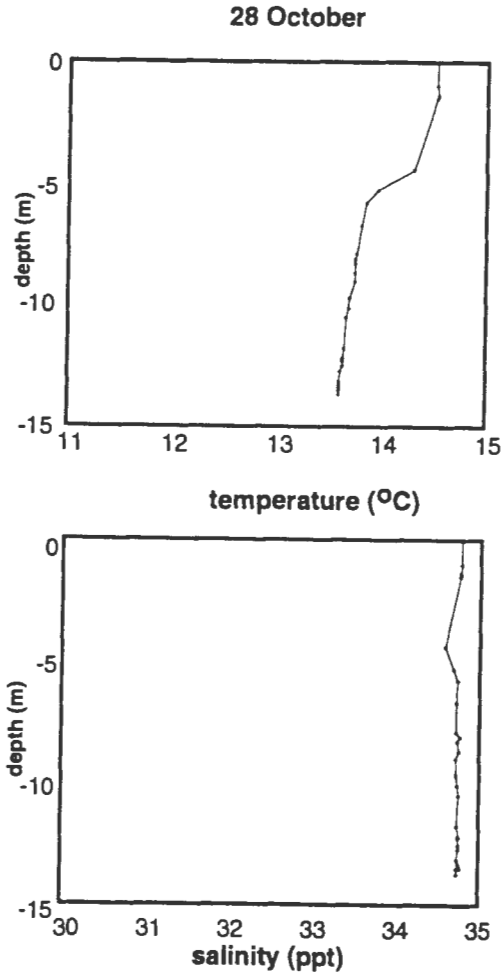

Figure 11 *Temperature and salinity profiles - Quarry Point.*

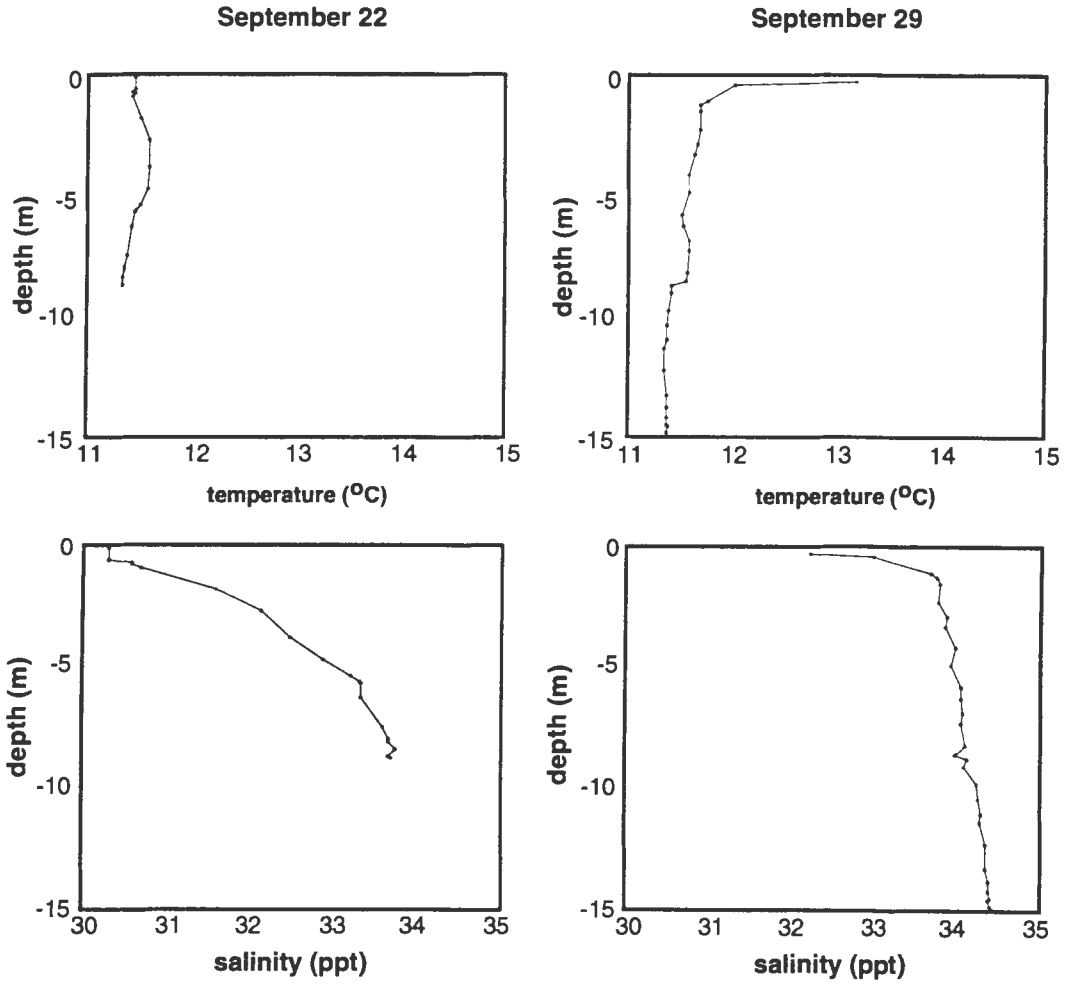

Figure 12 *Temperature and salinity profiles - Sullivan's Cove.*

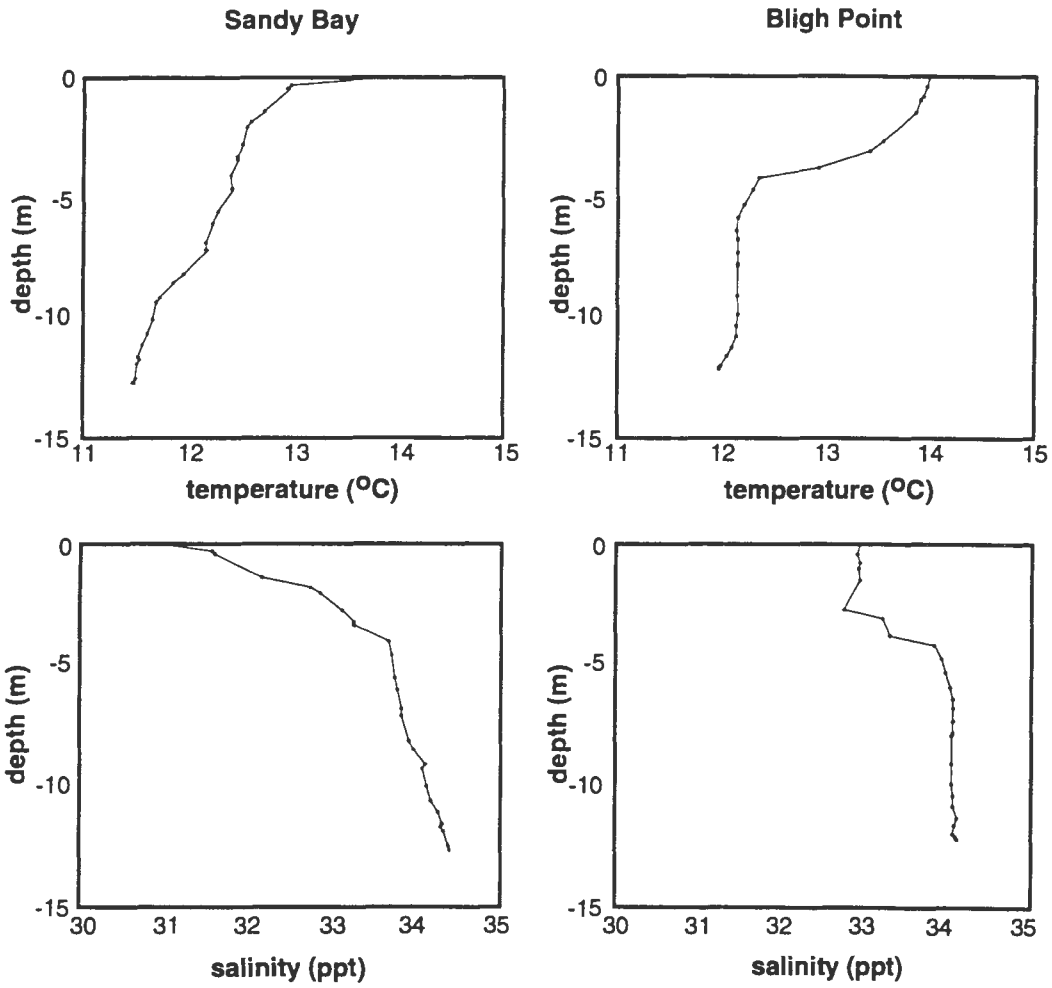

Figure 13 *Temperature and salinity profiles - Sandy Bay and Bligh Point.*

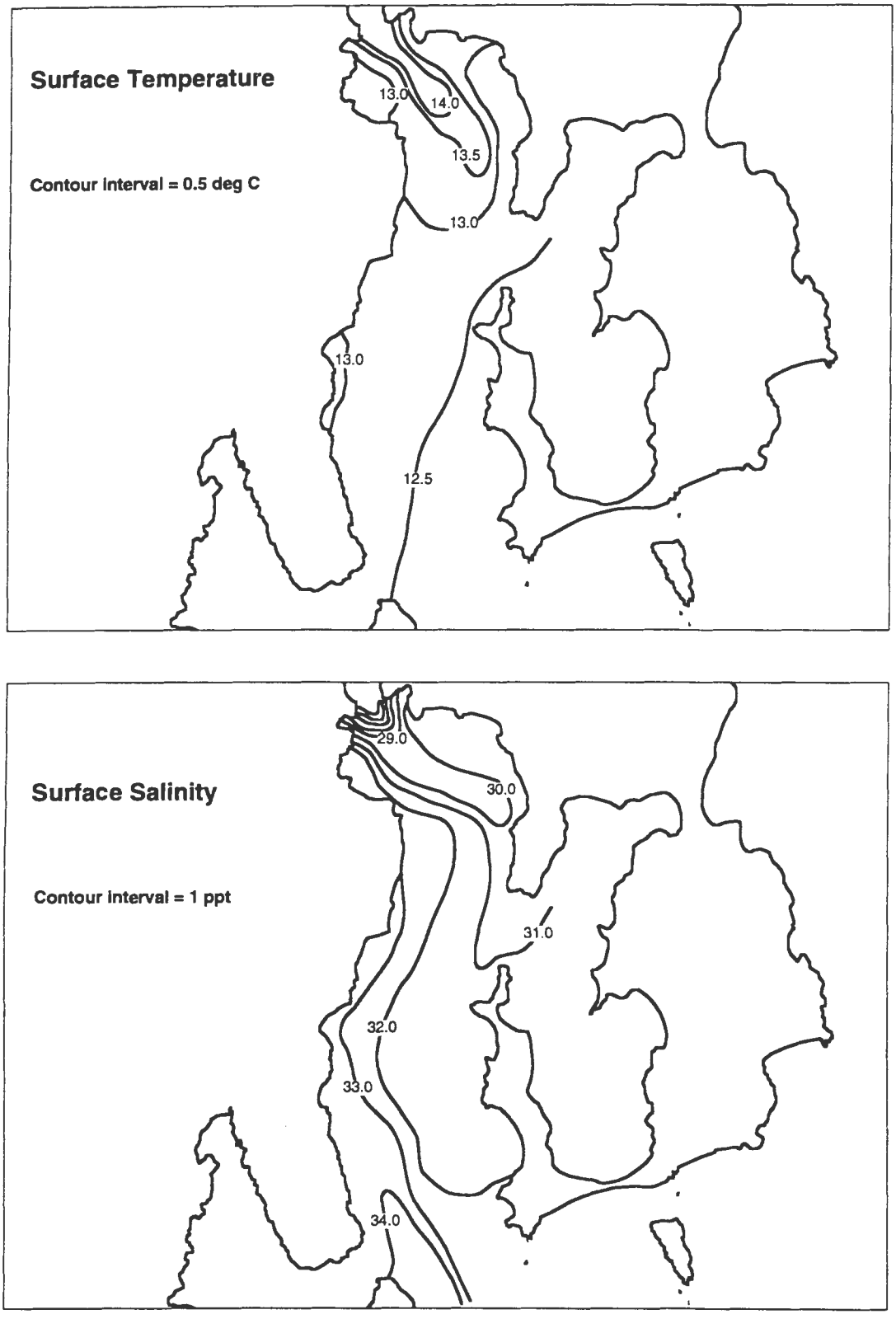

Figure 14 *Surface temperature and salinity - Derwent estuary, 14/10/93.*

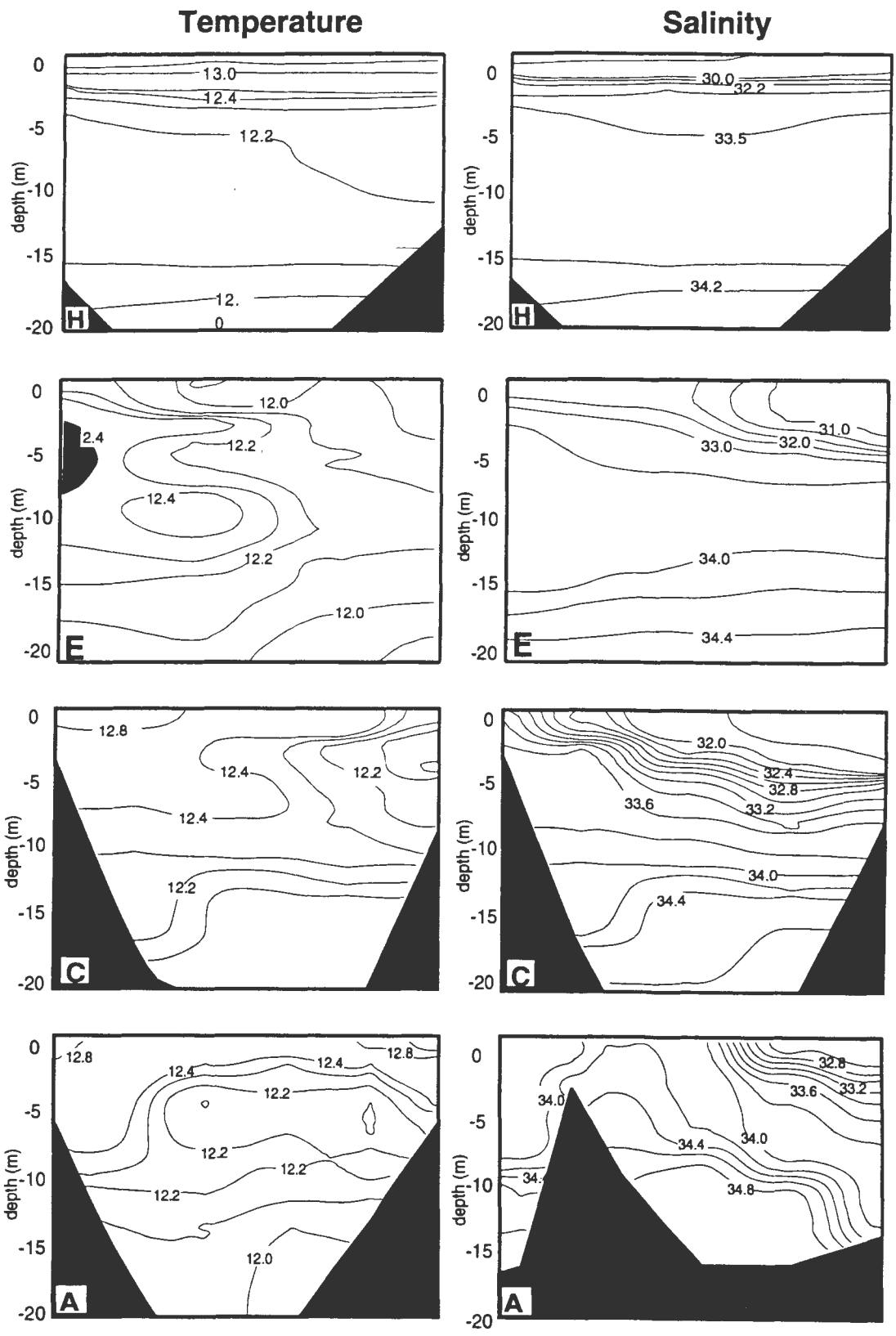

Figure 15 *Temperature and salinity profiles - Derwent estuary 14/10/93. See Figure 9 for location of transects.*

the eastern side of the River. High salinity (>34.2 ppt) water extended along the bottom to the Tasman Bridge.

6.2.2 VERTICAL DISTRIBUTION

No seastar larvae were found in samples from Spring Bay.

A. amurensis larvae were present at all sites sampled in the Derwent Estuary and D'Entrecasteaux Channel (Figure 16). The concentration of larvae was significantly different between sites (F-ratio = 24.252, P= 0.000) and depths (F-ratio = 26.289, P=0.000). At each site, the concentration of larvae was not significantly different between the surface and 5m, but larval concentration at both these depths were significantly higher than at 10 m (Table 6+7).

Table 11. *Tukey matrix of pair wise comparison probabilities for concentration of larvae between sites (MSE = 0.451, DF = 18).*

SITE	SULLIVAN'S COVE	SANDY BAY	BLIGH PT
Sullivan's Cove	-		
Sandy Bay	0.040	-	
Bligh Pt	0.000	0.000	-

Table 12. *Tukey matrix of pairwise comparison probabilities for concentration of larvae between depths (MSE = 0.451, DF = 18).*

DEPTH	SURFACE	5 M	10 M
surface	-		
5 m	0.506	-	
10 m	0.000	0.001	-

Sullivan's Cove was the only site where larvae were found at 10 m. There was a consistent trend of deceasing concentrations southwards down the river.

Larvae of all stages except advance brachiolaria were recorded. There were no significant trends between stage of development and either depth or site.

Sufficient data were available from only one site (Sullivan's Cove) to compare larval distribution between different sampling days. The concentration of larvae was significantly different between depths (F ratio = 11.707, P=0.002) but not between days (F ratio = 2.459 P=0.143). The concentration of larvae was not significantly different between the surface and 5 m, but larval concentration at both these depths were significantly higher than at 10 m (Table 13).

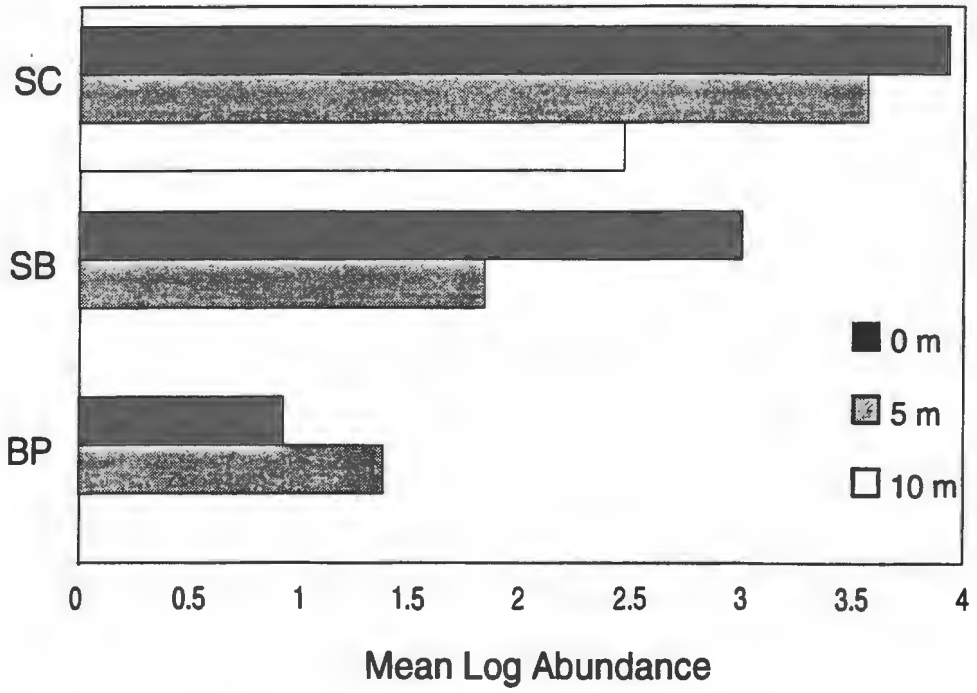

Figure 16 *Vertical distribution of seastar larvae - Derwent estuary; SC Sullivan's Cove; SB Sandy Bay; BP Bligh Point.*

Table 13. *Tukey matrix of pairwise comparison probabilities for concentration of larvae between depths at Sullivan's Cove on 22 + 29 September (MSE = 0.548, DF = 12).*

DEPTH	SURFACE	5 M	10 M
surface	-		
5 m	0.336	-	
10 m	0.001	0.018	-

6.2.3 HORIZONTAL DISTRIBUTION

No seastar larvae were found in samples from Spring Bay.

A. amurensis larvae were present at all sites sampled in the Derwent River (Figure 17). Standardised concentrations ranged from 8.6-152.7/m^3. Concentrations of larvae were highest in the vicinity of Hobart. Concentrations tended to decrease southwards, although high concentrations were also recorded near Kingston on the western side of the River.

Larvae of all stages, except advanced brachiolaria were recorded and were widely distributed throughout the Derwent (Figure 17). Bipinnaria and advanced bipinnaria were the most common stages, probably reflecting the lengthy duration (and thus increased opportunity for capture) of these stages. There were no obvious trends between position in the River and developmental stage except for gastrula which were highest on the western side towards the entrance to the D'Entrecasteaux Channel and tended to decrease towards Hobart.

6.3 DISCUSSION

6.3.1 VERTICAL DISTRIBUTION

Dispersal of pelagic larvae of marine invertebrates is a function both of their transport by estuarine and/or oceanic circulation and of the length of their planktonic larval stage (Sulkin and Van Heukelem 1986). Currents in estuarine, coastal and oceanic regions vary in direction, duration and velocity with depth (Pond and Pickard 1978) and thus position in the water column and its determinants (both physical and behavioural) are critical in the dispersal and advection of larvae.

There are no previously reported field studies on the vertical distribution of larvae of *A. amurensis*. The concentration of *A. amurensis* larvae within the upper 5 metres of the water column in the Derwent River suggests that wind driven circulation is likely to play a major role in larval distribution and, consequently, that modelling advection based on surface circulation patterns is

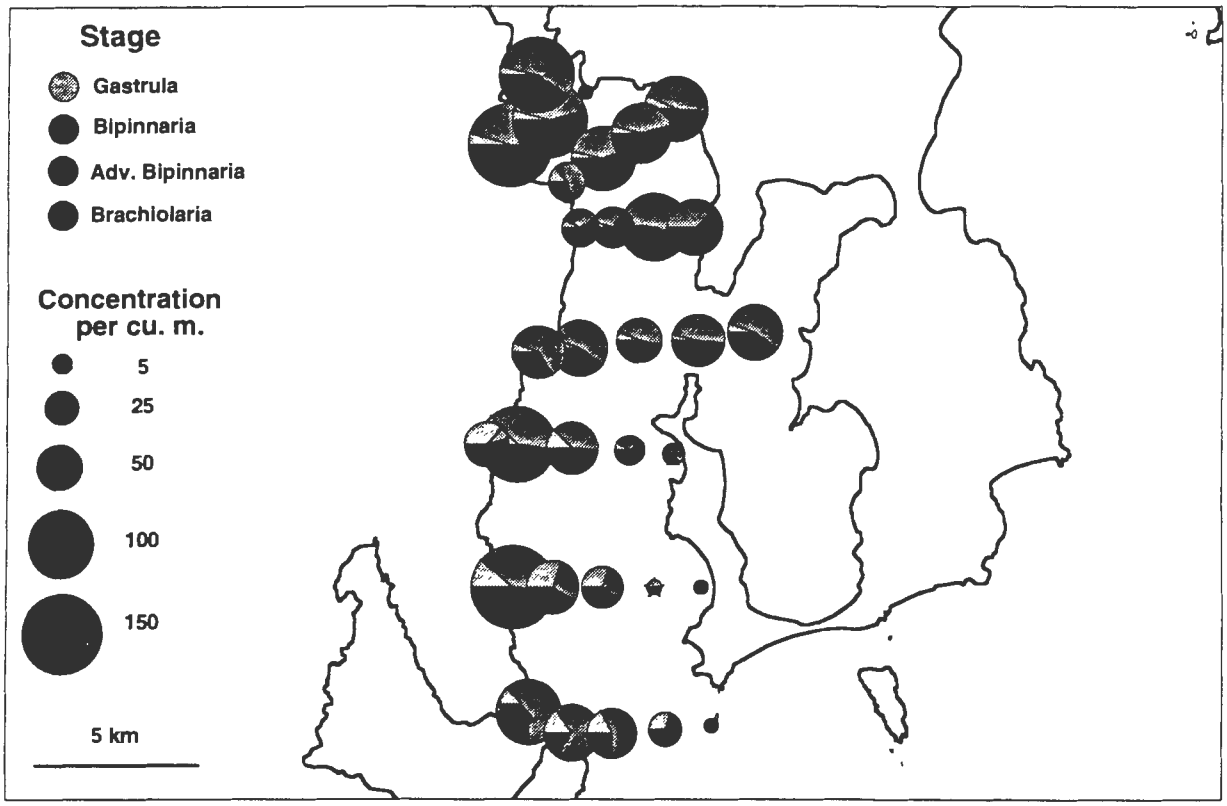

Figure 17 *Horizontal distribution of seastar larvae - Derwent estuary 14/10/93.*

appropriate. Observations on larvae of other echinoderms (including seastars) also suggests they commonly occur in surface waters (Sewell and Watson 1993, Pedrotti and Fenaux 1992, Rumrill 1987, Pennington and Emlet 1986) and in most cases, such surface orientation is believed to enhance advection of early life history stages.

We found no evidence of ontogenetic variability in vertical distribution, although brachiolaria larvae were rare and no late stage brachiolaria (settling stage) were found. The lack of brachiolaria larvae may reflect either active migration of this stage to the (unsampled) bottom boundary layer (which is presumably necessary for settlement, eg Scheltema 1986), advection of these stages out of the River, the short duration of this stage, or the larval population was not yet sufficiently old enough to have reached this stage at the time of sampling. *A. amurensis* spawn in the Derwent from July to November with a peak in spawning activity in August - October (Buttermore et al. 1994). Assuming a pelagic larval duration of at least 66-91 days, larvae derived from the main spawning period would not yet have developed into brachiolaria by the September sampling. The duration of the brachiolaria stage is variable, but it is certainly less than bipinnaria. Kasyanov (unpublished report) and Komatsu (pers comm.) report the brachiolaria stage lasting 10 - 20 days as apposed to 27-100 days for bipinnaria. Prolonging the brachiolaria stage in seastars is clearly possible (at least in the laboratory) if larvae are not presented suitable settlement cues/substrate (Barker 1977). Presumably this ability is advantageous in long lived larvae to maximise the chances of reaching a suitable habitat.

Neither settling cues nor substrate preferences are known for *A. amurensis*. The Derwent clearly offers suitable settlement habitat (based on the presence of large numbers of juveniles) and prolonging the brachiolaria stage may be of less importance for larvae within the estuary. Advection of larvae out of the estuary is possible, although the extent to which it occurs is difficult to assess. Clearly, based on the presence of juveniles and the variety of larval stages, at least some larvae complete their pelagic stage within the estuary. This point will be treated in more detail below, under horizontal distribution.

The concentration of *A. amurensis* larvae in surface waters of the Derwent suggests a reasonable degree of salinity tolerance. Surface salinities at vertical distribution sites ranged from 24.35-32.91 ppt and the highest numbers of larvae were recorded, during the broad scale survey, at sites with surface salinities as low as 23.15-25.00 ppt. In recent rearing experiments we have found *A. amurensis* larvae tolerate salinities of 28.0-32.0 ppt and there was a consistent (though not significant) trend towards more rapid growth in 28.0 ppt compared to higher salinities (CSIRO, unpublished data).

6.3.2 HORIZONTAL DISTRIBUTION

Juvenile and adult *A. amurensis* are extremely common in the Derwent River particularly in the vicinity of Hobart where concentrations of up to 9.44 per m^2 have been reported (Buttermore et al. 1994). The occurrence of reproductive adults and large numbers of small juveniles within the Derwent indicates that *A. amurensis* both spawns and recruits to the area. Furthermore, larvae of all developmental stages were widespread, but were particularly abundant around areas of maximum adult/juvenile concentrations. This strongly suggests that large numbers of larvae complete their development within the estuary. If this is correct, how are larvae retained in the Derwent for such extended periods?

Mechanisms facilitating the retention in, or transport from, estuarine systems have been widely reported for decapod, molluscan and vertebrate (fish) larvae (Norcross and Shaw 1984, Christy and Stancyk 1982, Wood and Hagris 1971). However, comparatively little is known regarding other taxa (Stancyk and Fellar 1986). Larval characters that favour retention within estuaries include: abbreviated development, short larval duration, location of larvae in subsurface layers not exposed to seaward flow or cyclic behaviour taking advantage of opposing tidal flows (Stancyk and Fellar 1986). The early life history of *A. amurensis* would therefore appear to be more suited to export and long distance dispersal rather than retention within the Derwent. Circulation patterns within the estuary may provide a clue to this apparent anomaly.

Lyne (1993) modelled the dispersal of *A. amurensis* larvae in southern Tasmania, assuming that they were distributed in the upper half of the water column and using wind data records from August/September 1988. One of the five initial seed populations was located within the Derwent in the vicinity of Hobart. Results of the model suggested that with a larval duration of 50 days, larval 'particles' were retained within the Derwent and, specifically, above the level of Ralphs Bay. This region corresponds to the area of maximum concentration of spawning adults and also to the region in which we recorded the greatest numbers of *A. amurensis* larvae during field sampling.

Relative stability of the Derwent estuary zooplankton community has been previously reported by Nyan Taw and Ritz (1978). They concluded that despite continual exchange between oceanic, coastal and inshore waters, there was only occasional penetration of estuarine plankton into the inshore region and little penetration of inshore plankton into the estuary. The dominant wind vectors over southern Tasmania during the winter/spring period (when *A. amurensis* larvae are in the water column) are from the south and south west. The Derwent estuary runs roughly north-south (below Hobart) and thus southerly winds would tend to facilitate retention of surface waters (and their zooplankton component) within the estuary.

Retention of surface zooplankters within the estuary seems anomalous given freshwater input and export from the system. Temperature and salinity profiles suggest that export of fresh water occurs predominantly on the eastern side of the Derwent. Interestingly, this was also the area in which *A. amurensis* larvae were least common. The lower numbers of *A. amurensis* larvae in this area may reflect either an active avoidance of the region or rapid export from the system. Active, horizontal migration is restricted to zooplankton taxa with increased swimming abilities (eg decapod larvae, Mileikovsky 1973) and is not a general feature of echinoderm larvae.

Despite the predominance of south or south westerly winds, results from modelling larval dispersal (Section 7) suggest that some larvae from the lower reaches of the estuary may be advected into Frederick Henry Bay. This may also be facilitated by periodic, short duration, reversals in wind direction that occur with the passage of weather systems over southern Tasmania (Lyne 1993).

6.3.3 INTRODUCTION AND SUBSEQUENT SPREAD OF *A. AMURENSIS*

A. amurensis larvae are ideally suited for uptake and translocation in ballast water, thus further supporting this as the most likely vector for introduction to Tasmanian waters. *A. amurensis* is highly fecund, has long lived pelagic larvae and, at least in the current study, larvae are abundant within the upper 5 m of the water column from where ballast water is most commonly drawn. In addition, larvae are obviously tolerant, and indeed may prefer, lowered salinity conditions found in estuarine areas that are typically the favoured locations for major ports.

Translocation of pelagic larvae in ballast water is well documented and is believed to have resulted in a large number of aquatic introductions (Carlton and Geller 1993, Carlton 1985). Williams et al (1988) identified 22 zooplankton species and 45 other planktonic taxa in ballast water of bulk cargo vessels sailing between Japan and Australia. Ballast water introductions have already resulted in the establishment, in Tasmanian waters, of the Japanese seaweed *Undaria pinnatifida* (Jones 1991) and the toxic dynoflagellate, *Gymnodinium catenatum* (Bolch and Hallegraff 1990).

Recent genetic analyses support a single rather than multiple introductions to Tasmanian waters (Ward 1994, Ward and Andrews submitted). Given the likelihood that *A. amurensis* was introduced in ballast water and the relative frequency of ballast water discharge at Triabunna by woodchip vessels originating from possible source regions in the north Pacific, it is reasonable to suggest that the species was initially introduced to that area and has spread southwards. However, as Ward and Andrews point out, there are some difficulties with this hypothesis. First, *A. amurensis* were initially recorded in the Derwent estuary in the early 1980s and were not detected in the vicinity of Triabunna until 1992. Second, only juvenile *A. amurensis* have been recorded

around Triabunna, and only in scallop spat bags suspended above the bottom. Third, the spread of *A. amurensis* southwards from Triabunna, assumes either migration by juveniles and/or adults or, more likely, advection of larvae. Advection southwards is, however, against the prevailing northward circulation predicted from circulation models (Lyne 1993). Fourth, the genetic similarity of east coast and Derwent populations suggest that if secondary colonisation has occurred, then it has done so without further loss of genetic variation and thus must have been foundered by a considerable number of recruits. Under wind conditions favourable to southerly advection, model larval 'particles' were transported offshore from the Triabunna region in a south easterly direction (Lyne unpublished data). Although it is conceivable that some larvae may eventually have been transported into Storm Bay and subsequently into the Derwent under such conditions, the number of larvae successfully completing this journey would be expected to be low. Thus one would anticipate some loss of genetic variability if such larvae were the founders of the Derwent population.

A second hypothesis, also discussed by Ward and Andrews (submitted), is that larvae were initially introduced to the Derwent estuary, either via shipping to Hobart, or perhaps to the, now closed, export terminal in the D'Entrecasteaux Channel. Either is possible, even though the frequency of visiting vessels discharging ballast from likely source localities is much lower (Kerr 1994 and pers. comm.) than at Triabunna.

The Derwent introduction hypothesis is attractive for several reasons. First, it is consistent with the documented chronology of sightings, although this may reflect either habitat differences or a higher frequency of encounter in the more heavily populated (and surveyed) Hobart region. Second, the Derwent offers an abundant supply of larvae that, given the right wind conditions may be exported from the estuary. Third, northward advection of larvae is consistent with both observed and modelled circulation patterns.

Yet to evoke the identical genetic result for east coast and Derwent River populations, the secondary colonisation (in this case of Triabunna) would require a considerable number of founders. Given the periodic reversal of the dominant south westerly wind vectors and the general leakage of larvae out of the Derwent predicted by the larval dispersal model,, it is conceivable that under favourable conditions, larvae may be flushed from the Derwent into Storm Bay. Subsequent return to the dominant south westerly pattern would result in the northerly advection of larvae into Frederick Henry Bay. Depending on wind conditions this may occur several times per season and thus provide a higher likelihood of large numbers of larvae being successfully transported. Tidal flow through Dunalley Canal (Figure 7) provides a short-cut route for larvae to be transported from Frederick Henry Bay to east coast waters. However, based on the modelled rate of dispersal (Section 7), it is unlikely that

larvae could be transported from the Derwent to the Triabunna area within one larval period. It is more likely that small colonies of *A. amurensis* have gradually been established from larvae exported from the Derwent and these are now producing larvae that are advecting through to the Triabunna area. The recent reports of adult *A. amurensis* from Frederick Henry Bay and Marion Bay on either side of Dunalley Canal (Buttermore et al. 1994, G. Edgar, pers. comm.) support this hypothesis.

Two issues are puzzling about the distribution of *A. amurensis* in southern Tasmania. First, why have only juveniles been recorded from Triabunna? Second, given the potential dispersal capabilities of such a highly fecund species that produces long lived, surface distributed, pelagic larvae, why has *A. amurensis* not spread further than largely southern Tasmanian localities?

The lack of *A. amurensis* at Triabunna, other than juveniles in scallop spat bags, may be due to recruitment originating from elsewhere (eg Blackman Bay or Frederick Henry Bay) and that a reproductive population has not yet been established in the area. Conversely, adults may be present but as yet undetected. They may be in deeper water, or in sufficiently small numbers that their presence has not been detected by the lower survey effort in the area. It is also be possible that either natural settlement substrates in the area are unsuitable for *A. amurensis* or that some other pressure (eg predation on newly settled larvae) is restricting colonisation in other than the protected habitat of spat bags. It is not yet possible to determine which, if any, of these is most likely, although, it is perhaps significant that all decrease the likelihood of Triabunna being the initial introduction point.

The relatively restricted distribution of *A. amurensis*, compared to its potential dispersal capabilities, may obviously be due to more than just strength and direction of larval advection. The timing of introduction to Tasmanian waters is unknown and indeed *A. amurensis* may have been present for a considerable period prior to being recorded. Some time lag can presumably be expected between the introduction of exotic species and their populations becoming sufficiently large to ensure their successful spread. It is possible that the population of *A. amurensis* has only recently achieved that 'critical mass'. Other factors such as density dependent spawning/fertilisation success, settlement success and environmental tolerances of all life history stages also pose potential restrictions.

If *A. amurensis* was initially introduced to the Derwent and retention of larvae within the estuary has been the dominant pattern, with only sporadic export during periods of suitable wind conditions, then this may also account for the relatively slow spread of the species to date. Subsequent colonisation of suitable habitats out side of the Derwent (eg Frederick Henry Bay) would be initially slow until sufficient numbers had been established to enable the development

of reproductive populations thus facilitating further spread ('slingshot' effect). If advection of larvae from the Derwent was sufficiently frequent then such colonisations could proceed without a detectable loss of genetic diversity.

Interestingly, another seastar (*P. regularis*) believed introduced to the Derwent in the vicinity of Hobart in the 1930's, has also spread slowly (Dartnall 1980, 1969). *P. regularis* is native to New Zealand and is a common component of the littoral fauna around much of the NZ coastline. The similarity of its native habitats and conditions led Dartnall (1969) to suggest that the species would become widespread in Tasmanian waters. *P. regularis* is extremely abundant in the Derwent estuary and has a similar early life history to *A. amurensis* with a larval duration of 9-10 weeks (Byrne and Barker 1991). However, *P. regularis* is still largely confined to southern Tasmania. The larval retention processes described above may also be operating to restrict the spread of this species.

The Derwent estuary introduction/larval retention hypothesis suggests that the natural rate of spread of *A. amurensis* may have been largely influenced by local meteorological and hydrological patterns. The establishment of populations outside the Derwent River may now facilitate the further spread of the species. The extent to which such new populations approach densities (and hence have the potential to impact native communities) similar to that in the Derwent may be, in part, dependent on the subsequent supply to, or retention of larvae in, these areas.

7. MODELLING

The original modelling study of advection and dispersal of *A. amurensis* larvae in southern Tasmanian waters by Lyne (1993) made a number of assumptions (based on available information) and used wind data derived from a station on the west coast of Tasmania. Further modelling was undertaken in this study to specifically examine aspects arising from the findings of laboratory and field investigations reported in Sections 5 and 6. The principal elements of these findings that were of relevance to the model were:

- **Larval duration**: The estimate of a 50 day larval duration used in the original model is less than the estimates of 66-91 days from laboratory studies (Section 5). Of interest is that the duration increases with decreases in the ambient water temperature.

- **Vertical distribution of larvae**: The original assumption of a surface-concentrated distribution is supported by field investigations which show that the larvae are primarily within the top 5m of the water column. This supports the assumption of a rapid surface, wind driven, drift pattern. When combined with the extended duration of the larval stage, this implies an extensive dispersal capability.

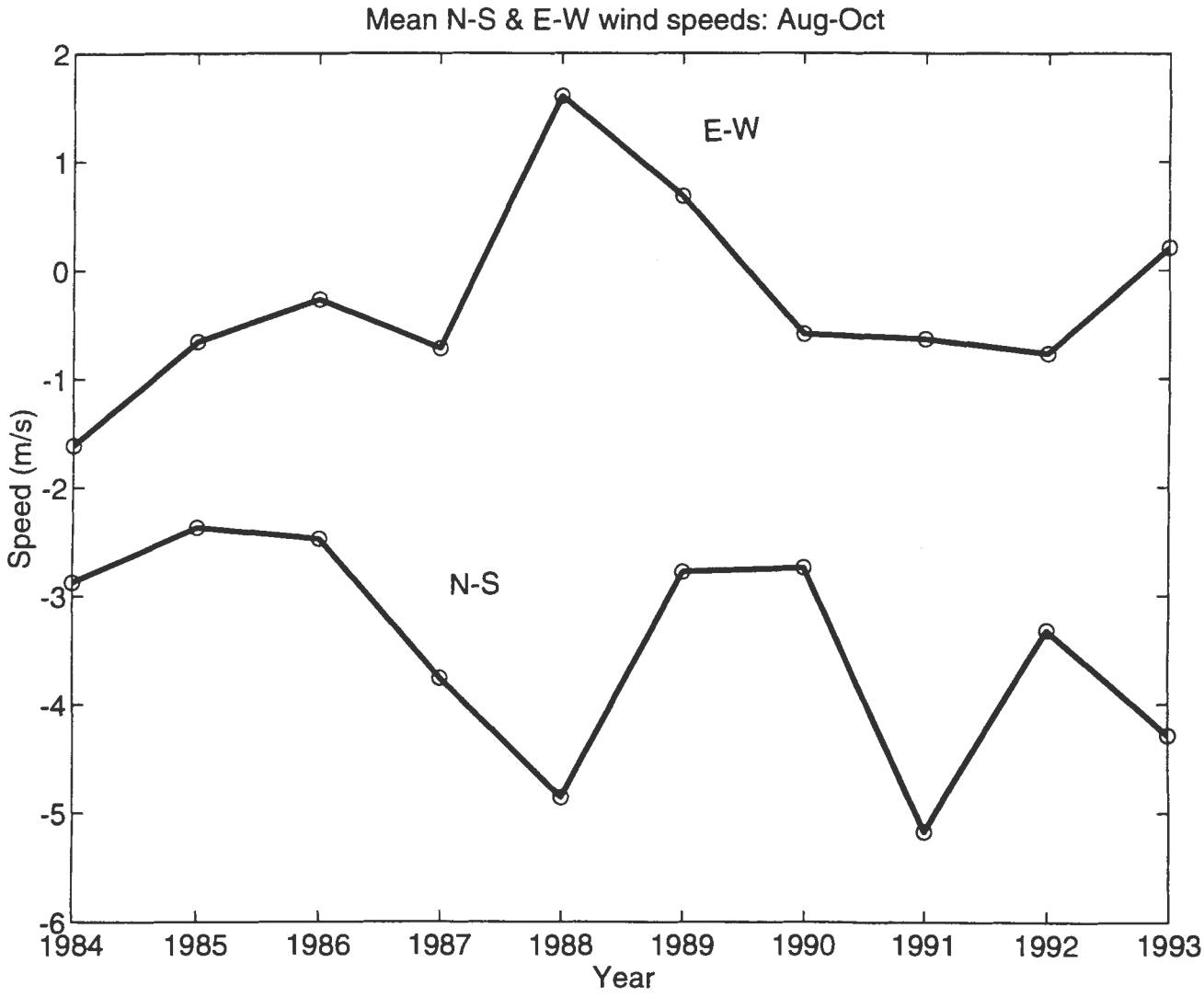

Figure 18 *Mean N-S and E-W wind speeds at Cape Bruny (August to October).*

- **Spatial distribution**: The spatial distribution of all life history stages suggests that the initial introduction point of *A. amurensis* in Tasmania was the Derwent system and that the species has subsequently spread from there to the Triabunna area. The primarily northwards dispersal of larvae predicted by Lyne (1993) also supports this finding. The presence of adults in Frederick Henry Bay, Norfolk Bay and Blackman Bay is consistent with the expected spread of the species via larval dispersal.

The aims of the present extensions of the modelling study were to investigate:

- The dispersal of larvae in the Derwent under a range of differing (historical) wind conditions in order to determine the rate of spread down the Derwent and the potential for the Derwent population to colonise Frederick Henry Bay.

- The potential for adults in Frederick Henry Bay (specifically those close to Dunalley) or in Blackman Bay to act as a seed population for juveniles found around Triabunna.

7.1 METHODS

The formulation and structure of the model were not modified from Lyne (1993) as the field investigations confirmed the major assumption of a surface-concentrated distribution. A more accurate simulation was sought by utilising wind data from the Derwent region and surrounding areas. The analysis by Pendlebury (1987) of wind frequency for the Derwent estuary shows the prevalence of katabatic and sea-breeze winds giving rise to diurnal and spatial variations due to the complex topography and roughness of the surrounding land. A proper simulation of the Derwent must take account of both these temporal and spatial variations. In addition, to investigate interannual effects in patterns of dispersal, a long and reliable series of wind data is required.

To take account of all these factors, would have required considerably more development of the existing model, so a number of simplifications were made. In particular, topographically-generated spatial variations were ignored although a factor of 20% random variation in the wind (vectorised random component) was used in the larval dispersion stage and 50% random variation for the egg stage - to allow for the fact that the egg stage may be more buoyant and hence closer to the more variable near-surface layer. Given the spatial variations in the Derwent, choosing a single station, from the area surrounding the estuary, would have biased the wind forcing. We were also concerned that long-term trends in the record may be affected by human developments along the estuary. Thus, we chose the record from Cape Bruny (Figure 7) to provide wind data for the model simulations.

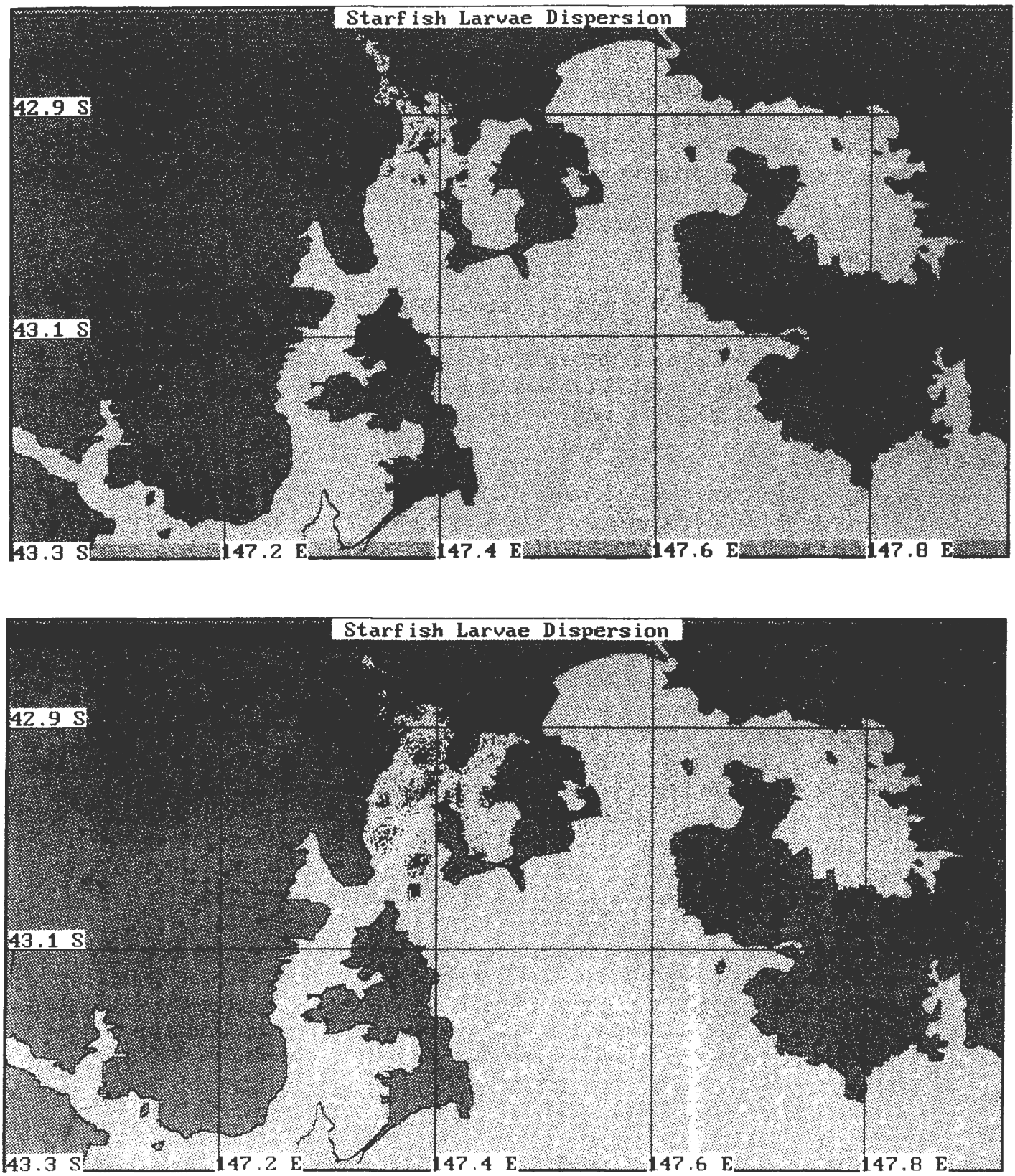

Figure 19 *Simulation of the drift of eggs and larvae of* A. amurensis *in the Derwent estuary for a 90 day period from seed areas marked by yellow squares (1984).*

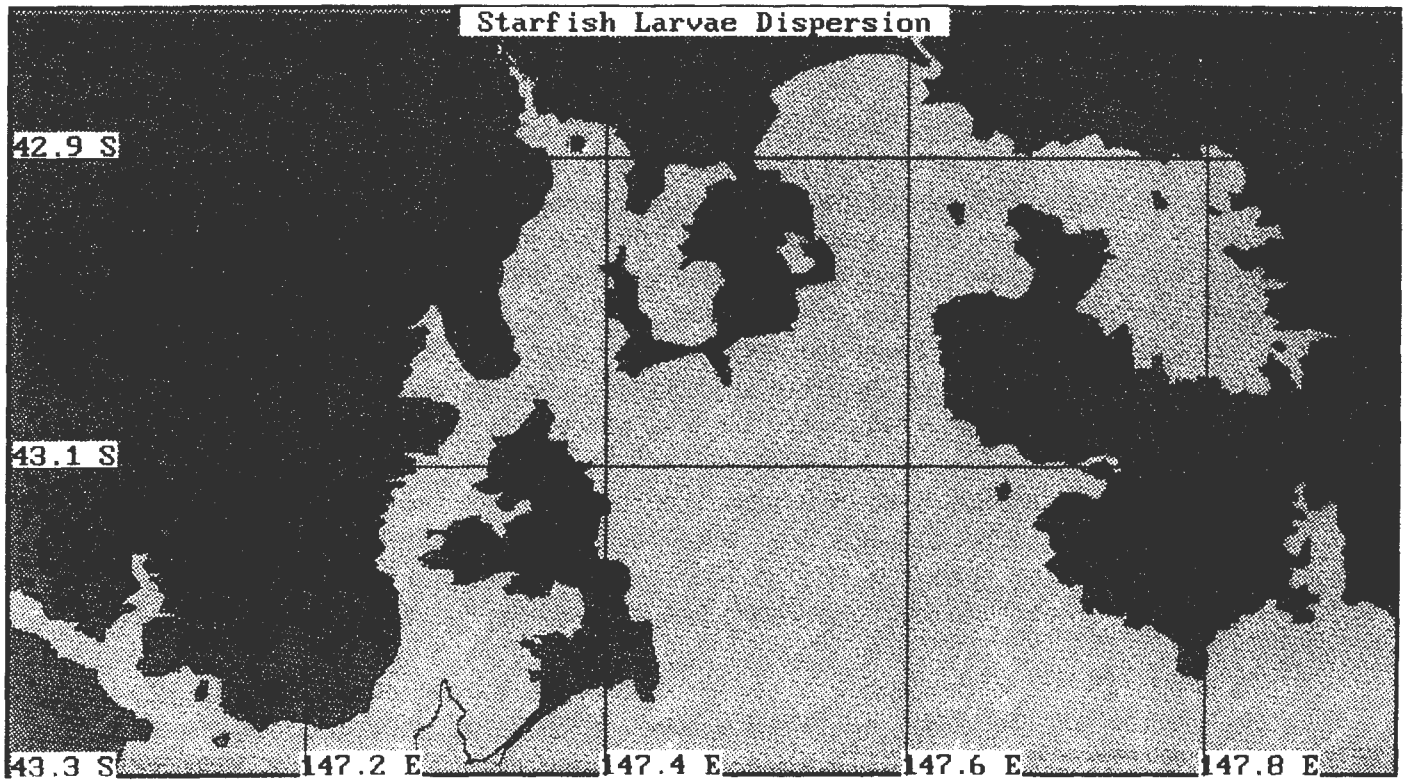

Figure 20 *Simulation of the drift of eggs and larvae of* A. amurensis *in the Derwent estuary for a 90 day period from the seed area marked by a yellow square (1988).*

The data from this station (Figure 18) for the period 1984 to 1993 shows that winds for the August-October period (straddling the main suspected period of larval duration) are primarily south to south-westerly. The La-Nina year (years in which waters off Tasmania are warmer than normal) of 1988 shows strong southerlies with an easterly average. The records from the years 1984 and 1988 were chosen to represent the range of conditions under which wind dispersal of larvae may take place. A 90 day period of larval duration was chosen to investigate the possible extreme range of larval dispersal.

7.2 RESULTS AND DISCUSSION

The simulations for the two years and for seed populations at various locations in the Derwent Estuary (Figures 19+20) show that larvae are primarily confined to the estuary. Even for a seed population placed at the mouth of the estuary most dispersal is northwards into the estuary, although a few seeds are advected into Frederick Henry Bay. A major caveat of the simulations is that is does not take into account net drift in the Derwent arising from the flow of warm fresh water downstream and to the east, and the flow of saline water upstream and to the west (Figure 14). Field observations suggest that larvae are primarily distributed to the west of the front separating these two water masses (Figure 17) and thus may be less influenced by fresh water exiting the system.

Of particular significance, is the similarity of the observed and predicted pattern of larval distribution and retention above the level of Ralphs Bay for seed populations in the vicinity of Hobart. This suggests that wind induced transport may play a dominant role in larval dispersal even within the estuary.

Whilst the Derwent simulations do suggest that larvae located near the mouth of the estuary can be advected into Frederick Henry Bay, it would require a number of years for populations to spread down the Derwent from Hobart (assuming that this port was the initial introduction point) and produce larvae capable of being exported from the system. Similarly, it is unlikely that larvae would disperse from the Derwent to northern most sections of Frederick Henry Bay in a single larval period. Thus colonies of *A. amurensis* are more likely to have gradually established and spread throughout the Bay.

The second set of simulations explored dispersal from near Dunalley Canal on the east coast (Figure 21). The simulation for 1984 shows that while much of the dispersion takes place in the corridor of Mercury Passage, some individuals do progress into Great Oyster Bay whilst others move well offshore and to the north. In contrast, the 1988 simulations show confinement of larvae to near-shore waters and a much more restricted spread up the coast due the coastal entrapment caused by the easterly winds. Both simulations however do show

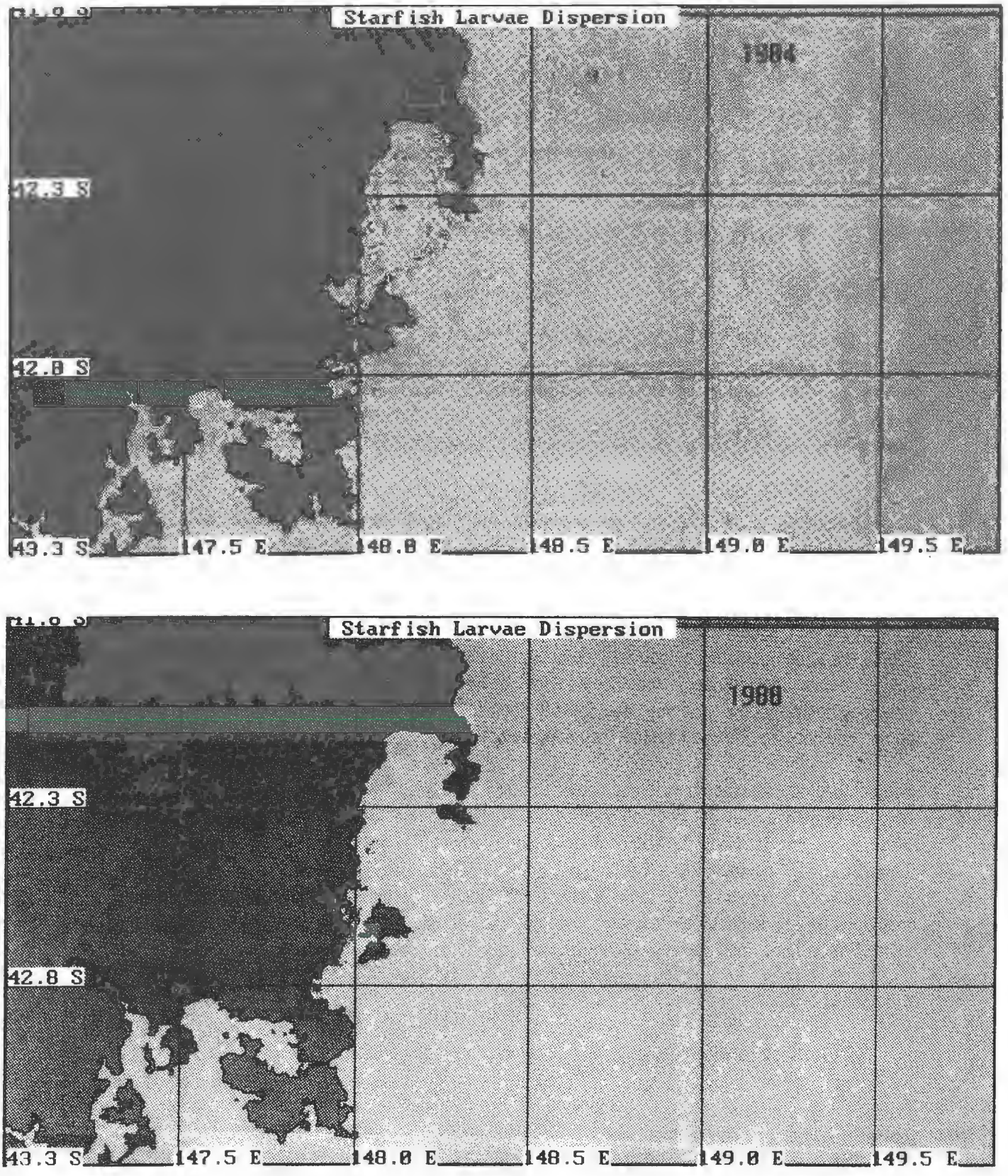

Figure 21 *Simulation of the drift of eggs and larvae of* A. amurensis *in east coast waters of Tasmania for a 90 day period from seed areas marked by yellow squares (1984 + 1988).*

that progress up to around Triabunna is possible within one larval duration period.

Whilst the Dunalley simulation suggests that larvae can spread up the east coast, the lack of sightings of juveniles or adults along exposed stretches of the east coast suggest that settlement and successful colonisation may depend on the suitability of habitat (eg. exposure conditions).

7.3 CONCLUSIONS

In summary, the modelling study provides support for the hypothesis of a dominant pattern of larval retention within the Derwent estuary, particularly for those larvae emanating from spawning near the port of Hobart. Furthermore, simulations suggest that the initial spread of *A. amurensis* southwards towards the mouth of the Derwent would have been slow and may have taken several years.

The model supports that with the establishment of adults in the lower reaches of the estuary, colonies in Frederick Henry Bay could have been derived from the Derwent and thus from an original colony established in the vicinity of Hobart.

The Dunalley simulation clearly demonstrates that the Triabunna juveniles could have emanated from colonies established near Dunalley. Even more disconcerting is the model predictions that in certain years, the Dunalley larvae may extend into Great Oyster Bay and beyond.

Based on these observations, the model predicts that (provided settlement requirements are met) colonies of *A. amurensis* may become established in the vicinity of the port of Triabunna and the northern reaches of Great Oyster Bay. Simulations undertaken by Lyne (1993) also suggest that if a spawning population does become established in Great Oyster Bay, then the dominant wind patterns during the spawning season would facilitate retention of larvae within that area and may subsequently lead to enhanced recruitment.

8. INTELLECTUAL PROPERTY

No commercial intellectual property arose from this work.

9. BENEFITS

The introduction of *A. amurensis* to Tasmanian waters is considered a problem of national significance. The potential for it to impact both the fishing industry (primarily shellfish fisheries and aquaculture) and marine ecosystems is considerable. This study provides basic biological details on early life history

that are necessary for interpreting patterns and rates of dispersal as well as for developing action plans for reducing the risk of spread of *A. amurensis*.

10. FURTHER DEVELOPMENT

Further development is recommended in four areas.

10.1. UNEQUIVOCAL LARVAL IDENTIFICATION

Although we can be reasonably confident that larvae collected in field sampling during this study were *A. amurensis*, based on the criteria outlined above, unequivocal identification of larvae is not yet possible. Characterisation of larvae will be necessary if further sampling is undertaken to assess patterns of dispersal, the window of availability of larvae to vessels ballasting in the Derwent or the identification of *A. amurensis* larvae in ballast water. Two additional identification techniques are available, monoclonal antibodies and genetic characterisation based on PCR amplification and analysis of known regions of the mtDNA genome.

Monoclonal antibodies are currently used in a wide range of diagnostic and research applications particularly in the identification of cell types. The technique has been applied to identification of Crown-of-Thorns starfish larvae with mixed results. The difficulty has been in testing for the species specificity of the antibodies produced (see Hanna et al 1994). Specificity cannot be guaranteed unless antibodies are screened against related species (eg to unequivocally identify *A. amurensis*, larvae of other seastar species in southern Australia waters would require screening). This would require both considerable expense to develop antibodies and also to collect spawn and rear larvae of other species to provide screening material (there are over 40 seastar species recorded from Tasmania alone).

PCR amplification of the mtDNA genome has the advantage of producing a species specific result without the need to screen all other seastar larvae. Analyses can be undertaken with extremely small amounts of material (eg a single larva) and the technique allows for the analysis of (alcohol) fixed material. The PCR technique is recommended for further characterisation of larvae. Alcohol fixed material has been archived during this project for such study.

10.2. MONITORING LARVAL AVAILABILITY IN THE DERWENT

The extremely high concentrations of *A. amurensis* larvae in the Derwent, particularly in the vicinity of port facilities, raises the possibility that ballasting vessels may take up larvae and translocate them from Hobart to other ports. Both the protracted spawning period and larval duration suggests that larvae

may be available in the Derwent over a lengthy period. Monitoring larval abundance at selected sites in the Derwent would provide a means of assessing the risk of uptake at any time and provide an active feed back into ballast water management decisions. Such a monitoring program would, by necessity, require the unequivocal identification of larvae.

10.3. SETTLEMENT MONITORING

Monitoring settlement of *A. amurensis* is recommended for two reasons. First, it is apparent, from the presence of *A. amurensis* juveniles in spat bags that competent larvae are present and settlement is possible on the Tasmanian east coast. Yet neither plankton samples nor diving surveys have located larvae or benthic populations in that area. The possible reasons for this are discussed above, but the observations suggest that artificial settlement collectors may provide a means of detecting the presence of competent larvae and thus provide a sensitive tool for monitoring the spread of the species. Determining settlement cues, substrate requirements and factors influencing mortality of newly settled starfish are related subjects warranting investigation. Second, monitoring the timing of settlement within the Derwent combined with monitoring the timing of spawning and larval availability represents the most effective way to estimate the larval duration of *A. amurensis* under local conditions. Further laboratory rearings, whilst informative for other reasons, are unlikely to provide additional information that can be directly applied to field conditions.

10.4. ENVIRONMENTAL TOLERANCES

The most rapid and unpredictable method by which *A. amurensis* is likely to spread in southern Australia is via translocation of larvae in ballast water. The uptake from Hobart and subsequent discharge of larvae into other Australian (or international) ports, however, does not mean that *A. amurensis* larvae will survive, settle and establish populations. Successful colonisation depends on settlement requirements, factors influencing post settlement mortality, and the environmental tolerances of both larvae and other life history stages. Studies on settlement cues and requirements are recommended above.

The distribution of larvae within the Derwent suggests that a wide range of temperatures and salinities may be tolerated by early life history stages. Observations of *A. amurensis* adults in the lower reaches of freshwater creeks entering the Derwent (eg Brown's River, Kingston - pers. observation) also suggests that other life history stages may similarly tolerate a range of conditions. Determining the salinity and temperature tolerance of all life history stages of *A. amurensis* would enable an assessment to be made of the geographic area in southern Australia that may be susceptible to colonisation by the species.

Work has recently commenced on detailing the temperature and salinity tolerances of *A. amurensis* larvae (Victorian Fisheries/CSIRO-Centre for Research on Introduced Marine Pests) and will continue in 1995.

11. STAFF AND ACKNOWLEDGMENTS

11.1. STAFF

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