

THE FISHERIES BIOLOGY OF THE CUTTLEFISH
***SEPIA APAMA* GRAY, IN SOUTH AUSTRALIAN WATERS**

K.C. Hall and A.J. Fowler



**THE UNIVERSITY
OF ADELAIDE**
AUSTRALIA



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FRDC FINAL REPORT

Project No. 98/151

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Non-technical summary

98/151 - The fisheries biology of the cuttlefish *Sepia apama* Gray, in South Australian waters

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Objectives of original FRDC proposal (1997)

- (1) to establish the general life history characteristics of *S. apama* in South Australian waters, including growth, age and reproductive biology;
- (2) to estimate the abundance of cuttlefish in the Point Lowly fishing grounds and its relationship to the commercial and recreational data;
- (3) to investigate egg densities, recruitment and the relationship with adult abundance, timing of spawning and its interaction with the fishery;
- (4) to investigate the structure and seasonal movement patterns of the exploited population and its relationship with other known populations in South Australian waters;
- (5) to investigate marketing strategies aimed at increasing the value of the current cuttlefish product and/or identifying alternative value-added products.

Outcomes achieved

The primary outcome of this project was a much better understanding of the population biology and life history of the giant Australian cuttlefish, particularly in the context of the spawning aggregation in northern Spencer Gulf. This information has contributed significantly to the development of management strategies for the species since 1998, which culminated in the closure of the main fishery in 1999. The results of this project have thus helped ensure the ecological sustainability of the species in South Australian waters. The uniqueness of the spawning aggregation means that it has special significance to the State, particularly as an eco-tourism attraction and for scientific investigation. Therefore, sustainability of the spawning aggregation will ensure considerable economic benefits into the future. This was also one of the first comprehensive studies of a southern hemisphere cuttlefish species and as such should prove useful for further studies on the biology and fisheries of other cuttlefish species.

Non-technical summary

The giant Australian cuttlefish has a broad distribution around Australia's southern coastline. Throughout this range there is one known location where the animals form a dense spawning aggregation from May to August each year. This is located at the Black Point / Point Lowly area of Northern Spencer Gulf in South Australia, an area characterised by sub-tidal rocky reef. This aggregation constitutes the only known spawning aggregation of cuttlefish in the world.

Historically the spawning aggregation attracted a small level of effort from commercial fishers. Until 1993 the total annual catch was very low, but increased to 263 tonnes in 1996/97 due to a substantial increase in fishing effort. Because 95% of the total State-wide catch came from the localised spawning aggregation and because of the opportunity for further substantial increases in fishing effort, there was considerable concern raised regarding the sustainability of the fishery. Yet, nothing was known of the population biology and life history of the species to facilitate management of the resource. This project was directed at providing such information.

Estimates of the abundance and biomass of *Sepia apama* at the aggregation area were obtained from regular sampling throughout the reproductive seasons of 1998 to 2001. The aggregation was determined to involve >170,000 animals, at densities of up to 85 individuals.100m⁻². In each year the numbers increased in early May, peaked by early June and then gradually declined until the end of August. The annual estimates of biomass decreased from 222 t in 1999 to 184 t in 2001, but this decline could not be attributed to the catches removed by the fishery prior to its closure mid-way through the spawning season of 1998. The cuttlefish were not evenly distributed amongst habitats or sites within the aggregation area, which has important implications for the implementation of a strategy of spatial closures.

At non-spawning times cuttlefish were widely distributed throughout northern Spencer Gulf at much lower densities than at the aggregation area. One individual that was caught and tagged 65 km south of the aggregation area was later recaptured at the spawning site, suggesting that the aggregated population is drawn from over a considerable area of northern Spencer Gulf.

From a tag/recapture program it was determined that some cuttlefish remained at the aggregation area for a minimum of six weeks. Their sex ratio was highly biased towards males, which meant that only 16-25% of the spawning biomass at the aggregation area was comprised of reproductive females. The males had a broad size distribution that included several modes, whereas for females the size distribution was condensed and unimodal. Some individuals were classified to year class on the basis of the microstructure of their cuttlebone. The internal structure of the cuttlebones consists of growth increments that relate to growth rate and which vary seasonally. By determining the number of slow and fast growth periods experienced by an individual provides a means of classifying it to a year class. The results suggested the presence of two year-classes for both sexes for the aggregation population and that size was related to age. Furthermore, analysis of the juvenile parts of the cuttlebones suggested two alternative life cycles:

- (1) the first was characterised by fast-growing individuals that returned to spawn in the winter following the hatching period, when they were only 6 – 7 months old;
- (2) the second comprised individuals that grew slowly throughout their first year, delayed maturity and returned to the aggregation area in the second winter after hatching, when they were 18 – 19 months old.

All individuals conformed to either the first or second life cycle, which means that there was no evidence that any individual participated in more than one reproductive season.

Egg deposition occurred throughout the long spawning season from May to August. The period for egg development varied with respect to the timing of deposition, with those produced early in the season taking up to 5 months, whereas the later ones requiring only two months of development time. Hatching then occurred throughout the two month period of early September to early November, through which the conditions at hatch varied considerably. The hatchlings were extremely cryptic and impossible to track in the field, which meant that aquarium experiments were required to study juvenile growth. These experiments determined that growth was extremely plastic, varying substantially under different conditions of water temperature and food availability. This could represent the basis of the two life cycles.

Reproductive indices confirmed that all individuals at the aggregation area, irrespective of size or age, were mature and spawning, whilst those in the wider Gulf population were immature and feeding. The spawning aggregation at Black Point / Point Lowly was comprised of individuals that conformed to the two life cycle types, which varied in their 'reproductive quality'. The larger, older individuals of both sexes had larger gonads than the younger, smaller ones, which suggests by delaying maturity the potential reproductive output of an individual is increased. Thus, any loss in fitness resulting from increased risk of mortality may be offset by an increase in fecundity related to individual size. There was a decline in condition throughout the spawning season, which supports the hypothesis of a semelparous spawning strategy, i.e. spawn once and then die.

A complex mating system was evident at the aggregation area. The sex ratio was highly biased towards males, with 3 to 6 males per female. Individuals of both sexes had multiple mating events throughout the reproductive season, forming only temporary pair bonds. Males of different sizes, representing the two year-classes, used different suites of behavioural tactics to achieve matings. The large males actively guarded females, whereas small ones made 'sneak' mating attempts. Both size groups achieved successful matings, although this may not be a good indicator of the rate of fertilisation success. There was no clear pattern to the choice of males selected by females for mating and no assortative mating with respect to size.

The population structure and species status of *S. apama* were determined for specimens collected across the species range, using a suite of genetic and morphological analytical procedures. Although there were some differences in results from the different techniques the overriding result was that the populations from WA, SA/Vic and NSW showed significant genetic differences. These differences were consistent with *S. apama* still being considered a single species, but with the populations having been separated in the past and having regained secondary contact. The consideration of microsatellite and morphometric data amongst samples collected in South Australia, i.e. the West Coast, Spencer Gulf, and Gulf St. Vincent also indicated a complex population structure, with genetic differences that suggest the existence of at least three stocks. Finer scale sampling may lead to a finer level of stock structure.

Keywords

Sepia, cuttlefish, cuttlebone, cephalopod, life history, population biology, fisheries, spawning aggregation

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1 General introduction

K.C. Hall, A.J. Fowler and D. McGlennon

Fishing is an ancient human activity of considerable economic, social and cultural importance (McGoodwin 1990). The global production of fisheries steadily rose from 2 million tonnes in 1850 to a peak of 93 million tonnes in 1997, is currently worth over US\$50 billion per year in international trade and supplies over 25% of the protein consumed by humans around the world (FAO 2000). However, many of the world's traditional finfish stocks are either fully exploited with no room for further expansion, overexploited, totally depleted or in recovery (FAO 2000). As a result, cephalopod stocks have gained importance in recent years as alternative sources to support the increasing demands on marine resources for human consumption and income (Piatkowski et al. 2001). Global landings of cephalopods have steadily increased over the last two decades while those of traditionally exploited species have started to level off or decline (Caddy and Rodhouse 1998; Pauly et al. 1998).

Cephalopods are a subgroup (Class Cephalopoda) of the Phylum Mollusca comprised of three subclasses, the completely shelled Ammonoidea (all extinct), Nautiloidea, of which there is a single extant genus, *Nautilus*, and the Coleoidea in which the shell is internal and either reduced or absent (Roper et al. 1984). The Coleoidea includes all squid (Order Teuthida), octopus (Order Octopodida), cuttlefish and sepiolids (Order Sepiida) (Sweeney and Roper 1998). The squids are further divided into two suborders, Myopsina and Oegopsina. Myopsin squids tend to be residents of coastal or continental shelf waters, often forming dense inshore spawning aggregations over large benthic egg masses. Oegopsin squids are a more diverse assemblage including many oceanic species, which apparently complete most of their life cycle offshore forming large dense migratory schools over shelf-break or upwelling areas and which lay eggs in large neutrally buoyant masses (Boyle 1990). Octopus, cuttlefish and sepiolid species tend to be demersal or benthic and are mainly found in coastal continental shelf waters. Most of the commercially important cuttlefish species are from the one highly speciose genus, *Sepia*, which is found throughout the world in all temperate and tropical coastal waters, excluding those of the Americas (Roper et al. 1984).

As interest in cephalopods is relatively recent, the ecology, fisheries biology and even basic life history information of most species is less well known than for other marine groups (Voss 1983). Furthermore, recent research indicates that many of the established fisheries models used for longer-lived finfish species appear inappropriate for most cephalopod species (Pierce and Guerra 1994; Lipinski 1998). In general, cephalopods have much shorter life spans than finfish, with many species living for less than

12 months, and spawning only once at the end of their life cycle (O'Dor 1998). As such there may be no overlap between successive generations and no continuation of spawning biomass (Pierce and Guerra 1994). Hence, recruitment supplies most of the spawning biomass of each generation, which can vary highly in response to environmental variation (Rodhouse 2001). In comparison, for many finfish species, spawning biomass and reproductive effort are spread across a number of overlapping generations, which serves as a reserve of reproductive potential that buffers against years of poor recruitment (O'Dor 1998).

Oceanic squid species account for most of the recent increases in global cephalopod fishery production, generally resulting from the discovery and exploitation of new resources rather than the expansion of existing fisheries (Boyle and Boletzky 1996). Cuttlefish catches have remained relatively constant over the last decade at the global level of around 240,000 tonnes per year (FAO Eastfish 1997). Masked in this apparent constancy, however, is the decline of some traditional fisheries with the concomitant development of new ones. Historically, countries that exploit cuttlefish have also been the main consumers, and include those of south-east Asia and the European countries that border the Atlantic Ocean and Mediterranean Sea (Roper et al. 1984). More recently, some distant-water fisheries such as those of the northwest coast of Africa and P.D.R. of Yemen have developed (Sato and Hatanaka 1983), and other nations such as India and England have increased their production of cuttlefish solely for export (Silas et al. 1985; Dunn 1999). Most of this additional product is destined for traditional markets rather than new ones, to offset declining catches in local fisheries. The cuttlefish product has yet to gain the wider market acceptance and demand that squid marketed as calamari has gained in recent years.

Most of the global cuttlefish landings are taken as by-catch to other target species in multi-species bottom trawl fisheries (Denis and Robin 2001), but can account for a large proportion of the total catch (Roper et al. 1984). Trawl catches dominate the fisheries of the English Channel (Dunn 1999), European Atlantic Ocean (Denis and Robin 2001), Mediterranean Sea (Wurtz et al. 1991; Sánchez and Martín 1993), northwest Africa (Bakhayokho 1991), Indian Ocean (Narasimham et al. 1993), P.D.R. of Yemen (Aoyama and Nguyen 1989) and Japan (Natsukari and Tashiro 1991). Bottom trawl fisheries usually target cuttlefish distributed offshore over feeding grounds. A few specialised small-scale artisanal or traditional fisheries use highly selective methods such as purpose-designed traps, set nets, jigs, spears or live cuttlefish lures to target adult cuttlefish while they are inshore over spawning grounds. Examples of these fisheries, include the traditional trap fishery of Japan (Watanuki et al. 1993; Natsukari and Tashiro 1991) and jig fisheries of the Philippines (Watanuki et al. 1993) and

Vizhinjam, India (Nair 1985) and the recent introduction of these methods to northwest Africa (Bakhayokho and Ito 1991) and United Kingdom (Dunn 1999).

Despite the long history of some cuttlefish fisheries that date back to second century A.D. (Boycott 1958), most recent advances in cephalopod fisheries assessment apply to squid species. Only one species of cuttlefish, *Sepia officinalis*, has been well studied but generally by laboratory-based research. Hence, little is known about the population dynamics of wild cuttlefish populations (Boletzky 1983). Although, life history traits of cuttlefish appear to be quite species-specific (summarised in Table 1.1 for the main commercial species) and tend to vary across the distribution of a single species depending upon local environmental conditions, some common features are apparent. Most species are demersal or benthic, inhabit coastal and continental shelf waters and undergo seasonal migrations between feeding and spawning areas (Roper et al. 1994). Temperate species tend to spend the winter months in colder, deeper waters and migrate inshore during the spring and summer to spawn in shallower, warmer waters (e.g. *S. officinalis officinalis*; Boletzky 1983). Conversely, subtropical and tropical species tend to spend the summer offshore in cooler, deeper waters and then migrate into coastal waters to spawn during the cooler seasons of spring and/or autumn (e.g. *S. officinalis hierredda*; Bakhayokho 1983). Although, mature individuals may be found throughout the year in a number of species, one or more "peaks" in spawning are usually observed, presumed to coincide with optimal temperature conditions.

Cuttlefish spawn relatively few, large, yolky eggs, which females attach individually to the substrate in clusters. The requirement for suitable substrate is thought to drive the inshore migration of cuttlefish for spawning. Most species are assumed to be semelparous, dying soon after spawning. The time taken for eggs to develop is usually negatively related to water temperature (Boletzky 1974b; Bouchaud and Daguzan 1989). Development is direct, with no larval phase, and juveniles closely resemble the adult form at hatching (Boletzky 1974b; Nixon and Mangold 1998). Juvenile growth rates in captivity vary with water temperature and food availability (Motschaniwskyj and Martinez 1998; Koueta and Boucaud-Camou 1999; Domingues et al. 2001).

The time taken to reach sexual maturity can vary between different locations within the one species (e.g. all six species studied off the shores of India by Silas et al. 1985b) but is generally less than one year. Male cuttlefish tend to be more precocious than females, maturing at a smaller size and presumably younger age (e.g. Guerra and Castro 1988).

| Species | Common name | Main areas of exploitation | Main fishing methods | Life span | Max Size (ML, weight) | Age, size at maturity | Spawning season | Habitat | Refs* |
|---|--------------------|---|--|---|---|--|--|---|-------------------|
| <i>S. officinalis officinalis</i> Linnaeus, 1758 | Common cuttlefish | English Channel European Atlantic, Mediterranean, NW Africa | Trawl, artisanal live cuttlefish lures, traps, spears | Alternating 1-2 years | 450mm, 4kg (temperate); 300mm, 2kg (subtropical) | < 1 year M: 60-110mm F: 80-250mm | all year, peak in spring- summer | Sandy, muddy bottoms Shore to 200m Inshore spawning migration to hard bottom | 1,2,3,4, 5,6,7 |
| <i>S. officinalis hierredda</i> Rang, 1837 | Common cuttlefish | NW Africa | Trawl, artisanal jigs & traps | 1-2 years | M: 440mm F: 370mm | 5-6 months M: 120-140mm F: 130mm | spring & autumn | Sandy, muddy bottoms Shore to 120m Inshore spawning migration to <16m | 1,8,9 |
| <i>S. elegans</i> Blainville, 1827 | Elegant cuttlefish | European Atlantic, Mediterranean, NW Africa | Trawl | 1.5 years | M: 65mm F: 80mm | ~1 year M: 25-50mm F: 30-60mm | all year; peak in spring- summer | Muddy bottoms to 430m Inshore spawning migration to 40-70m | 1,10,11 12,13 |
| <i>S. orbignyana</i> , Ferussac, 1826 | Pink cuttlefish | Mediterranean, NW Africa | Trawl | | 100mm, 100g | M: 40-50mm F: 70-80mm | all year; peak in summer- autumn | Muddy bottoms Shelf & slope to 450m No migrations reported | 1,10,12 13 |
| <i>S. bertheloti</i> , Orbigny, 1839 | African cuttlefish | Mediterranean NW Africa | Trawl, artisanal traps & lines | 1-2 years | M: 175mm F: 130mm | | summer-autumn | Open bottom habitats to 160m | 1 |
| <i>S. esculenta</i> Hoyle, 1885 | Golden cuttlefish | Japan, Philippines, China, Hong Kong, Thailand | Trawl, set nets, seine, artisanal traps | ~ 1 year | 190mm, 0.6kg | M: 83-115mm F: 90-120mm | Spring | Sandy bottom 10-100m Inshore spawning migration | 1,14,15 |
| <i>S. lycidas</i> Gray, 1849 | Kisslip cuttlefish | Japan, Hong Kong, SE Asia | Trawl, set nets, seine, traps, live cuttlefish lures | ~ 1 year | 380mm, 5kg | | spring-summer | 10-100m Inshore spawning migration to 15-30m | 1,14,16 |
| <i>S. latimanus</i> Quoy & Gaimard, 1832 | Giant cuttlefish | Japan, Philippines, SE Asia | Jigs, set nets, spears | | 500mm, 20kg | | summer-autumn | Coral reefs to 30m | 1,17 |
| <i>S. pharaonis</i> Ehrenberg, 1831 | Pharaoh cuttlefish | India, P.D.R. Yemen, Philippines, Hong Kong, SE Asia, Northern Australia | Trawl, artisanal traps, jigs, lures & spears | 2-3 years, males longer than females | M: 430mm F: 350mm (smaller in subtropical) | M: 110-150mm F: 120-170mm | all year, peaks in spring & autumn | Shore to 110m Inshore spawning migration | 1,16,17 18,19 |
| <i>S. aculeata</i> Orbigny, 1848 | Needle cuttlefish | India, Hong Kong, SE Asia | Trawl, inshore set nets & seines | | M: 245mm, 1.3kg F: 200mm | M: 70-130mm F: 90-170mm | all year, peaks in spring & autumn | Shore to 60m Inshore spawning migration to 5-20m | 1,17,18 20,21 |
| <i>S. prashadi</i> Winckworth, 1936 | Hooded cuttlefish | India, Red Sea | Trawl | | 140mm | M: 67mm F: 72mm | | Shore to > 40m | 1,18 |

*References: 1. Roper et al. 1984; 2. Boletzky 1983; 3. Denis and Robin 2001; 4. Dunn 1999; 5. Sánchez and Martín 1993; 6. Le Goff and Daguzan 1991; 7. Guerra and Castro 1988; 8. Bakhayokho 1983; 9. Bakhayokho 1991; 10. D'Onghia et al. 1996; 11. Guerra and Castro 1989; 12. Jereb and Ragonese 1991; 13. Wurtz et al. 1991; 14. Natsukari and Tashiro 1991; 15. Watanuki et al. 1993a; 17. Chotiyaputta 1993; 16. Watanuki et al. 1993b; 19. Silas et al. 1985b; 20. Nair et al. 1993; 21. Rao 1997; 22. Rao et al. 1993.

Table 1.1 Life history characteristics of the main commercial cuttlefish species of the genus *Sepia*.

Life span may vary within a species but generally range from 1 to 3 years. Two alternative types of life cycles have been proposed for *S. officinalis* in the Mediterranean Sea, with some individuals reaching maturity within one year and others taking two years and attaining a larger size (Le Goff and Daguzan 1991; Gauvrit et al. 1998). However, in other areas only one type of life cycle was evident (e.g. in the English Channel region).

Lu (1998a) described 26 species of cuttlefish found in Australian waters. He defined four distinct species assemblages, based on latitude: (1) a northern assemblage consisting of 9 species with wide distributions that overlap with other Indian and Western Pacific Ocean species; (2) a southern assemblage consisting of 8 species, all of which are endemic to Australian waters; (3) a group in the eastern overlap zone comprised of 5 species; and (4) a group of 3 species in the western overlap zone. A number of the species are taken as by-catch to multi-species bottom trawl fisheries in Victoria, New South Wales, Queensland and Western Australia with a total catch of around 288 tonnes in 2000 (Anonymous 2001; Kennelly and McVea 2001; Penn 2001; Haddy J. pers. comm. 2002). The only species that is specifically targeted by commercial fishers in Australia is the giant Australian cuttlefish, *Sepia apama*. A small jig fishery for this species has operated in South Australia for over 20 years, with a peak reported catch of 262 tonnes in 1997 (Chapter 2).

S. apama is the largest cuttlefish species in the world, with a maximum recorded size of 520 mm mantle length (Gales et al. 1990) and over 12 kg weight (Kassahn unpub. data). It is widely distributed across temperate southern Australia from Moreton Bay in southern Queensland to Point Cloates in Western Australia, and northern Tasmania (Lu 1998a). Before this study, little was known of the biology of *S. apama*. Adults were reported to migrate into shallow coastal waters to spawn during the austral winter from May to August (Lu 1998b), where females attach their eggs to the underside of rocks, ledges and caves in the subtidal rocky reef habitat. The juveniles hatch after 3-5 months (Cronin and Seymour 2000). The life cycle and habitats used by juveniles and adults prior to spawning were largely unknown.

A large spawning aggregation of *S. apama* occurs every winter over a restricted area of rocky reef in the Black Point to Point Lowly region of northern Spencer Gulf (Norman et al. 1999). This is the only known dense aggregation of spawning cuttlefish in the world. Elsewhere, less concentrated spawning and egg-laying by *S. apama* occur over areas of rocky reef. Similarly, many other *Sepia* species increase in numbers in coastal waters during peak spawning periods (Boletzky 1983); however, no localised aggregation of comparable density to that of *S. apama* has ever been reported.

1.1 The study of populations

A population may be defined as a group of organisms of a single species inhabiting a certain region at a particular time, which can all potentially interbreed (Krebs 1994). A "truly distinct population" is a completely self-sustaining unit, which is reproductively isolated from other populations of the same species (Dobzhansky 1950; Boyle and Boletzky 1996). However, only a few dispersing individuals are required to connect populations genetically, and hence render them not "truly distinct" by the strict definition. Hence, the term "local population" is often used to divide genetically homogenous populations with a wide distribution into geographically useful units or local assemblages, each of which is thought to be largely self-sustaining (Andrewartha and Birch 1984). The number and distribution of local populations of *S. apama* throughout the whole range has not been determined. Therefore, for the purposes of this study the spawning population present in the main aggregation area of Black Pt to Point Lowly (henceforth referred to as the aggregation area) each year from April to August, is considered to be a local population. *S. apama* are also distributed throughout the northern Spencer Gulf during most of the year. Hence, the aggregation population is assumed to be a part of the wider northern Spencer Gulf population (henceforth referred to as the NSG population) at other times of the year, the geographical boundaries of which are currently unknown but set for practical reasons for this study to include all waters north of the latitude 34° S line (see Fig. 2.3).

Population dynamics is the quantitative study of the variation in the characteristics of a population, over time and the primary processes and their interactions that influence it (Bradley and Jones 1969; Krebs 1994). The main characteristic used to describe a population is size, expressed as either abundance (numbers) or biomass (weight). The main processes that influence the size of a population are depicted in Fig. 1.1. Increases in abundance and biomass are produced by: the reproduction of adults, which adds new individuals (recruits); immigration from elsewhere; and growth of individuals (Beverton and Holt 1957). The abundance and biomass are reduced by: natural mortality by predation, disease and senescence; fishing mortality; and emigration.

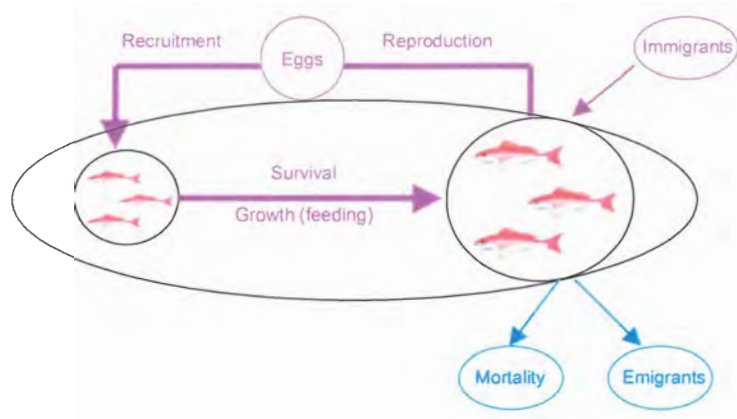


Figure 1.1 Factors affecting the population dynamics of a fish population. The biomass of the population is increased by growth, immigration and recruitment (indicated in purple), and is reduced by mortality and emigration (indicated in blue).

The study of population dynamics is generally dichotomous in nature. The main purpose is to describe the general trends in characteristics and processes for the whole population, based on the summation or averages of individual values (Begon and Mortimer 1986). However, the composition of a population is often quite complex due to the heterogeneity of individuals that comprise it. Hence, an understanding of the patterns of variation of individual life history traits is also important. Life history traits of individuals that can influence population processes include life span and type of life cycle, growth rates, the size or age of sexual maturity, the frequency, duration and fecundity of reproductive events, individual movement patterns and the relative allocation of resources for growth, reproduction and survival over the life cycle of an individual (Begon and Mortimer 1986). The life history traits of an individual are thought to be influenced by natural selection, such that the combinations of these traits, known as "life history strategies", which maximise the fitness of an individual in a given environment, will be favoured by evolution over time.

1.2 Background – original FRDC proposal (1997)

A small fishery for cuttlefish has existed in South Australia for over ten years. Until 1993, reported catches were very small, i.e. less than 4 tonnes per annum. However, in recent years catches have increased significantly due to a rapid rise in effort. A catch of 34 tonnes was reported in 1994/95, 71 tonnes in 1995/96 and 263 tonnes in 1996/97. The value of the fishery was estimated at \$335,000 for the 1996/97 financial year.

Cuttlefish are fished commercially in South Australia under the general marine scalefish fisheries licence. There are currently 500 marine scalefish fishermen in South Australia, all of whom are eligible to take cuttlefish if they wish. Until recently, the number of licence holders reporting cuttlefish catches remained relatively low, i.e. 25 fishermen or less. However, in the last three financial years commercial interest in the cuttlefish has grown with 38 fishermen reporting cuttlefish catches in 1994/95, 49 fishermen in 1995/96 and 62 fishermen in 1996/97.

The majority of the catch (>95%) arises from the exploitation of one localised population near Point Lowly in the northern Spencer Gulf. The cuttlefish are targeted in the area when they aggregate to spawn during the winter months (May to August). Knowledge of this aggregation has existed for many years and it has been fished historically for bait and aquaculture feed. However, the lack of either an established domestic or export market has underpinned additional development of the fishery. It is suspected that recent increases in catch have resulted from the development of a potential market.

The cuttlefish species currently being exploited is believed to be *S. apama* Gray, whose distribution is reported to extend across southern Australia from Ningaloo in Western Australia to Port Jackson in New South Wales. It is one of the largest species of cuttlefish in the world, reaching a mantle length of 50 cm and over 5 kg in weight. No scientific studies on the biology of the species were found in the literature.

Other species of *Sepia* support substantial fisheries in other countries either as target species, in conjunction with other cephalopods or as by-catch of trawl fisheries. Markets for these products exist in Japan and other Asian countries, and selected European countries. For example, the EEC and Japan imported 65 – 75,000 and 50 - 80,000 metric tonnes respectively, in recent years. Prices for frozen, trawler-caught cuttlefish of a size similar to *S. apama* are currently \$US5-7.kg-1 (FAO Eastfish 1997).

The cuttlefish fishery in South Australia is still in its infancy with only one localised population being exploited. The fishery has the potential to expand and exploit other populations both within South Australia and other southern Australian states, if an appropriate market for the product can be established. Cuttlefish from the South Australian fishery were reportedly sold for between AUS\$0.50-2.00.kg-1 in 1996/97. Because of their large size and high quality (i.e. hand-jigged as opposed to trawled), *S. apama* may represent a niche export marketing opportunity for South Australian fishermen.

1.3 Need – original FRDC proposal (1997)

This project aims to provide the biological knowledge to sustainably manage a new species in fisheries production. It also aims to maximise returns for a species, which until recently, yielded low returns to fishers but has significant potential in export markets.

Targeted fishing effort on the cuttlefish *S. apama* has rapidly increased in northern Spencer Gulf during recent years. Annual reported catches have increased from less than 4 tonnes to more than 250 tonnes within 5 years. Licence holders in the fishery have expressed strong concern over the sustainability of the fishery, which is restricted to a small area near Point Lowly. The fishery is currently managed within the broad management framework of the commercial marine scalefish fishery, which provides for no restrictions on either effort or catch for cuttlefish. More stringent management controls, specific to this species, need to be introduced before the commencement of the 1998 fishing season.

There is a complete lack of information on the general biology and life history characteristics of *S. apama*, despite its large size and common occurrence in southern Australian waters. Other commercial cuttlefish species are short-lived and semelparous (i.e. spawn once and then die), a life cycle common to many cephalopods. With this type of life cycle, overfishing in a single year can cause a stock to collapse (Rodhouse and White 1995). In addition, the exploited population is understood to be a spawning aggregation, which would further increase the potential to deplete the stock.

An improving market for this species, including the potential for an export market, suggests that exploitation levels will continue to increase. Additionally, other populations within South Australia and other southern States (currently unfished) may attract fishing effort. There is, therefore, an urgent need to establish the general life history characteristics of *S. apama* in South Australian waters, and to gather baseline biological data upon which the impacts of fishing on this species may be assessed and any necessary management controls may be based.

Currently, South Australian cuttlefish are attracting a much lower market price than other cuttlefish species of a similar size being sold in Japanese and European markets (AUS\$1-2 as compared with \$US5-7.kg-1). Therefore, there is a definite need to investigate possible marketing strategies, which would increase the price of the product and/or identify alternative value-added products (especially if they utilise other parts of the cuttlefish that are currently being discarded). An even more exciting opportunity may exist with the live fish trade.

1.4 Objectives - original FRDC proposal (1997)

- (1) to establish the general life history characteristics of *S. apama* in South Australian waters, including growth, age and reproductive biology;
- (2) to estimate the abundance of cuttlefish in the Point Lowly fishing grounds and its relationship to the commercial and recreational data;
- (3) to investigate egg densities, recruitment and the relationship with adult abundance, timing of spawning and its interaction with the fishery;
- (4) to investigate the structure and seasonal movement patterns of the exploited population and its relationship with other known populations in South Australian waters;
- (5) to investigate marketing strategies aimed at increasing the value of the current cuttlefish product and/or identifying alternative value-added products.

1.5 Alterations to original proposal

The aspects of this project pertaining to the life-history and population dynamics of the cuttlefish in northern Spencer Gulf were done as a PhD research project by Karina Hall, whilst the population structure/genetics component was done by Karin Kassahn as an Honours project. Karina Hall found that when designing her research project and her thesis that the list of objectives that were provided in the original proposal did not represent a series of aims around which she could develop a logical sequence of chapters. Rather she redefined the nominated objectives, to facilitate the research process. Thus the main objectives of the study became: to describe the population characteristics of the spawning population of *S. apama* in the aggregation area in each year and to determine any changes that may relate to fishing; to investigate the general life history traits of *S. apama* in the northern Spencer Gulf, which influence the population dynamics of the spawning aggregation; and the third aim addressed by Karin Kassahn was to determine the population structure for *S. apama* at different spatial and temporal scales using an array of molecular genetic methods and morphological analyses. The population-level research in northern Spencer Gulf involved a number of specific objectives, which were:

- (1) to analyse the catch and effort data from the commercial fishery to elucidate spatial and temporal trends in the exploitation of *S. apama* in South Australia that influence the population dynamics of the spawning aggregation;
- (2) to determine the abundance and biomass of the spawning population in the main aggregation area using underwater visual survey methods, and describe patterns of temporal and spatial variation in abundance or biomass which relate to fishing or movement of individuals;
- (3) to describe the sex, size and age composition of the spawning population and relate these to the life cycle and potential life span of *S. apama* in northern Spencer Gulf;
- (4) to investigate growth and survival rates of the early life cycle stages;
- (5) to investigate the reproductive biology of the species to estimate individual fecundity and elucidate the reproductive strategy of adults;
- (6) to describe the reproductive behaviour of adults, the mating system and possible mechanisms of sexual selection in operation.

The final modification to the original objectives relates to changes to the fishery management regime that occurred subsequent to the development of the original proposal and after the research project was underway. As part of the project development it was considered desirable that any sustainable fishery for *S. apama* at the aggregation area should be based on a better market price for the product than was being obtained at that time. Consequently, Objective 5 of the original proposal was to investigate marketing strategies and the possibility of value adding for the product, which was to be done at a workshop that involved appropriately qualified personnel. However, from mid-way through the spawning season of 1998 the fishery at the aggregation area in northern Spencer Gulf was closed to fishing, and has subsequently remained closed. Thus, the running of a workshop on marketing strategies was considered inconsistent with the on-going closure of the fishery, and so the marketing workshop was abandoned as an appropriate and viable objective.

1.6 Report Format

An analysis of the commercial fishery data is presented in Chapter 2. These data were obtained from commercial fishers' returns and are thus treated separately from the remainder of the report, which was based on fisher-independent data. General methods used throughout the rest of the research are presented in Chapter 3. Chapters 4 to 9 address specific topics relating to various population or life

history characteristics such as population size, age composition and reproductive biology for the biological research that was done in Northern Spencer Gulf. Chapter 10 summarises the results from the analysis of population structure based on specimens collected across the geographic range of the species, and that were analysed using a suite of molecular and morphometric analyses. Finally, Chapter 11 provides a general discussion of the findings of the research with respect to the population dynamics of the spawning aggregation and the general life history of *S. apama* in the northern Spencer Gulf, and the consequences of this life history for the population structure.

2 South Australian cuttlefish fishery

K.C. Hall and D. McGlennon

2.1 Introduction

For unexploited stable populations, losses due to natural mortality (the removal of individuals due to natural causes such as disease, senescence and predation) and emigration are theoretically balanced by the gains due to recruitment and immigration, and the population size fluctuates around a stable average (Beverton and Holt 1957). However, for exploited populations fishing mortality, the removal of individuals by the activity of fishing, can also reduce population size. If the level of exploitation is too high, the number of adult fish may be reduced to a level at which recruitment cannot replace the numbers lost and the average population size decreases (King 1995). Therefore, understanding the population dynamics of an exploited population requires an analysis of the historical pattern of fishing. Historical catch and effort data obtained from the logbook records of commercial fishing operators are the most common form of data available on the patterns and levels of exploitation (Hilborn and Walters 1992). However, the quality of these data is obviously subject to the reliability of the fishers involved and should thus be accepted cautiously (Gulland 1983).

2.1.1 Description of the fishery

The commercial cuttlefish fishery in South Australia is managed under the broad management framework of the multi-species marine scalefish fishery (PIRSA 1999). There are around 470 marine scalefish licence holders, all of whom are endorsed to take cuttlefish. However, only a small percentage of these, usually less than 50 fishers, report catches of cuttlefish in any given year, and even fewer actually target the species. Cuttlefish are targeted using lines and squid jigs during late autumn and winter over shallow inshore reefs where mature adults move to spawn. Multi-purpose marine scalefish fishing vessels of 5-8 m in length are typically used, each with up to 4-5 fishers. Larger vessels with sleeping accommodation in combination with smaller dinghies were also used in the mid-1990's as the fishery expanded. Whole fresh cuttlefish are maintained on ice on-board the vessels, and after unloading are either transported fresh overland to processing facilities or frozen for bait. Therefore, catch figures are usually reported as fresh whole weights.

Small quantities of cuttlefish are also taken as by-catch by commercial fishers whilst targeting other marine scalefish species, in particular the southern calamari, *Sepioteuthis australis*, as similar gear is used for both species. The prawn trawling fleets operating in the South Australian Gulfs also catch cuttlefish as by-catch during trawl operations, but none are kept and most are assumed to be discarded alive (Carrick 1997). Cuttlefish are rarely targeted by recreational fishers and are usually only taken as by-catch when southern calamari is targeted (McGlennon and Hall 1997). Therefore, the commercial targeted catch and by-catch appear to be the only significant sources of fishing mortality of cuttlefish in South Australia. Although the catch is not reported according to species, *S. apama* accounts for most of the catch (McGlennon and Hall 1997).

2.1.2 Management of the fishery

Until 1998, there were no specific management restrictions on the taking of cuttlefish in the State. As the commercial fishery rapidly developed between 1994 and 1997, concerns were raised over the level of effort that was being concentrated on the spawning aggregation in northern Spencer Gulf. As a precautionary measure, before the start of the 1998 spawning season a time and area closure was introduced over approximately 50% of the reef habitat in the aggregation area (Fig. 2.1a). As the 1998 fishing season progressed, further concern was raised over the level of spawning biomass that was protected by this closure due to an increase in fishing effort outside the closed area. Consequently, the closed area was expanded to include most of the main spawning grounds (Fig. 2.1b) for the remainder of the spawning season, i.e. from 11 June until 30 September 1998. In the subsequent three years (1999 to 2001), the second closure was implemented for the duration of each spawning season, i.e. from 1 March until 30 September.

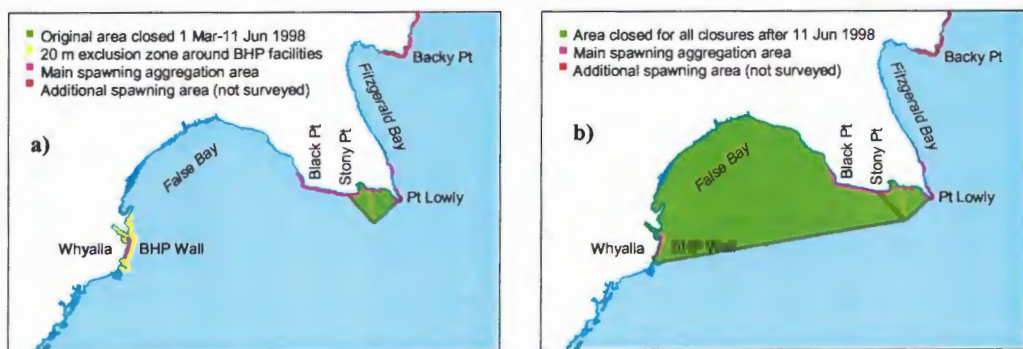


Figure 2.1 Maps of the main spawning aggregation area for *S. apama* in the northern Spencer Gulf; and (a) the boundaries of the original area closed to fishing from 1 March to 11 June 1998; and (b) the area closed from 11 June to 30 September 1998 and for the full season (1 March to 30 September) in each of 1999, 2000 and 2001.

2.2 Aims

The aim of this chapter was to analyse the commercial catch and effort data to determine spatial and temporal trends in the exploitation of *S. apama* in South Australia over the history of the fishery and the potential effects of this exploitation on the population dynamics of the species. Particular attention was paid to the main aggregation area in northern Spencer Gulf and the influence of the time and area closures on the pattern of exploitation in recent years.

2.3 Materials and methods

As part of their licence conditions, commercial fishers are required to submit a monthly catch and effort return detailing all fishing activities involving marine scalefish species. These data are collated by the Statistics Unit of SARDI Aquatic Sciences and have been maintained in a large catch and effort database since 1983. All data relating to cuttlefish were extracted from the database for the period from January 1984 to June 2001. The summaries of data based on the returns of fewer than 5 fishers are confidential and so could not be included in the final presentation of results.

Spatial and temporal trends in the total catch, targeted catch and effort and targeted catch-per-unit-effort (CPUE) were analysed by calendar year and where possible on a monthly basis. Note the total catch also includes non-targeted catch, taken as by-catch to fishing operations that targeted other species. Catches are reported according to a spatial breakdown of the State's marine waters into 58 fishing blocks (Fig. 2.3). The main spawning aggregation area in northern Spencer Gulf is located within Block 21, and for this reason the data for Block 21 are presented separately and compared to those for the remainder of the State. Block 21 covers a large proportion of the northern Spencer Gulf region; however there is minimal reef outside of the main aggregation area likely to facilitate cuttlefish spawning. Therefore, catch statistics presented for Block 21 are assumed to be strongly indicative of the catch statistics for the aggregation area.

2.4 Results

2.4.1 Total catch, targeted catch and effort

Between 1984 and 1993 the total catch of cuttlefish taken by commercial fishers in South Australia was less than 5 t per annum, taken by 5 or fewer fishers. However, catches increased dramatically by over 700% from 1994 to 1997, to a peak of 262 t in 1997 (Fig. 2.2). The sudden drop in catch between

1998 and 2001 is attributable to the early closure of the aggregation area in Block 21 to fishing during the second week of June in 1998, and the almost total closure of the area for the duration of the spawning seasons in 1999, 2000 and 2001. Non-targeted by-catch accounts for a small proportion of the total catch; thus, targeted catch shows a similar trend over years as that for total catch (Table 2.1).

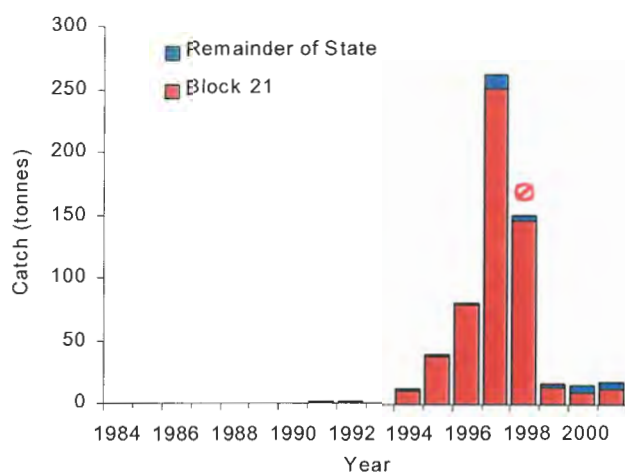


Figure 2.2 Annual total catch of cuttlefish for South Australia, indicating the proportions taken from Block 21 and the remainder of the State. The main fishery in Block 21 was closed early in 1998 (X) and from March to September in each of 1999 to 2001.

Table 2.1 Comparison of non-targeted and targeted catch, and the number of fishers for South Australia and Block 21. Data for 2001 includes Jan-Jun only.

| Year | Non-targeted Catch | | Targeted Catch | | | | % of State Targeted Catch |
|--------------------------|--------------------|----------------|----------------|----------------|----------------|----------------|---------------------------|
| | State | | State | | Block 21 | | |
| | Catch (tonnes) | No. of Fishers | Catch (tonnes) | No. of fishers | Catch (tonnes) | No. of Fishers | |
| 1996 - Unrestricted | 5.3 | 33 | 77.3 | 15 | 77.2 | 13 | 93% |
| 1997 - Unrestricted | 8.6 | 30 | 253.5 | 33 | 246.1 | 28 | 97% |
| 1998 – Closed early | 4.3 | 28 | 145.9 | 30 | 145.7 | 24 | 100% |
| 1999 – Closed all season | 2.9 | 29 | 13.6 | 10 | 13.5 | 7 | 99% |
| 2000 – Closed all season | 5.4 | 32 | 10.5 | 7 | N/A* | <5 | N/A* |
| 2001 – Closed all season | 5.1 | 12 | 12.2 | 13 | 11.8 | 6 | 97% |

* Data for 2000 in Block 21 could not be included for confidentiality reasons.

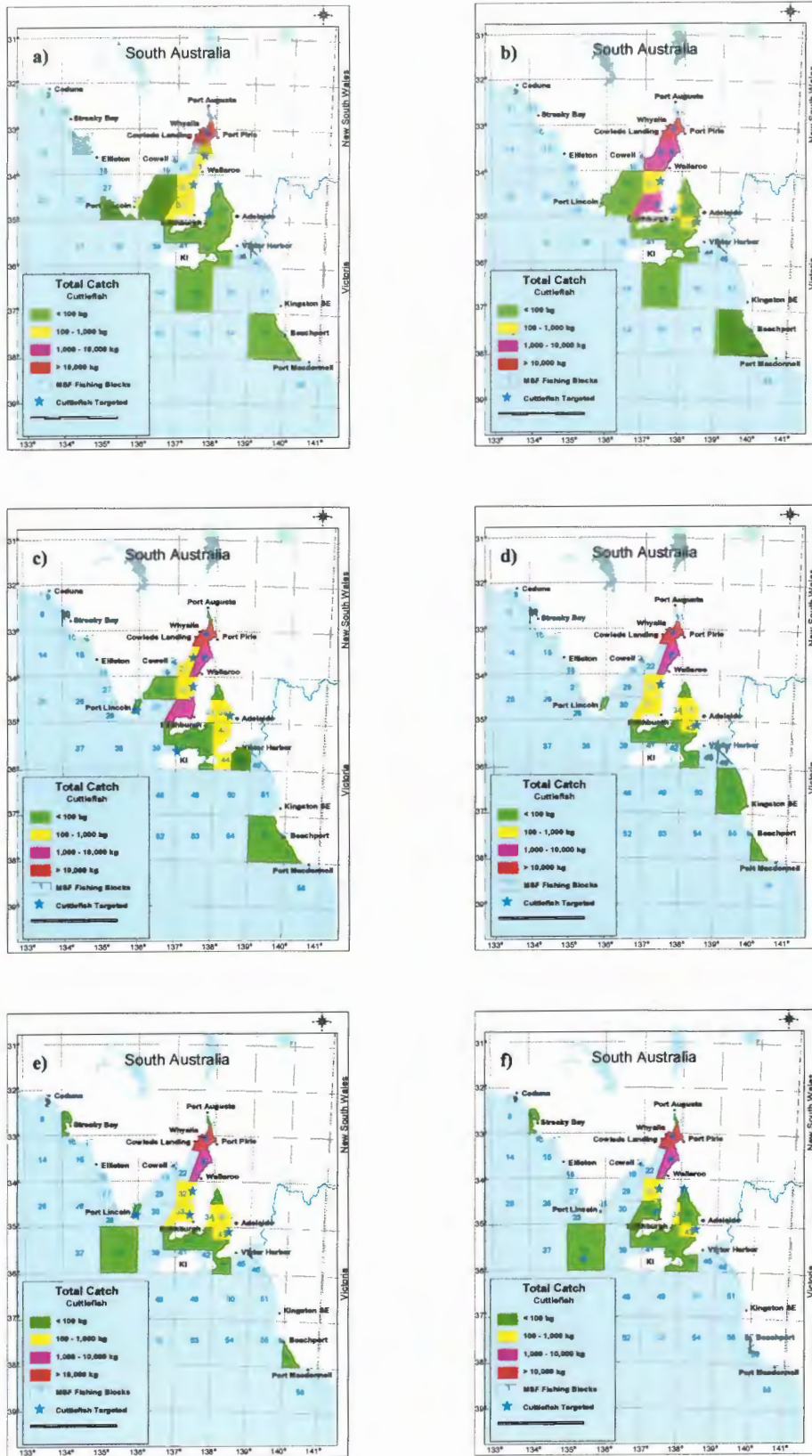


Figure 2.3 Map of South Australia showing the boundaries of marine scalefish fishing blocks, blocks where cuttlefish were targeted in 1996 to 2001 (a-f) and the magnitude of catches reported.

Most of the targeted catch for the State (90-100%) in each year was taken from Block 21 (Fig. 2.3; Table 2.1). Even while the main spawning aggregation area was closed to fishing in 1999 to 2001, more than 97% of the targeted catch was still taken from Block 21. Other areas around the State where cuttlefish have been targeted or have been taken as by-catch over the last six years and the general magnitude of catches reported are shown in (Fig. 2.3). The total catch is unevenly distributed throughout the remainder of the State and cuttlefish have been targeted in only a small number of areas outside of Block 21, primarily within the two Gulfs.

Up to 63 fishers reported catches of cuttlefish in the State in any given year. Prior to 1994, most fishers reported small non-targeted by-catches and only 5 or fewer fishers actively targeted cuttlefish. Between 1994 and 1996 this number trebled to 14-15 and then doubled again to 33 in 1997 and 30 in 1998. As the main aggregation area was closed to fishing in 1999 to 2001, the number of fishers declined through this period. Also, only 7 of the 39 fishers who reported catches in 2000 actually targeted cuttlefish.

The temporal trends in annual estimates of targeted fishing effort (Fig. 2.4) mirror those of total catch (Fig. 2.2). Fishing effort in Block 21 increased from 1993 to a peak of 841 fisher days in 1997, then declined between 1998 and 2001. There was minimal targeted effort on cuttlefish in Block 21 after the main aggregation area was closed to fishing. Targeted fishing effort throughout the remainder of the State was always low compared with Block 21 with a maximum of 50 fisher days in 1997.

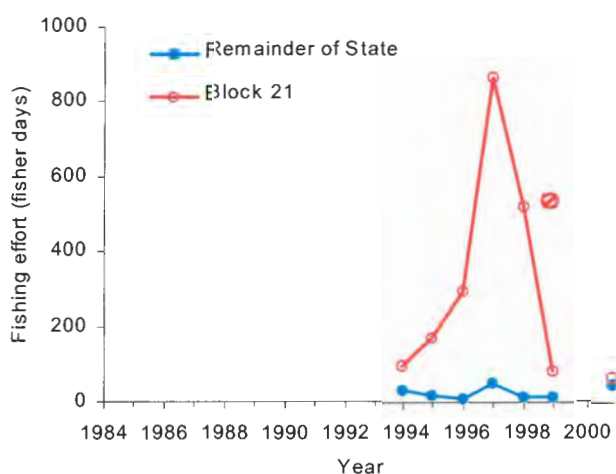


Figure 2.4 Annual targeted effort for cuttlefish in Block 21 and the remainder of the State. The main fishery in Block 21 was closed early in 1998 (⊗) and all season in 1999 to 2001. Data for Block 21 in 2000 could not be included for confidentiality reasons.

2.4.2 Catch-per-unit-effort

Annual CPUE for Block 21 increased from 115 kg per fisher day in 1995 to a peak of around 280 kg per fisher day in 1997 and 1998 (Fig. 2.5). In 1999, when the main aggregation area was closed to fishing CPUE decreased by 40% to 159 kg per fisher day and remained around this level in 2001 (data for 2000 could not be included for confidentiality reasons). These reduced values, however, were still higher than the CPUE values recorded in the remainder of the State. In 1997, a much higher CPUE was also recorded for other areas of the State compared to other years.

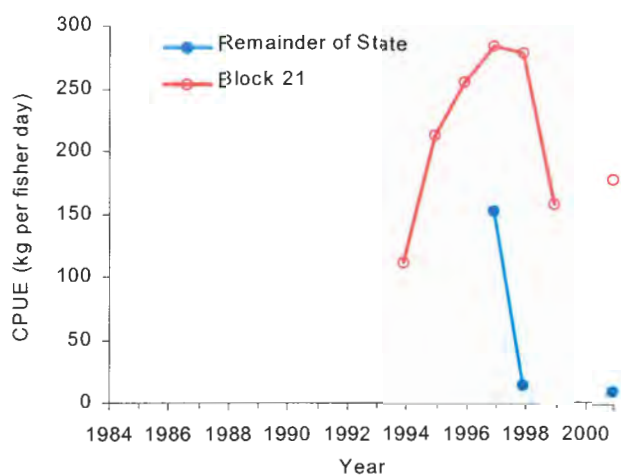


Figure 2.5 Annual CPUE for cuttlefish in Block 21 and the remainder of the State. Data for Block 21 in 2000 and the remainder of the State for 1994 to 1996 and 1999 to 2000, were not included for confidentiality reasons.

2.4.3 Monthly catch and effort

The cuttlefish fishery of South Australia is very seasonal, reflecting its dependence on the increased densities of cuttlefish in coastal areas during the winter months. This is particularly prevalent in the targeted catch reported from Block 21. Between 74 and 97% of the State annual catch is taken from May to July each year (Fig. 2.6a).

The highest monthly catch rates were also reported in May and June (Fig. 2.6b). In 1996, CPUE increased from May to June, and then declined in July. In comparison, in 1997 and 1998 there was a general decline in CPUE from the start of the season. Catch rates started lower in May 1998 than May 1997 and never rose as high as the peak values recorded in June of 1996 and 1997. The CPUE values recorded in June of 1999 and 2001 were well below those for the same month in previous years.

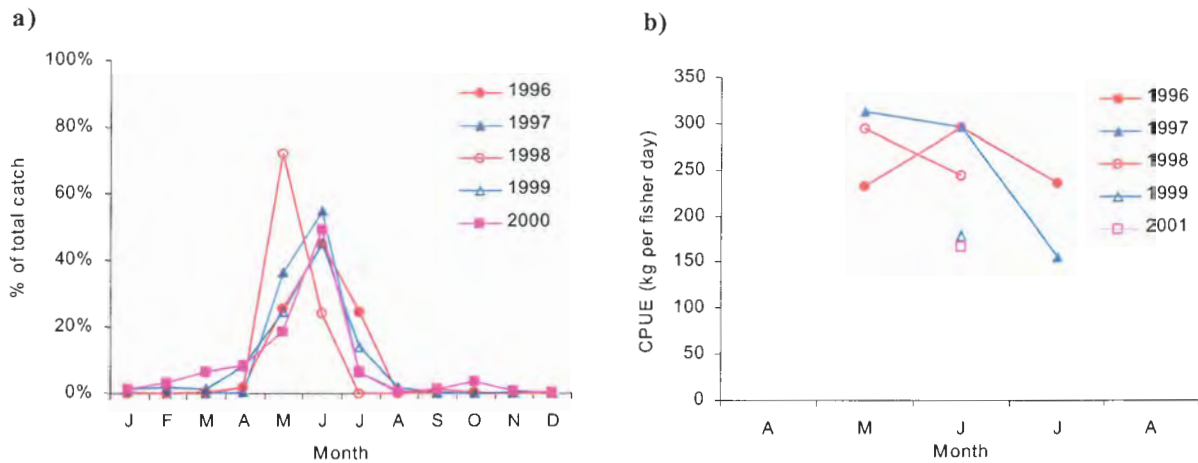


Figure 2.6 Percentage of total catch taken in each month (a) and monthly CPUE (b) (1996 to 2001). Data for 2001 was not included in (a) as data for the full year were not available to calculate percentages. Data for 2000 were not included in (b) for confidentiality reasons.

2.5 Discussion

S. apama was historically one of the least exploited species managed in the marine scalefish fishery in South Australia. However, with establishment of an export market for the species there was a dramatic increase in the number of fishers who targeted the species between 1994 and 1998 resulting in a rapid rise in total catch. The extra catch and effort was not evenly distributed across the State, but rather was concentrated in Block 21, which supports the main spawning aggregation area. During the three years of 1999 to 2001, when most of this area was closed to fishing during the spawning season, the total commercial catch and effort was greatly reduced, and yet over 90% of the targeted catch of the State was still taken from Block 21. This indicates that the exploitation of cuttlefish in South Australia outside of Block 21 is minimal and ultimately the fishery is largely dependent upon the one spawning aggregation.

The lower CPUE values recorded in 1999 and 2001 suggests that fishers were unable to maintain high catch rates outside the main aggregation area in Block 21. Certainly, the CPUE values obtained for the remainder of the State were much lower than for Block 21 in all years when data were available. This suggests that the abundances and densities of cuttlefish populations elsewhere around the State are lower than in the aggregation area. Anecdotal observations by fishers and divers support this. However, this interpretation relies on the assumption that CPUE is directly proportional to the relative abundance of the population, and does not allow for biases caused by non-random distribution of fishing effort and

variation in the catchability of fish or efficiency of fishing gear (Hilborn and Walters 1992). In most cases that have been tested, CPUE has not been a reliable indicator of abundance (Harley et al. 2001). In our case, the proportion of search time incorporated in the CPUE values from different locations is unknown and unlikely to be equal. Therefore, these results should be treated as an indication only and further verification using independent means of abundance estimation is required.

The fishery is not only highly localised spatially, but temporally as well. Although cuttlefish catches are taken throughout most of the year, most of the annual catch is taken between May and July, the time when cuttlefish aggregate over shallow inshore reef areas to spawn. The highest monthly catch rates were reported in May and June each year and decreased to July. A decline in catch rates during short intense fishing seasons on densely aggregated populations are common and are thought to correspond to the depletion in abundance caused by fishing (Beddington et al. 1990). Here, however, the cuttlefish leaving the aggregation area may confound this.

Spawning aggregations are prime targets for fishing, as individuals that are sparsely distributed naturally group together in large numbers for the purpose of spawning (Domeier and Colin 1997). The aggregations are often predictable in both location and timing and the catchability of fish may be higher due to hunger developed during migration or the depletion of local food resources (Domeier and Colin 1997). Thus, spawning aggregations are considered to be particularly vulnerable to exploitation and some reef fish species that aggregate to spawn have been susceptible to local extinctions caused by fishing (Beets and Friedlander 1998). Other effects of heavy fishing pressure on the population dynamics of some reef fish spawning aggregations have been: (1) decreased abundances of spawning numbers; (2) decreased mean size of spawning adults; and (3) changes in the sex ratio of the spawning population (Sala et al. 2001). Since cephalopod species are generally shorter-lived than most reef fish species and there is little carry-over of standing biomass from year to year, the effects of intense or selective fishing pressure on the population structure may not be perpetuated nor accumulated in subsequent years. However, this also means that a sufficient proportion of adults must spawn each year prior to removal by the fishery to ensure adequate recruitment to maintain the population level.

Few studies have investigated the long-term effects of fishing on cephalopod spawning aggregations (Hanlon 1998). Many loliginid squid species form dense feeding or spawning aggregations in shallow coastal waters, which are targeted by commercial fishing operations (Hanlon 1998). Feeding aggregations of the Japanese common squid (*Todarodes pacificus*) have been fished for many years in Japanese coastal waters. An analysis of the historical catch and CPUE data from 1958 to 1986 suggested that the population size had decreased drastically after 1968 and that the migration range had

become narrower (Nakata 1993). The population structure had also possibly changed as the population size had declined. Few other fisheries have such a long time series of data, and stock assessment methods are generally still being developed. However, preliminary recruitment modelling results based on CPUE data for the South African chokka squid *Loligo vulgaris reynaudii* suggest that the historical biomass may have been heavily depleted by fishing (Roel and Butterworth 2000).

In this study, lower monthly catch rates were reported for Block 21 in May 1998 compared to May of the two previous years, which then declined more rapidly as the season progressed. This suggests that the initial abundance was lower in 1998 than in previous years, possibly as a result of the large catches removed in 1996 and 1997. However, given that cephalopod populations are subject to large natural population fluctuations and that the data cover only a short time period, further evidence is required before any such conclusions can be made. Furthermore, the issues involved in using CPUE data to infer trends in abundance are again pertinent, which means that such interpretations of the results should be treated with caution. Hence, an independent means of determining the spatial and temporal patterns in the abundance and biomass of the spawning aggregation was necessary and the results of that research are presented in Chapter 4.

In conclusion, the analysis of the historical commercial catch/effort data for cuttlefish in South Australia indicated that the main aggregation area in northern Spencer Gulf sustained a low level of exploitation for over 15 years, and then experienced a short but intense period of highly concentrated exploitation from 1996 to 1998. Following this, the area was closed to fishing for the last three years resulting in a significant decline in catch and effort in the fishery. It is highly possible that the level of fishing mortality experienced during the peak fishing years affected the population dynamics of the spawning aggregation, and as such should be considered when interpreting temporal and spatial variation in abundance and biomass of the spawning population.

3 General materials and methods

K.C. Hall

3.1 Spencer Gulf

Spencer Gulf is a large semi-enclosed sea on the southern coast of Australia (Nunes Vaz et al. 1990). It extends approximately 325 km inland from the southern continental shelf in an elongate triangular shape (Fig. 3.1), and is 130 km across at its widest point (Noye 1984). It is relatively shallow with a mean depth of 22 m, and consists primarily of large shallow sedimentary tidal flats dissected by narrow deeper channels. High evaporation with low rainfall and minimal runoff or groundwater supply combine to form an inverse estuary with salinity increasing from oceanic values at the entrance to hypersaline conditions towards the head of the Gulf, where salinity can reach 48 ‰ in late summer (Nunes Vaz et al. 1990). Water temperatures within the Gulf vary considerably with season, from around 12°C in mid-winter to 28°C in mid-summer (Nunes Vaz and Lennon 1986).

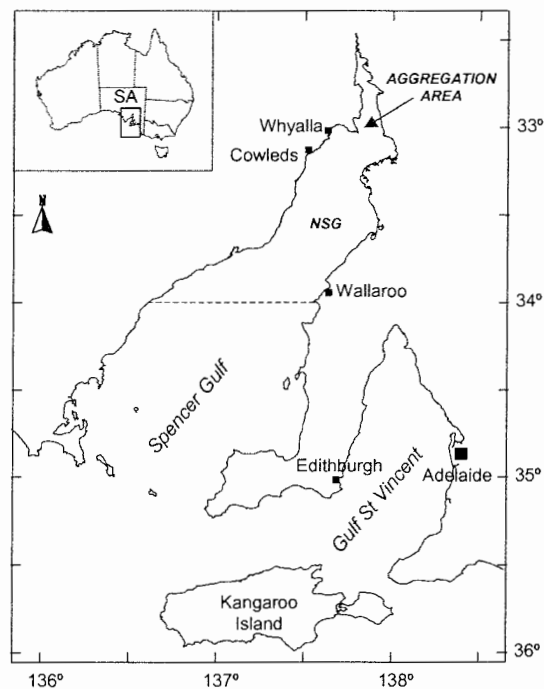


Figure 3.1 Map of the South Australian Gulf system showing the shape and orientation of Spencer Gulf and the location of the aggregation area and northern section of the Gulf.

The Gulf is relatively sheltered with only short periods of increased wave activity from locally generated wind waves of up to 2 m height during storms (Noye 1984). A fortnightly spring-neap tidal cycle exists with large vertical amplitude at spring tides (3.1m at Whyalla) to practically no variation in tide height for 24 h at neap tides. This causes regular fortnightly periods of minimal tidal water movement, known locally as “dodge tides” (Noye 1984). The shallow depth and long narrow configuration of the Gulf create maximum tidal current speeds of up to 1 m.s⁻¹. Usually tidal currents are oscillatory in nature with no net movement; however, the combination of shallow waters with complex shorelines can produce secondary non-linear tidal flows, such as the gyre formed during the ebb flow just south of Point Lowly, with significant mean flow in an easterly direction for most of the tidal cycle (Noye 1984). Residual circulation patterns in the Spencer Gulf due to thermohaline currents are slower (0.25 m.s⁻¹) and basically clockwise (Fig. 3.2a), with an inflow of low salinity water along the western side and an outflow of increased salinity water moving along the eastern side (Green 1984).

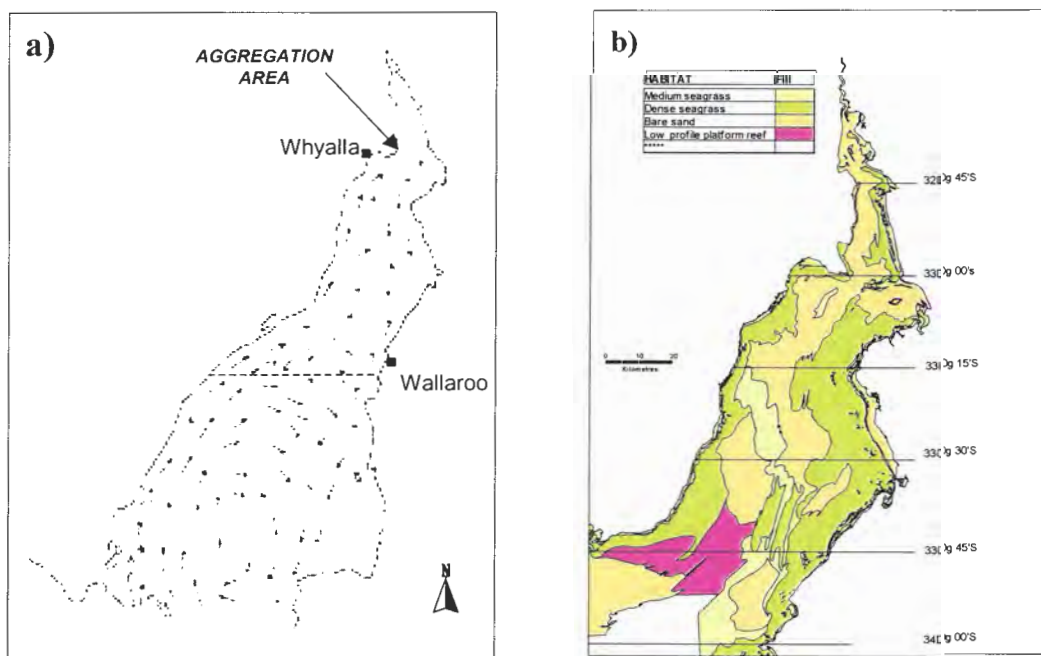


Figure 3.2 (a) Residual circulation patterns in Spencer Gulf (divided into upper and lower Gulf regions). Figure reproduced from Bullock (1975). (b) Map of northern Spencer Gulf showing main habitats.

Spencer Gulf has a total area of 22,610 km². Seagrass meadows and other soft substrate habitats such as sand comprise most of the seabed of the Gulf, and reef habitat accounts for just 1,442 km² (6.4%) (Edyvane 1999) (Fig. 3.2b). Most of the reef habitat consists of low profile platform reef often covered by sand with the only significant areas of calcareous or rock reef outcrops in southern Spencer Gulf.

The only reef areas in northern Spencer Gulf, i.e. north of Wallaroo (lat. 34° S) are a few small outcrops around Point Lowly, Backy Point and Plank Point.

3.2 Sampling locations

Field sampling for this study was concentrated primarily in the coastal waters between Black Point and Point Lowly in northern Spencer Gulf where the dense spawning aggregation of *Sepia apama* occurs every winter (Fig. 3.1). The aggregation area occurs along approximately 8 km of coastline (with a subtidal reef area of 0.64 km²). The coastline consists of a platform of plate-like fragments of dense quartzite bedrock (Fig. 3.3) (Hails and Gostin 1978), which extends out beyond the intertidal zone, gradually becomes low-relief subtidal rocky reef out to 70-130 m offshore (ca 8 m depth) and then ends in bare sand and seagrass. *Sepia apama* aggregate over the hard substrate in 2-8 m depth.



Figure 3.3 Photograph of fragmented bedrock habitat of Black Point.

Very few cuttlefish were present at the aggregation area from September to March, i.e. outside the spawning period. To provide a comprehensive analysis of the life history, samples from other areas of northern Spencer Gulf were obtained whenever possible, using other means. Throughout the text, samples from the main aggregation area are referred to as "aggregation samples" and those from the northern Spencer Gulf as "NSG samples". The latter were restricted to the northern region of the Gulf (i.e. north of latitude 33°55' S; Fig. 3.1) to comply with the definition of the NSG population (Chapter 1).

3.3 Sampling dates

3.3.1 Aggregation area

The sampling dates for different methods were determined by the timing of events at the aggregation area (Fig. 3.4). Anecdotal reports indicate that adult cuttlefish begin to aggregate in the area around the end of April/early May. Thus, sampling in the aggregation area commenced in March 1998 before the start of the spawning season. The first habitat surveys were completed at this time to describe and quantify the areal extent of main hard substrate habitat types and to verify the absence of cuttlefish. The adult population was surveyed at regular intervals (actual times depended on weather and equipment/personnel availability) from the end of April until end of August each year from 1998 to 2000 (Fig. 3.4). A single sampling was completed in 2001 to finalise a number of data sets, including an additional set of habitat transects. Eggs were monitored in situ from May until the last had hatched in November.

3.3.2 Northern Spencer Gulf

Adult cuttlefish departed from the aggregation area around the end of August every year, and were rarely sighted or caught in other coastal areas from September to March. From November to June cuttlefish were often caught in deeper offshore Gulf waters (10-20 m depth) as by-catch of trawling for the western king prawn, *Penaeus latisulcatus* (Carrick 1997). Samples were also obtained from an annual research trawl survey for juvenile snapper (*Pagrus auratus*), that was done in February and/or April each year. Other samples were provided by a commercial pot fisher who targeted blue swimmer crabs (*Portunus pelagicus*), just south of the aggregation area in April or May. Since the importance of these NSG samples only became apparent as the study progressed, they were collected opportunistically rather than systematically. These samples were often used for multiple purposes such as tagging and genetic sampling, and were thus not always available for laboratory processing and analysis.

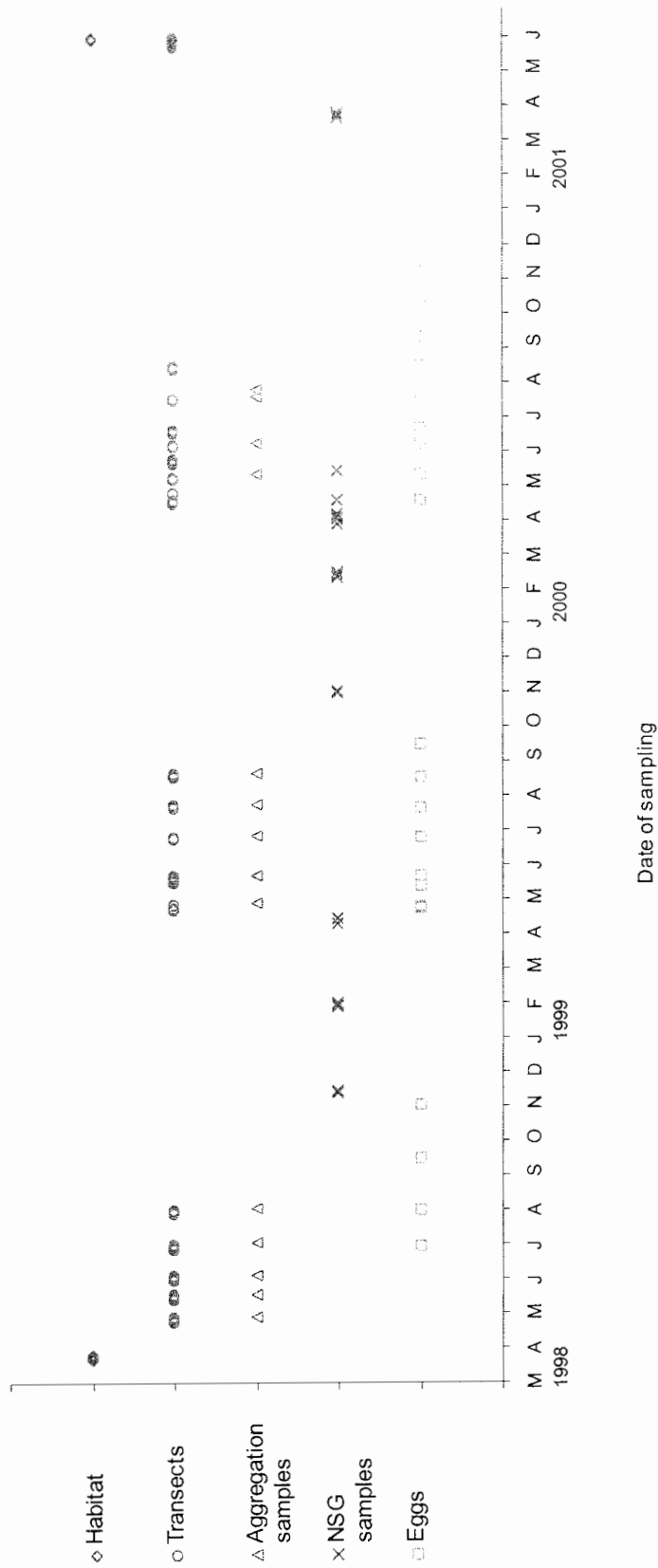


Figure 3.4 Timing of field sampling for different purposes from March 1999 to June 2001 (see text for details).

3.4 Sampling methods

3.4.1 Aggregation area

Underwater visual survey techniques have proven to be a particularly effective, non-destructive method for estimating the abundances of various species of coral reef fish (Cappo and Brown 1996).

Consequently, an underwater visual strip transect method (cf., McCormick and Choat 1987) done on SCUBA was used to document the spatial and temporal changes in density, sex ratio, and size composition of *S. apama* within the aggregation area throughout each spawning season.

Furthermore, on the last day of each sampling trip (Fig. 3.4), a sample of 30 cuttlefish was collected from a random location at Black Point, re-selected each time. Individuals within a 20 m radius of the boat anchor were captured individually using a hand-net while snorkelling. Equal numbers of both sexes were sampled. The length and sex of each individual were estimated underwater and the behaviour of the animal immediately before capture was noted. Each animal was assigned an identification code corresponding to the one recorded underwater, and placed in a separate labelled bag to ensure accurate identification later in the laboratory. Sex and length estimates noted underwater were compared to those measured in the laboratory to assess the accuracy and precision of underwater estimates. These samples were used for laboratory analysis for age determination and describing the reproductive biology (Table 3.1).

In 1999, an additional sample of 30 cuttlefish was also collected at each sampling time via hand-line and squid jig, at a second random location at Black Point. All animals were kept for measurement in the laboratory to determine the composition of the catch potentially removed by commercial fishing operations. On collection these cuttlefish were immediately placed on ice to promote rapid death and were kept chilled in an ice slurry until returned to the laboratory.

A sample of hatchlings was also collected from the aggregation area in September or October each year. They were captured by hand as soon as they emerged from the egg capsules and were used for age and size analysis (Table 3.1). In 1998 and 2000, the hatchlings were stored in 70% ethanol, whereas in 1999 they were stored frozen.

Table 3.1 Details of samples collected – date, location, sample method, total number of each sex collected, number used for ageing and reproductive biology analysis, and type of storage prior to processing.

| Date | Location | Sample method | Total M : F | No sex | Ageing M : F | Repro M : F | Fresh M : F | Frozen M : F |
|---------------|-------------|----------------|----------------------|-----------|------------------|-----------------------|----------------|-----------------|
| 30 Apr 1998 | Aggregation | Jig | 32 : 1 | | | 32 : 1 | All | |
| 19 May 1998 | Aggregation | Jig / Hand net | 17 : 13 | | 17 : 13 | 17 : 13 | All | |
| 5 Jun 1998 | Aggregation | Hand net | 8 : 22 | | | 8 : 22 | All | |
| 4 Jul 1998 | Aggregation | Hand net | 24 : 6 | | | 24 : 6 | 16 : 6 | 8 : 0 |
| 4 Aug 1998 | Aggregation | Hand net | 21 : 10 | | 14 : 10 | 21 : 10 | 6 : 6 | 15 : 4 |
| | Aggregation | Hatchlings | | 10 | 10 | 10 | | Ethanol |
| 15 Nov 1998 | NSG | Prawn Trawl | 15 : 16 | | 15 : 16 | 15 : 16 | All | |
| 2 Feb 1999 | NSG | Snapper Trawl | 15 : 19 [‡] | 2 | 6 : 4 | 6 : 4 | Parts All | Parts All |
| 14 Apr 1999 | NSG | Prawn Trawl | 40 : 44 | | 22 : 21 | 40 : 44 | All | |
| 20 April 1999 | Cowleds | Crab Pot | 18 : 11 | | | 18 : 11 | | All |
| 3 May 1999 | Aggregation | Hand net | 17 : 3 | | | 17 : 3 | | All |
| 28 May 1999 | Aggregation | Hand net | 23 : 13 | | 23 : 13 | 23 : 13 | All | |
| 2 Jul 1999 | Aggregation | Hand net | 23 : 7 | | | 23 : 7 | All | |
| 29 Jul 1999 | Aggregation | Hand net | 19 : 11 | | 19 : 11 | 19 : 11 | All | |
| 26 Aug 1999 | Aggregation | Hand net | 7 : 14 | | | 7 : 14 | All | |
| | Aggregation | Hatchlings | | 6 | 6 | 6 | | All |
| 7 Nov 1999 | NSG | Prawn Trawl | 2 : 2 | | 2 : 2 | 2 : 2 | All | |
| 20 Feb 2000 | NSG | Snapper Trawl | 28 : 14 | | 28 : 14 | 28 : 14 | | All |
| 5 Apr 2000 | NSG | Prawn Trawl | 42 : 40 | | 15 : 12 | 42 : 40 | | All |
| 13 Apr 2000 | NSG | Snapper Trawl | 121 : 69* | 5 | 11 : 8 | 11 : 8 | Parts All | Parts All |
| 27 Apr 2000 | Cowleds | Crab Pot | 9 : 10 | | | 9 : 10 | | All |
| 23 May 2000 | Cowleds | Crab Pot | 11 : 8 | | | 11 : 8 | | All |
| 19 May 2000 | Aggregation | Hand net | 14 : 15 | | 14 : 15 | 14 : 15 | All | |
| 17 Jun 2000 | Aggregation | Hand net | 14 : 14 | | | 14 : 14 | All | |
| 3 Aug 2000 | Aggregation | Hand net | 15 : 19 | | 15 : 19 | 15 : 19 | All | |
| | Aggregation | Hatchlings | | 12 | 12 | 12 | | Ethanol |
| 4 Apr 2001 | NSG | Snapper Trawl | 104 : 93 | | 52 : 51 | 104 : 93 [#] | All | Gonads |
| TOTALS | | | 639 : 474 | | 253 : 207 | 520 : 398 | | |

[‡] All cuttlefish over 10 mm ML were used for a genetic study; * all cuttlefish over 10 mm ML were tagged and released alive to investigate movement patterns in the northern Spencer Gulf; # all cuttlefish were processed for reproductive indices but none were processed for condition indices; shaded samples were used for size and sex composition data in conjunction with transect data from the aggregation area. For location of aggregation area and Cowleds refer to Fig. 3.1.

3.4.2 Northern Spencer Gulf

The prawn trawlers use standard paired otter trawl equipment with a cod end mesh size of 45 mm. All cuttlefish caught by the port net during a 30 min trawl were kept, bagged and chilled overnight in an ice slurry before being transported back to the laboratory for dissection the following day. The locations from where samples were collected varied according to the prawn survey sampling design and the vessel sampled.

In February and/or April of 1999, 2000 and 2001 a systematic trawl survey was done by researchers from the South Australian Aquatic Sciences Centre, throughout the northern Spencer Gulf to document the recruitment of 0+ snapper. The *MRV Ngerin* was fitted with an otter trawl and fine mesh net (cod end mesh size 12 mm). Trawls were for a set duration of 10 min each, at an average speed of 3.2 kn. This resulted in a swept-area of 8880 m² per trawl. Sampling stations covered most of the channel areas in the northern Spencer Gulf but exact locations varied between sampling trips (Chapter 4). These data provided a snapshot of the distribution and abundance of *S. apama* just prior to the spawning season.

In 1999 and 2000, samples were only partially processed on board before being frozen. In February 1999 all cuttlefish with mantle lengths greater than 100 mm were used for genetic analysis. Those captured in April 2000 were kept alive and used for tagging. Therefore, although they could be included in the size and sex structure analysis they were unavailable for the reproductive biology analysis and age estimation (Table 3.1). In April 2001, most of the laboratory processing was completed on board while the specimens were fresh, but as fine-scale measurements of weight were not possible at sea, reproductive organs were removed and frozen for later weighing back in the laboratory.

One commercial fisher regularly targets blue swimmer crabs along the coast at Cowleds Landing, approximately 20 km south of Whyalla. Custom-made crab pots are soaked overnight and hauled during the early morning. The catches of cuttlefish as by-catch in these pots become more numerous around April and May, coinciding with when the cuttlefish migrate to the aggregation area for spawning. Those captured were assumed to be on their migration route, and the samples obtained in April and May each year were used for analysis of reproductive biology.

3.5 Aquarium experiments

Adult *S. apama* collected from the wild, were maintained in aquaria for age validation experiments (Chapter 6) and spawning experiments (Chapter 8) in 1998 and 1999. Eggs were also collected from the wild in 2000, for development and hatching experiments. The hatchlings were reared for juvenile growth and age validation experiments (Chapter 7), that were done at the South Australian Aquatic Sciences Centre (SAASC) in Adelaide, in either outdoor or indoor aquarium facilities. In both aquarium set-ups, large circular 400 L fibreglass tanks were used, supplied with flow-through seawater, at a rate of approximately $50 \text{ mL}\cdot\text{s}^{-1}$. The seawater originated 1 km off the metropolitan coast of Adelaide, in Gulf St. Vincent and passed through a settlement tank and primary sand-filter (filtration to $30 \mu\text{m}$) before entering the aquarium system. Salinity remained between 36-37 ‰ throughout all experiments and bottom aeration was provided in each tank at a rate of approximately $300\text{-}600 \text{ mL}\cdot\text{min}^{-1}$.

In the outdoor set-up, the tanks were exposed to the natural photoperiod, although shaded to reduce light intensity. Where required constant water temperature was maintained by continual heating with electric heaters. A probe directly linked to the aquarium computer system continuously logged ambient water temperature. In the indoor set-up, the tanks were subjected to a 12 h light:12 h dark photoperiod and water temperature and salinity were controlled by a computer-automated system.

3.6 Laboratory processing

All individuals collected from the field or used in aquarium experiments were dissected fresh, within one or two days of capture or death. In some instances storage by freezing was required before dissection (Table 3.1); however, this precluded some measurements be taken. Total wet weight (TWt) and dorsal mantle length (ML) of all specimens were recorded before dissections were started. All wet weight measurements were made on a digital Sartorius scale, to the nearest 0.01 g or for smaller measures a Mettler electronic balance, to the nearest 0.0001 g. Length measurements were made with a standard 30 cm ruler to the nearest 1mm, callipers to the nearest 0.1 mm or a Leitz dissecting microscope fitted with an ocular micrometer to nearest 0.01 units and converted to mm using appropriate calibrations.

Image analysis equipment was used to make fine scale microscopic measurements. The system comprised a dissecting microscope fitted with a digital video camera connected directly to a computer

with video grab software and SigmaScan Pro[®] image analysis software. Digital images were saved and distances estimated using pixel counts from the images converted to mm using appropriate calibrations.

3.7 Statistical analysis

All statistical analyses were completed using SPSS Version 10.0 for Windows or JMP IN Student Version 4.0. Most were assessed at the $\alpha = 0.05$ significance level unless otherwise stated. Details of the tests used and data manipulations required for each test are presented in individual chapters.

4 Abundance and biomass

K.C. Hall, A.J. Fowler, M.C. Geddes and D. McGlennon

4.1 Introduction

A reliable sampling methodology to monitor population size in space and through time is fundamental to studies of population biology (Andrewartha and Birch 1954). It is rarely possible to count or measure all individuals of a population (Seber 1982). Therefore, the population area is usually divided into smaller sampling units and data are collected from a randomly chosen subset to produce estimates of relative abundance or biomass (Cochran 1977; Andrew and Mapstone 1987). If the total area occupied by the population is known, areal expansion can be used to estimate the total population size from the average density or weight-per-unit area obtained from the sample units (Seber 1982). In some instances, direct counts or measures of individuals are not possible, even over small sampling units, and indirect methods must be used. These usually rely on indices of relative abundance estimated from other variables known to vary in proportion to population size.

Due to the short life span of most cephalopod species the assessment of population sizes generally needs to be done on a shorter time-scale than for many finfish species (Pierce and Guerra 1994). The standing biomass in each year often relies largely on recruitment levels due to the minimal overlap between generations; hence population sizes may fluctuate broadly in response to environmental variation and show little relationship to previous levels. Therefore, the most useful forms of population assessment usually involve: (1) within-season estimates of abundance or biomass using incomplete data sets collected for real-time management; (2) pre-season or recruitment indices which reliably forecast the population entering the fishery; or (3) the correlation of long-term population size data sets to changes in environmental variables over time, to predict future population levels based on forecasts of environmental fluctuations.

Most data on the size of cephalopod populations have been derived using indirect methods. The most common of these uses landings or catch-per-unit-effort (CPUE) data from commercial fisheries as an index of relative abundance. Although, fisher-dependent data have many limitations (Chapter 3 and reviewed by Harley et al. 2001), they may be more indicative of abundance in instances where the species of interest is taken as by-catch rather than as targeted catch (e.g. Pierce et al. 1994). However, in most cases proportionality of CPUE to abundance is assumed and not tested and biases or changes in

the pattern of fishing effort are rarely accounted for (Roel et al. 2000). Fisher-dependent CPUE data are often used for depletion estimates of population size using a modified Leslie-DeLury method (e.g. Rosenberg et al. 1990; Augustyn et al. 1993) or for relating long-term patterns to environmental variables (e.g. Robin and Denis 1999; Ueta et al. 1999; Bellido et al. 2001; Waluda et al. 2001).

Another indirect method gaining popularity for coastal squid aggregations is hydro-acoustic surveys with echo-integration that convert target strength data to biomass or density estimates (reviewed by Starr and Thorne 1998). This method has proven useful for dense aggregations of many coastal Japanese squid species, and *Loligo vulgaris reynaudii* off southern Africa (Sauer et al. 1993; Augustyn et al. 1993) and *L. opalescens* off the west coast of America (Vaughan and Recksiek 1978; Jefferts et al. 1987). However, when squid are very thinly dispersed or in extremely dense schools the technique can be less reliable (Augustyn et al. 1993; Starr and Thorne 1998).

Direct estimates of abundance or biomass of cephalopod populations are less common, and usually take the form of catch-per-unit-effort data from fisher-independent research surveys using trawl, jigging or other fishing methods as relative indices of abundance (e.g. Dare 1981; Lang 1991; Yatsu et al. 2000) or coupled with swept-area calculations and areal expansion to determine absolute estimates (e.g. Murata 1989; Augustyn 1991; Wurtz et al. 1991). The surveys usually allocate effort evenly or according to a random stratified design over most of the population area, thus eliminating the problems of biased fishing effort associated with fisher-dependent CPUE data. However, surveys are still subject to selective biases associated with fishing gears and may be limited in coverage of the water column and/or population range. Thus, they may be useful in determining population size estimates over offshore feeding grounds but as many cephalopod spawning aggregations occur in inshore waters over rough untrawlable bottom these methods are rarely used to directly estimate the size of spawning aggregation populations (Augustyn 1991).

Sepia apama occurs in shallow reef areas during the spawning season, which are unsuitable for trawling but are easily accessible for SCUBA diving. Therefore, underwater visual transect methods were chosen as a non-destructive method of density estimation for the spawning population. Furthermore, since the area of hard substrate constitutes a conspicuous and finite manageable area, it was possible to estimate the habitat area and calculate an estimate of total abundance. This is the first use of this technique for the study of a cephalopod population.

In 1996 and 1997, large quantities of *S. apama* were removed from the aggregation area by commercial fishers during the spawning season. Surveys of the population using underwater transects did not begin until 1998, when an area that included approximately 50% of the reef in the aggregation

area was first closed to fishing. The fishers were already present in the last week of April and started fishing as the first cuttlefish arrived. After 32 days of fishing most of the area remaining open to fishing was also closed; all but the eastern side of Pt Lowly (Chapter 2). In the following three years (1999 to 2001), this larger closure remained in place for the duration of each spawning season. Therefore, relative estimates from the fished and unfished areas, i.e. the original areas left open or closed to fishing at the start of the 1998 spawning season, were compared between years, and absolute estimates from 1998 were compared to those from subsequent years to identify differences that may have related to fishing.

4.2 Aims

The focus of this chapter was to design and implement a sampling regime based on underwater visual transects to achieve two broad aims: (1) to provide estimates of cuttlefish density for spatial and temporal comparison within and between different closure areas and years, to understand the population dynamics of the spawning aggregation; and (2) to provide annual estimates of spawning population abundance and biomass for use in fisheries management. These aims were further broken down into a number of specific objectives as follows:

- (1) to describe the main types of habitat present in the aggregation area and estimate the area covered by each;
- (2) to estimate the densities of cuttlefish in different habitat types, sites and closure areas within and between spawning seasons;
- (3) to estimate the total abundance and biomass of cuttlefish in the aggregation area each year from 1998 to 2001 and estimate the relative proportions that occurred within the closed area introduced at the start of the 1998 spawning season, and that removed as catch due to fishing;
- (4) to determine any within-season or between-season changes in total abundance and biomass or the spatial distribution of cuttlefish in the aggregation area, which may relate to the effects of fishing;
- (5) and to investigate the movement patterns of individuals within the spawning aggregation area that have potential implications for the interpretation of spatial and temporal distribution data and assumptions involved in the estimates of total population size.

4.3 Materials and methods

4.3.1 Habitat assessment and area estimation

The subtidal rocky reef habitat in the aggregation area was first surveyed in March 1998 before cuttlefish began arriving in April. The purpose of the assessment was three-fold: (1) to provide a broad description of the main habitat types present; (2) to determine if habitat was sufficiently variable to warrant stratification of sampling according to habitat type; and (3) to estimate the areas covered by the main habitat types. Since this was not the main focus of the research, a rigorous quantitative assessment of the habitat according to established quadrat or line-intercept transect methods was not practical (Underwood and Kennelly 1990), and a more general descriptive method was used.

An aerial photograph (South Australian Department of Natural Resources, 1996; Survey 5079; scale 1:18,700; Fig. 4.1) was used to identify the main sections of reef coastline. These sections were ground-truthed on snorkel to verify the presence or absence of hard substrate. In each 1 km section of reef coastline, two haphazardly located habitat transects were completed. A 50 m fibreglass tape measure was anchored at the mid-intertidal level, as delineated by the calcareous tubes of *Galeolaria caespitosa* on rocks, and swum out normal to the coastline. At each 5 m interval the depth, main substratum type and predominant algal and sessile invertebrate species were noted. Consecutive 50 m lengths were sampled in this way until hard substrate ended and was replaced by bare sand or seagrass.

In June 2001, four additional random transects were completed in each site to increase the precision of the estimates of habitat area. These transects were laid out normal to the shoreline, and the distance along the tape measure (and depth) at which one habitat type ceased and the next began was noted.

4.3.2 Spatial and temporal allocation of sampling

Cuttlefish densities were sampled in the aggregation area from April to August each year from 1998 to 2001. As different time and area closures for commercial fishing were implemented over this four year period, the aggregation area was divided into three sub-areas defined according to the extent of commercial fishing allowed (Fig. 4.1). The "closed-closed area" referred to the area originally closed to fishing in 1998 and closed again in all subsequent years; the "open-closed area" referred to the area originally left open to fishing for the first half of the 1998 season and then closed, and closed again in all subsequent years; and the "open-open area" referred to the area left open to fishing in all years (Fig. 4.1).

Within these areas, the hard substrate was not always continuous. Therefore, the coastline was further subdivided into a number of separate sites, with the boundaries arbitrarily determined by geographic features such as sand patches and underwater channels. This resulted in four sites in the open-closed area, four in the closed-closed area and three in the open-open area (Fig. 4.1). On average, sites consisted of 600 m of coastline, but ranged from 280 m to 1.2 km. One additional site outside of the main aggregation area was also monitored. This site, referred to as the "BHP Wall", was 15 km away near Whyalla and consisted of an artificial habitat provided by the Broken Hill Propriety Ltd (BHP) pellet plant wall. It was known to local divers as another area of high density of cuttlefish during the spawning season and had always been protected from fishing by a 20 m exclusion zone maintained by the BHP industry. Hence, it represented an additional area that was closed to fishing.

The four main habitat types identified in the aggregation area formed relatively homogeneous zones parallel to the shoreline, but not all were present at all sites. Therefore, a fully balanced factorial sampling design with replication at all levels of spatial variation (i.e. all combinations of area, site and habitat) was not possible. Thus, two different sampling designs were used to address the two main objectives separately. To compare the relative abundances of cuttlefish between and within all levels of spatial variation, a reduced balanced design was implemented in 1998. This consisted of two habitat types, sampled at three sites within two of the areas, the closed-closed area and the open-closed area. This sampling was completed on 6 sampling occasions approximately 3 to 4 weeks apart throughout the spawning season, each accomplished in 4 to 6 days. At each sampling time four replicate transects were completed within each habitat within each site within each area.

The results of such sampling indicated that cuttlefish were not evenly distributed throughout the aggregation area and that densities varied greatly between different habitat types. Therefore, to obtain more accurate estimates of total abundance and biomass, a stratified random sampling design incorporating all sites and habitats was implemented at a single sampling occasion each year between 1999 and 2001. The timing was chosen to coincide with the maximum level of biomass in the aggregation area. This relied on a subjective decision of when the peak in the season occurred. However, the relatively long residence times of individuals in the area suggested this might occur over a range of dates (weeks), which would allow for some error in judgement. A stratum was defined as a single habitat type within a site, with 20 strata in total. The area of each stratum (henceforth referred to

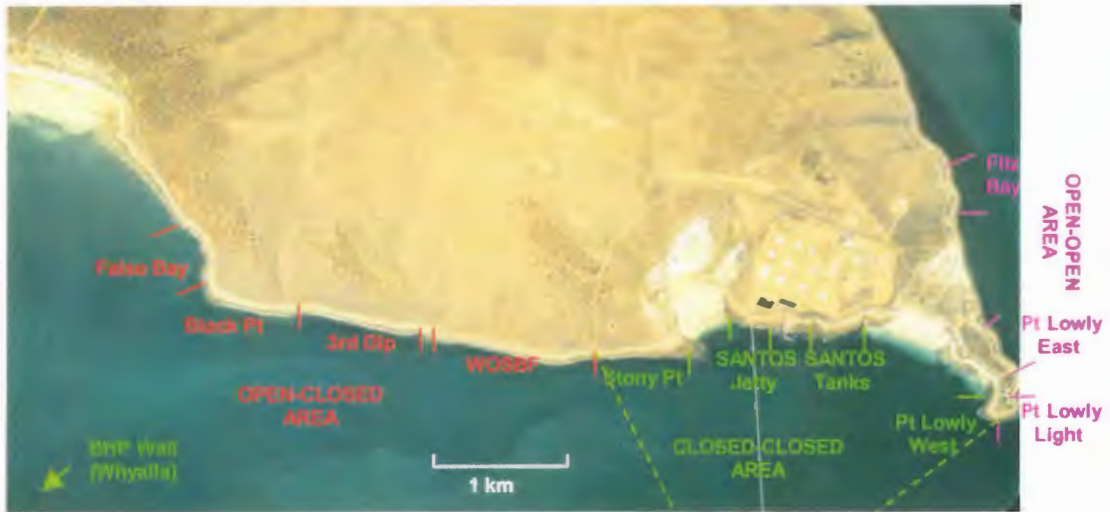


Figure 4.1 Aerial photograph of the main spawning aggregation area from Black Point to Point Lowly, with the location of sampling sites indicated. Sites in the open-closed area are indicated in red, closed-closed area in green and open-open area in pink.

as "stratum-area") was estimated using the habitat widths from transects multiplied by shore lengths derived from the aerial photograph and nautical charts (RAN Chart No. AUS 136; scale 1:75,000). Four transects were completed in each stratum, allowing for relative comparisons between strata.

Unfortunately, some strata were not sampled in every year, specifically those under the SANTOS jetty and near the Point Lowly lighthouse, due to problems with access. The former required special permission from SANTOS to obtain access and the latter was subject to strong tidal currents. Hence, these strata were not included in the final estimates of abundance and biomass.

In 1999 and 2000, temporal sampling was reduced to incorporate just one stratum within each area. This was undertaken to verify whether the peak time occurred at the same time in each area, and between different years. The analysis and interpretation of these results was obviously limited by the lack of replicate strata within each area. In 2001, no temporal sampling at all was completed due to time limitations.

4.3.3 Transect methods for density estimation

Strip transects of 50 x 2 m were used to quantify the density of cuttlefish. These dimensions were chosen for logistical reasons that relate to total dive time (maximum of three 1.5 h dives per day) and number of replicates possible. All transects were completed by the same individual, thus eliminating possible variation due to different counters. Sampling was done during daylight hours between 09:00 and 16:00, and only when visibility exceeded 4 m.

No transects crossed a habitat or site boundary, but were otherwise randomly allocated within each stratum at each sampling occasion. A 50 m fibreglass tape was anchored at the start point and unwound gradually as each transect was swum roughly parallel to shore. The habitat within 1m of both sides of the tape was searched and any cuttlefish encountered were recorded, their length estimated (dorsal mantle length to the nearest 1 cm; ML), and sex and behaviour noted. Males were distinguished by having longer arms, distinctive skin patterns, and characteristic behaviours and postures (Fig. 4.2). The time to complete the transect depended on the number of cuttlefish encountered and complexity of habitat searched.

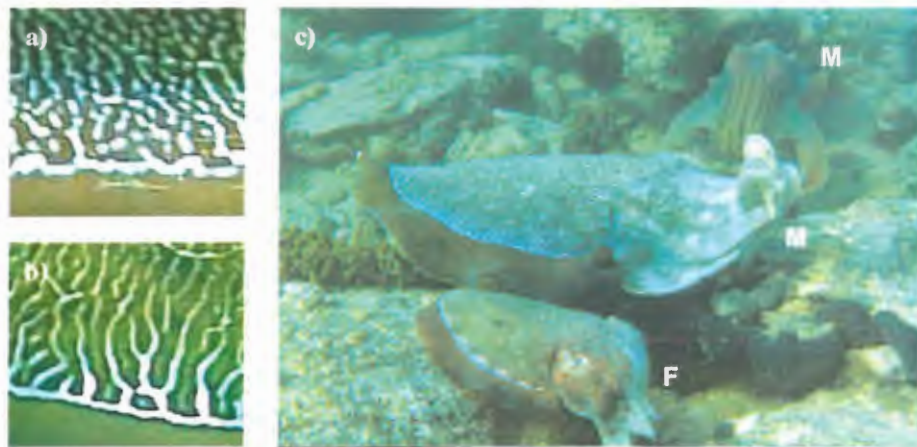


Figure 4.2 Pictures showing the distinctive skin patterning (a-b) and body proportions (c) of *S. apama* used to assign sex underwater. The basic skin pattern of females (a) has many white dots and broken lines, whereas, males (b) tend to have very solid white lines. Females (F) have shorter arms than males (M) and have a humped head profile at times when egg-laying (c).

4.3.4 Statistical analysis

Analysis of the transect data was complicated by a number of factors including: (1) the large number of zeros recorded at the start and end of each season and for many transects in the algal habitat; (2) the non-normality and non-linearity of the count data, (3) the potential serial correlation of repeated measures from a single area; and (4) the necessity to block certain variables. Therefore, complex statistical modelling was required, which was completed by Julian Taylor of Biometrics SA (Appendix I). The analysis was completed in two parts, corresponding to the two different sampling designs used to address the main objectives of the study. For both models, the significance of fixed effects were tested sequentially using a Wald statistic, which has an asymptotic chi-squared distribution. The blocking variables, random effects and cubic smoothing spline terms with their associated variance/correlation parameters were tested for inclusion in the models by testing the null hypothesis that the associated variance component was zero, using restricted maximum likelihood theory (Appendix 1). The data describing temporal trends across all years for one stratum within each area were not analysed due to the lack of replication in the sampling design.

4.3.5 Abundance estimation

Areal expansion of density data was used to obtain an estimate of abundance for each stratum, by multiplying the average density of cuttlefish by the estimate of stratum-area. Total abundances in the fished area and the closed area were estimated by combining the abundance estimates for all strata in the fished and closed areas, respectively.

4.3.6 Length and weight estimation

The estimated ML of each cuttlefish encountered on transects was used to calculate the average weight-per-transect. First, the accuracy and precision of the ML estimates were considered by plotting the difference between paired values of ML that were estimated underwater and that measured in the laboratory. The methods used to collect the latter measurements were detailed in Chapter 3. Student's t-tests were used for each data set to determine if the estimated lengths were significantly different from actual lengths (Zar, 1999). For those occasions on which a significant difference was detected, the estimated lengths were corrected by subtracting or adding the mean difference from the original estimates. The corrected ML values were converted to weights (TWt), using length-weight relationships, from the population sampling (Chapter 3). A separate length-weight relationship was determined for each sex in each year, and an analysis of covariance used to test for significant

differences between years. The estimated TWt values were summed for each transect in a stratum, and the mean weight-per-transect calculated.

4.3.7 Biomass estimation

An estimate of biomass for each stratum was calculated by multiplying the average weight-per-transect by the stratum-area. Total biomasses in the fished and closed areas were estimated by summing across appropriate strata. The cumulative commercial catch was added to the estimate of biomass for the fished area to provide an estimate of total biomass for that area. This was combined with the estimate of biomass for the closed area to provide an estimate of total biomass for the entire aggregation area.

4.3.8 Uncertainty estimation

Many different sources of error were incorporated in the estimates of uncertainty for the estimates of biomass and abundance, including measurement or method error and those associated with the sampling design. To estimate this uncertainty the procedure outlined by Taylor (1982) for the propagation of uncertainties through serial calculations was used. The uncertainties in the estimates of biomass were higher than those for abundance due to the added sources of measurement error involved in the calculation of weights from visually estimated lengths.

4.3.9 Movement studies

The estimates of total abundance and biomass relied on a number of assumptions. The first was that most animals arrived in the aggregation area before any started to leave, such that there was a peak in abundance and biomass at some point that could be sampled and estimated. If there was a constant turnover of cuttlefish in the area the method would underestimate the true abundance and biomass of cuttlefish using the aggregation area. Secondly, the method relied on the assumption that there was no net movement of cuttlefish from the closed-closed area to either the open-closed area or open-open area over time.

A tagging study was done in the 2000 spawning season with two aims: (1) to determine the movement patterns of cuttlefish within the spawning grounds during the season; and (2) to determine the length of time spent in the aggregation area by individual cuttlefish. The BHP Wall was chosen as the site for tagging due to the high density of animals within a small area, which was considered manageable for subsequent searching for tagged individuals. A total of 178 males and 19 females were tagged on 17-18

May 2000. Due to the low number of females tagged, an additional 35 females were tagged on 15 June 2000.

Cuttlefish were caught using a hand line and squid jig or by hand net whilst on snorkel. They were quickly but carefully removed from the squid jig or net and immediately processed on deck. The ML was measured (nearest 1 mm), and the sex determined by examination of the buccal area for the presence or absence of a sperm receptacle (present in females but absent in males). Each individual was tagged through the posterior lateral fin using a Hallprint polyethylene streamer tag on one side and a custom-made tag on the other (Fig. 4.3). The custom-made tags consisted of a flexible polyethylene Hallprint tag fitted with fishing line through both ends. The line was passed through the lateral fin using a sewing needle and secured in place with knots on the underside of the fin. The tagged cuttlefish was immediately returned to the water over the side of the boat. The tagging procedure was rapid (approximately 2 min in duration) and resulted in only two mortalities.

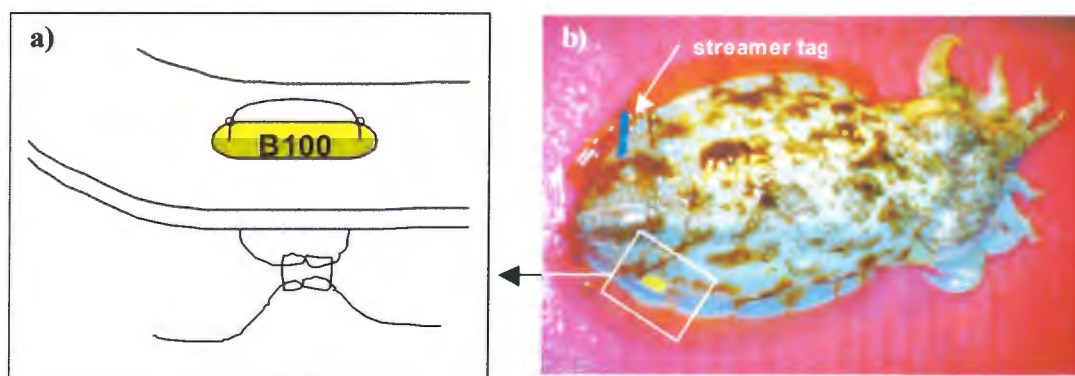


Figure 4.3 (a) Diagrammatic representation of a custom-made polyethylene cuttlefish tag fixed through the lateral fin with fishing line. (b) Photograph of a tagged cuttlefish indicating location of tags.

Tagged animals were searched for during all subsequent transect dives and during dedicated search dives on 25 May, 16 June and 29 June 1999 in Sites from Black Point to Stony Point, and on 2 June, 15 June, 28 June and 24 July 1999 along the BHP Wall. During the search dives, two divers swam in a zig-zag pattern over adjacent broad strips of the reef. Tag numbers were readable underwater, without need to disturb or catch cuttlefish. One tagged animal was returned by a recreational fisher.

4.3.10 Abundance and distribution of the non-spawning population

The sampling methods used to survey the NSG population were outlined in chapter 3. These surveys only covered trawlable habitat and no abundance estimates were obtained for coastal or hard bottom areas during non-spawning times.

A tagging study was also done in northern Spencer Gulf during the non-spawning time to investigate the larger-scale movement patterns of *S. apama*. All cuttlefish larger than 100 mm ML caught during the SARDI juvenile snapper survey in April 2000 (Chapter 3) were kept alive onboard, tagged and released. On capture, cuttlefish were immediately placed in one of three 300 L holding tanks that were supplied with constant flow-through seawater. They were then tagged using the same methods described above. After tagging the cuttlefish were retained for at least 1 h to ensure adequate recovery from the trawling and tagging procedure. Groups of tagged cuttlefish were released periodically as close to their collection site as possible. In some instances some cuttlefish were released some distance from the capture site to avoid predation by dolphins that followed the research vessel. Overall, 110 males and 61 females were tagged between 10 – 15th April 2000.

4.4 Results

4.4.1 Habitat assessment

Four main habitats were identified, which formed distinct zones parallel to the shoreline. The first included from just below zero datum to approximately 1 m depth, and consisted primarily of relatively bare boulders or solid bedrock steps with associated limpets and calcified tubes of *Galeolaria caespitose*. The second habitat started with the onset of filamentous algal mattes, *Gigartitina brachiata*, at a depth of 1 m and extended to 4-5 m depth. It consisted of broken bedrock/reef habitat with large flat slabs dominated by sea urchins, *Helocidaris erythrogramma*, sponges and low turfing algae. This will henceforth be referred to as the “urchin habitat” (Fig. 4.4a). Fine red algal species such as *Asparagopsis taxiformis* and many brown Dictyotales species such as *Lobophora variegata* were the main algae present. Further offshore from 4-5 m to 7-8 m depth, the reef was more patchy, covered in dense tall stands of furoid brown and green algae such as *Scabaria agardhii*, *Cystophora expansa*, *Caulocystis* spp. and *Sargassum* spp., and interspersed with sand and bivalves (predominantly razorfish, *Pinna bicolor*, and hammer-oysters, *Malleus meridianus*). This habitat will be henceforth referred to as the “algal habitat” (Fig. 4.4b). Beyond 7-8 m depth, hard substrate ceased and sand and

seagrass dominated. The border between the urchin and algal habitats was usually delineated by a distinct “front” of urchins between the relatively bare slab reef and tall stands of algae.

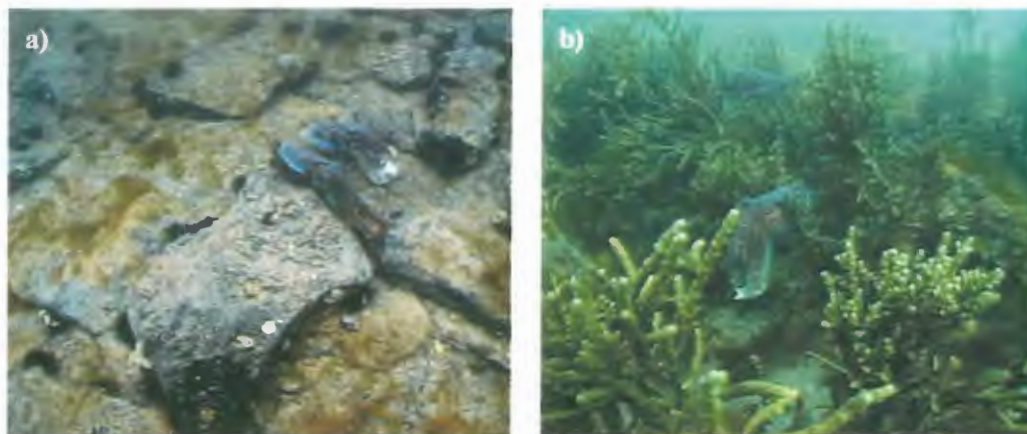


Figure 4.4 Underwater photographs showing the contrast in appearance of (a) the "urchin habitat" and (b) the "algal habitat".

At Fitzgerald Bay, Point Lowly East and False Bay (Fig. 4.1), there was only one habitat type, i.e. the reef extended only 10–45 m offshore and 3–4 m in depth, and was a more complex high relief reef that dropped off steeply into sand and seagrass. This habitat will be henceforth referred to as the "reef habitat". Only one habitat was identified at the BHP Wall, comprised of large angular slag boulders forming a steep gradient from 1 to 6 m depth and ending in bare sand at the bottom. This habitat will be referred to as the "boulder habitat".

4.4.2 Stratum-area estimates

Individual stratum-area ranged from 0.3 to 7.1 ha (Table 4.1). The total shore length of habitats in the open-closed area was longer than in the closed-closed area but the habitats did not extend as far offshore, particularly in the case of the reef habitat. At all sites the urchin habitat tended to be wider than the algal habitat, except for two sites in the closed-closed area, i.e. SANTOS Tanks and Pt Lowly West, where the algal habitats were over 100 m wide (Table 4.1).

The total stratum-area closed to fishing in 1998 (closed-closed area) was estimated at 26.3 ha (43% of total) and that left open (open-closed and open-open areas) was estimated at 34.8 ha (remainder of total).

Table 4.1 Details of strata in each area, including habitat types present, shore length, habitat width and stratum-area, based on habitat transects completed in June 2001 (refer to Fig. 4.1 for Site locations).

| Strata | Site | Habitat | Shore length (m) | Habitat width (m) | Stratum -area (hectares \pm SD) | % of total |
|--------|---------------------------------|---------------|------------------|-------------------|-----------------------------------|------------|
| 1. | BHP Wall | Boulders wall | 558 | 6 | 0.3 \pm 0.0 | 0.5% |
| 2. | False Bay | Reef drop off | 598 | 31 | 1.9 \pm 0.7 | 3.1% |
| 3. | Black Point | Urchin | 972 | 73 | 7.1 \pm 1.7 | 11.6% |
| 4. | | Algal | | 27 | 2.6 \pm 1.1 | 4.2% |
| 5. | 3 rd Dip | Urchin | 879 | 63 | 5.6 \pm 1.0 | 9.1% |
| 6. | | Algal | | 24 | 2.1 \pm 0.9 | 3.5% |
| 7. | WOSBF | Urchin | 1290 | 43 | 5.6 \pm 0.6 | 9.2% |
| 8. | | Algal | | 45 | 5.8 \pm 0.3 | 9.6% |
| 9. | Stony Point | Urchin | 748 | 83 | 6.2 \pm 0.3 | 10.1% |
| 10. | | Algal | | 33 | 2.5 \pm 0.1 | 4.0% |
| 11. | SANTOS Jetty | Urchin | 281 | 65 | 1.8 \pm 0.2 | 3.0% |
| 12. | | Algal | | 13 | 0.4 \pm 0.1 | 0.6% |
| 13. | SANTOS Tanks | Urchin | 411 | 95 | 3.9 \pm 0.3 | 6.4% |
| 14. | | Algal | | 142 | 5.9 \pm 0.3 | 9.6% |
| 15. | Point Lowly West | Urchin | 318 | 69 | 2.1 \pm 0.6 | 3.5% |
| 16. | | Algal | | 102 | 3.2 \pm 0.5 | 5.3% |
| 17. | Point Lowly Light | Urchin | 281 | 49 | 1.4 \pm 0.1 | 2.2% |
| 18. | | Algal | | 26 | 0.7 \pm 0.4 | 1.2% |
| 19. | Point Lowly East | Reef drop off | 449 | 27 | 1.2 \pm 0.7 | 2.0% |
| 20. | Fitzgerald Bay | Reef drop off | 430 | 18 | 0.8 \pm 0.2 | 1.3% |
| 1-3-16 | TOTAL CLOSED-CLOSED AREA | | 2,316 | 605 | 26.3 \pm 1.0 | 43% |
| 2-8 | TOTAL OPEN-CLOSED AREA | | 3,740 | 187 | 30.7 \pm 2.7 | 47% |
| 17-20 | TOTAL OPEN-OPEN AREA | | 1,159 | 120 | 4.1 \pm 0.8 | 10% |

4.4.3 Density estimates

1998 data

There was a distinct temporal trend in the average density of cuttlefish in all sites and habitats surveyed in 1998 (Fig. 4.5). There were few cuttlefish present at any site until the last week of April. From then on the density increased, peaked by the end of May/early June and then gradually fell again until the end of August, when nearly all cuttlefish had left the area.

Cuttlefish were not evenly distributed over all strata, as some sites and habitats consistently supported higher densities than others (Fig. 4.5). In particular, more cuttlefish were found in the urchin than the algal habitat, at all sites and times sampled. The highest densities recorded were over 100 cuttlefish.100 m⁻² at the BHP Wall (Fig. 4.5*d*), whilst at the aggregation area the maximum density was 85 cuttlefish.100 m⁻².

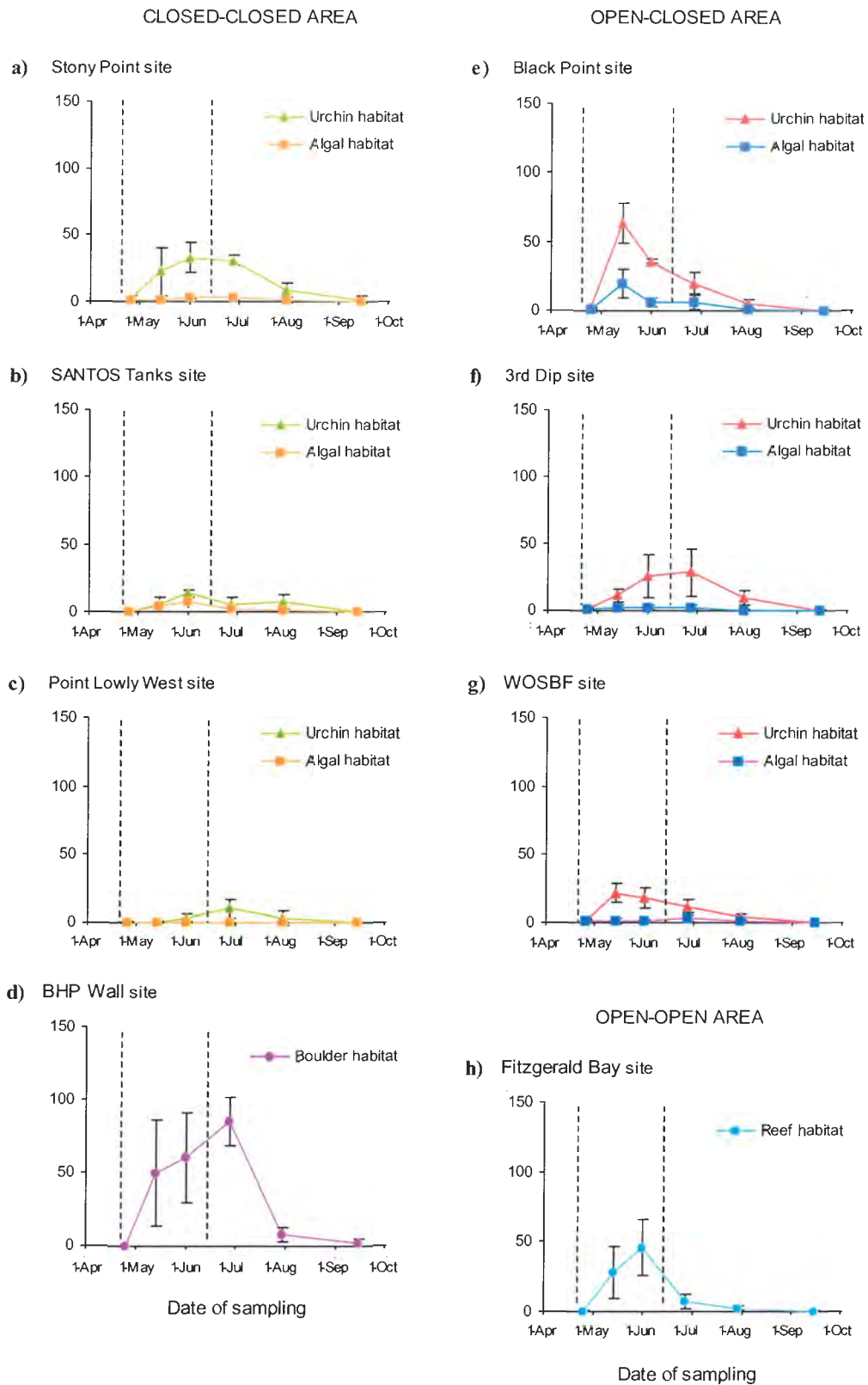


Figure 4.5 Average density of *S. apama* (cuttlefish.100 m⁻²) in each habitat at each site throughout the 1998 spawning season; closed-closed area (a-d), open-closed area (e-g) and open-open area (h). The dashed lines indicate the start and end of the fishing period in the areas originally open.



Figure 4.6 An example of the high density of *S. apama* in the urchin habitat at some sites. This photo was taken at the Black Point site in May 1999.

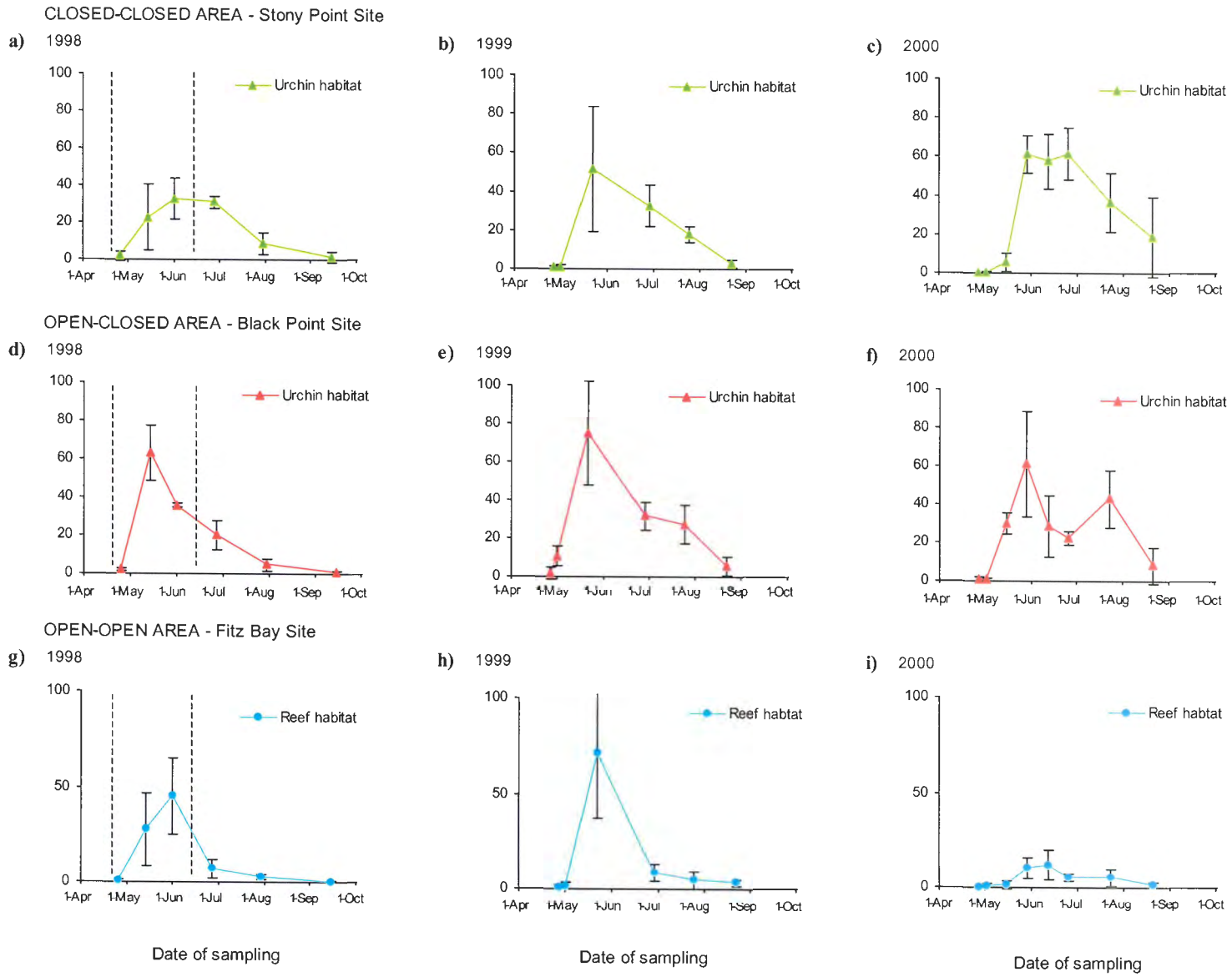
Stony Point was the only site in the closed-closed area that recorded an obvious increase in density during the peak period (Fig. 4.5a). Density rose gradually at the start of the season, stabilised and then gradually declined. At the BHP Wall, densities rose throughout the season until early July, reaching the highest density recorded at all sites, before crashing by early August.

In the open-closed area, Black Point recorded a higher peak density than any site in the closed-closed area (Fig. 4.5e) despite being subjected to the most intense fishing pressure (pers. obs.). However, after this, the densities decreased rapidly throughout the rest of the season. The same trend was recorded for the WOSBF site, although, the densities were lower than at Black Point (Fig. 4.5g). The temporal trend at the 3rd Dip (Fig. 4.5f) was more similar to that of Stony Point than the other two sites in the open-closed area. However, this site was exposed to lower fishing pressure than either of the other two open-closed sites (pers. obs.). Even though it was in the area left open to fishing, it was rarely fished. The open-open area in Fitzgerald Bay was also rarely fished, until the closure of the open-closed area later in the season, when a decrease in density was observed (Fig. 4.5h)

Temporal trends across all years

The temporal trends in densities were only determined for one stratum in each area in 1999 and 2000. These were: the urchin habitat at Black Point in the open-closed area; the urchin habitat at Stony Point in the closed-closed area; and the reef habitat at Fitzgerald Bay in the open-open area (Fig. 4.7).

Figure 4.7 Temporal variation in the average density of cuttlefish (cuttlefish, 100 m⁻²) at three sites sampled over three years (1999 to 2000): Stony Point in the closed-closed area (a-c), Black Point in the open-closed area (d-f) and Fitzgerald Bay in the open-open area (g-i).



In 1999, the densities at Black Point and Stony Point displayed a similar temporal trend when neither site was fished (Fig. 4.7*b,e*). At Black Point the density increased faster during the first two weeks of May and reached a higher level than in 1998 (a peak of 75 cuttlefish.100 m⁻²), followed by a steep decrease until July and a more gradual decline from then on. This temporal trend was similar to that of 1998 when the site was fished. At Stony Point the density also increased rapidly during May, reached a higher level than in 1998, and gradually declined throughout the remainder of the season. Densities at Fitzgerald Bay increased and then decreased rapidly, remaining low for the rest of the season (Fig. 4.7*h*). This site was still open to fishing in 1999.

In 2000, different temporal trends were evident for Black Point and Stony Point (Fig. 4.7*c,f*). The timing of the spawning season was later than in the two previous years, with cuttlefish not increasing in numbers until the second week of May, two weeks later than in 1998 and 1999. At Black Point there was a more gradual increase in numbers during May to a lower density than in 1999. Then there was a rapid decline in numbers during June followed by a second peak in July. At Stony Point numbers increased very rapidly during the last two weeks of May to a higher density than in 1999, and then remained high throughout June and slowly declined through July and August, such that there were still over 10 cuttlefish.100 m⁻² remaining in late August. Densities at Fitzgerald Bay were very low throughout the season in 2000 (Fig. 4.7*i*), which was once again open to fishing.

4.4.4 Statistical analysis - 1998 data

The results of the statistical analysis supported the graphical interpretation of density data presented above (Appendix I). The habitat within site effect was highly significant due to the large difference in counts between the algal and urchin habitats. The non-linearity for each habitat within each site also differed significantly, which indicated the temporal patterns in density differed between the algal and urchin habitats within each site. Numbers increased in the urchin habitats during the season but remained close to zero in all algal habitats throughout the season. The linearity was also significantly different within habitats between sites. This difference was evident in the graphical interpretation of the density data, where the temporal pattern in the urchin habitat at one site varied from that observed at another site. All of the above effects were significant at the $p = 0.0000$ level (Appendix I).

4.4.5 Abundance estimates

The estimates of total abundance increased from 88,634 in 1998 to over 170,000 in the following three years (1999 to 2001) (Table 4.2). Most of the increase in abundance after 1998 was in the open-closed area, which was fished for half of the season in 1998 but closed from then on. The abundances in the closed-closed area increased comparatively less over the same time period.

Table 4.2 Comparison of estimates of abundance for the four spawning seasons from 1998 to 2001.

| Area | Year | | | |
|-------------------------------------|---------------|----------------|----------------|----------------|
| | 1998 | 1999 | 2000 | 2001 |
| TOTAL ORIGINAL CLOSED AREA | 33,064 | 42,381 | 47,413 | 53,628 |
| ± SD | ± 7,612 | ± 20,430 | ± 7,401 | ± 10,275 |
| % error of estimate | 23% | 48% | 16% | 19% |
| TOTAL ORIGINAL FISHED AREA | 55,570 | 140,260 | 123,692 | 123,534 |
| ± SD | ± 11,685 | ± 27,704 | ± 35,747 | ± 18,679 |
| % error of estimate | 21% | 20% | 29% | 15% |
| TOTAL Whole aggregation area | 88,634 | 182,642 | 171,106 | 177,161 |
| ± SD | ± 13,945 | ± 34,422 | ± 36,505 | ± 21,318 |
| % error of estimate | 16% | 19% | 21% | 12% |

4.4.6 Length and weight estimates

The mean difference between estimated and actual ML ranged between -19.5 mm to +7 mm (Fig. 4.8). Lengths were underestimated more often than overestimated, with consistent overestimation only occurring in June 2001. The mean difference was significant in most cases (paired t-tests; p values 0.0000 to 0.2317; Fig. 4.8); therefore, all ML values estimated underwater were corrected by the corresponding mean difference before being used for any further analysis.

The TWt of each cuttlefish collected from the aggregation area and NSG samples was plotted against ML for both sexes in each year (Fig. 4.9). Individuals collected from the aggregation area later in the spawning season were not included, so as to avoid potential confounding associated with a decline in condition toward the end of the season. Both variables were log-transformed to obtain the linear regression relationship for each sex in each year (Fig. 4.10), which were compared between years using an analysis of covariance (ANCOVA). For both sexes the log-linear relationships were significantly different between years. Therefore, different relationships were used to calculate the annual estimate of biomass in each year.

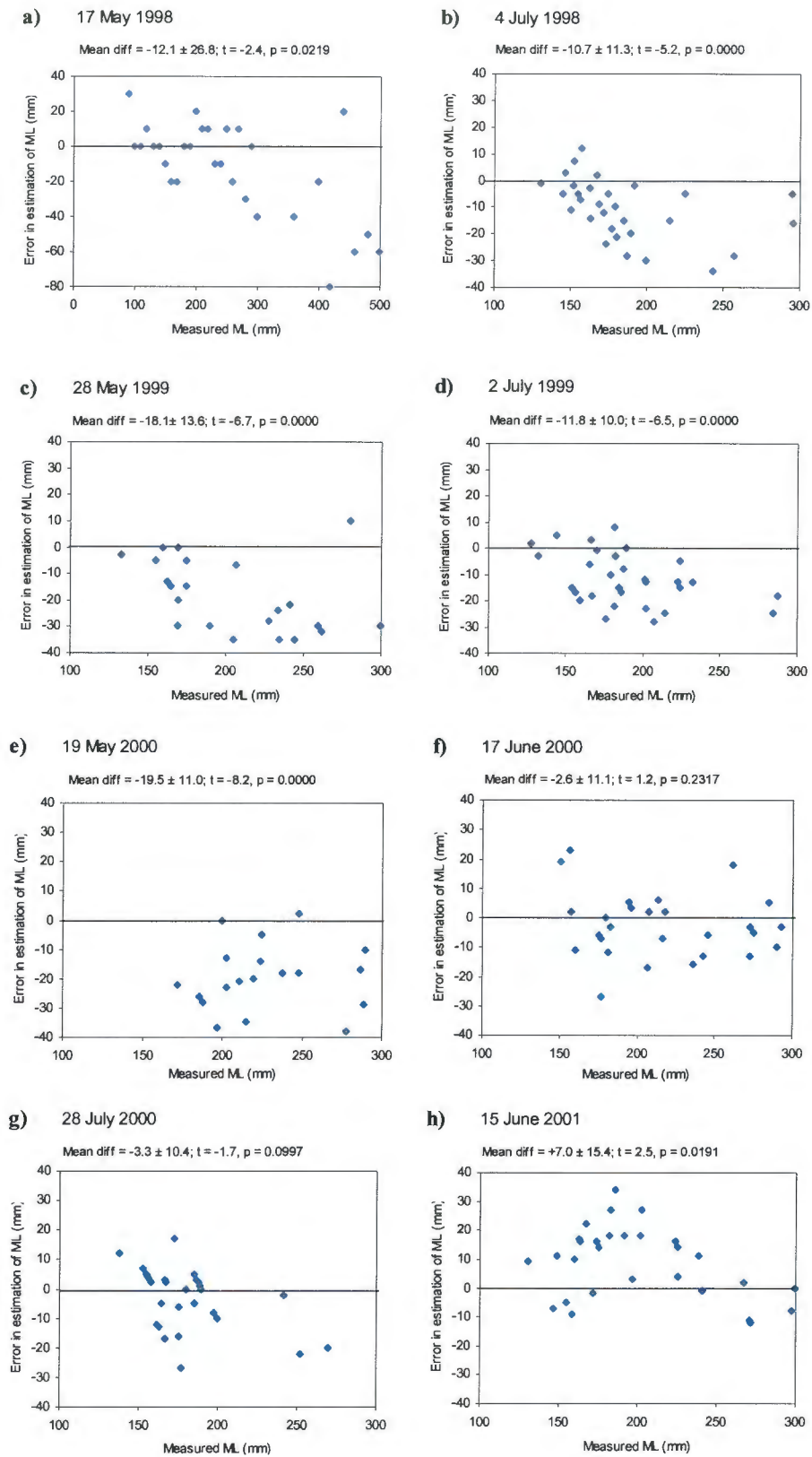


Figure 4.7 Residual plots of error in the estimation of ML underwater on various sampling dates in 1998 to 2001; paired t-test results indicated above each graph.

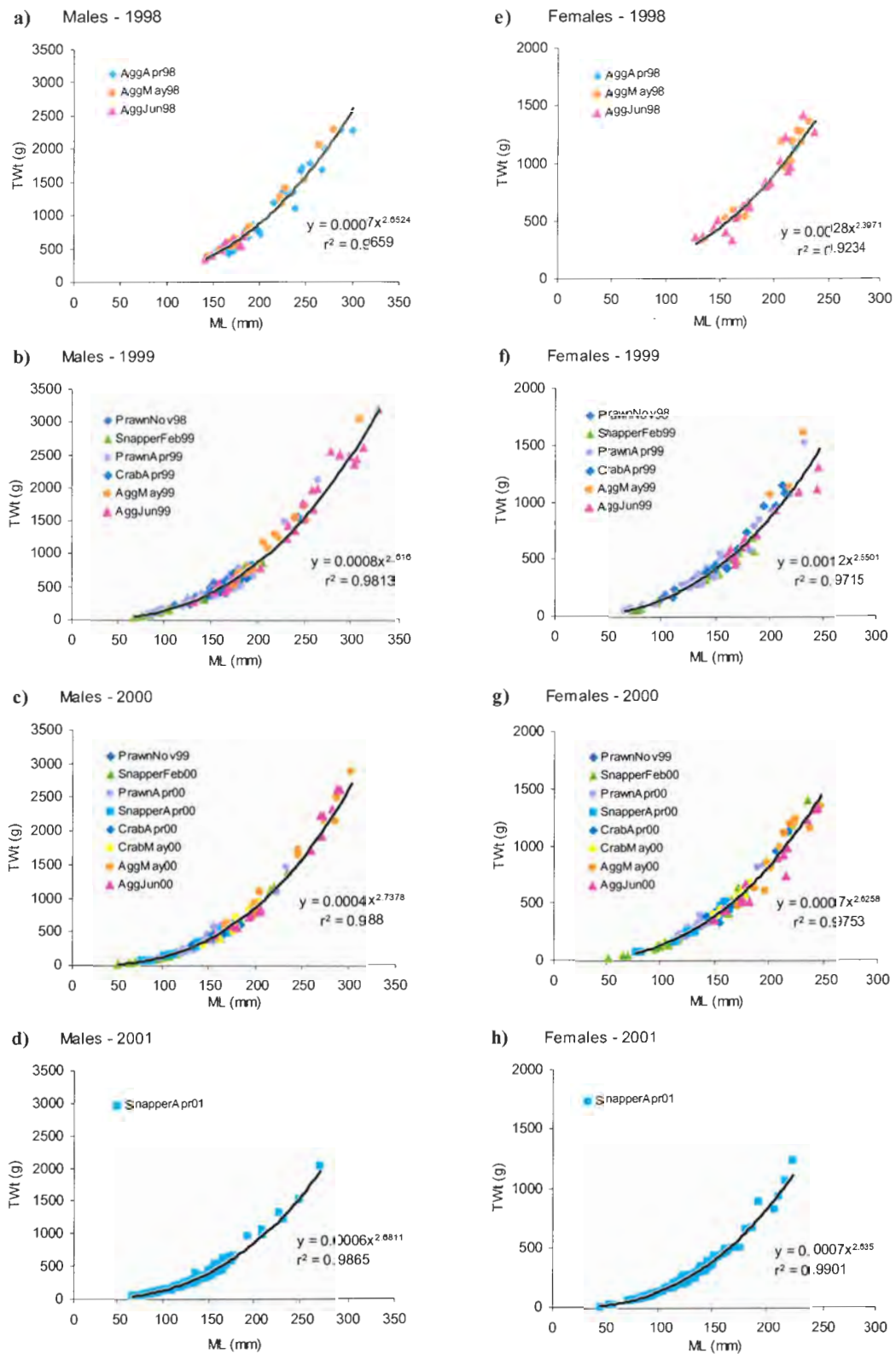


Figure 4.8 Annual length-weight relationships for male (a-d) and female (e-h) *S. apama*, constructed from NSG and aggregation samples from early in the spawning season (i.e. April to June).

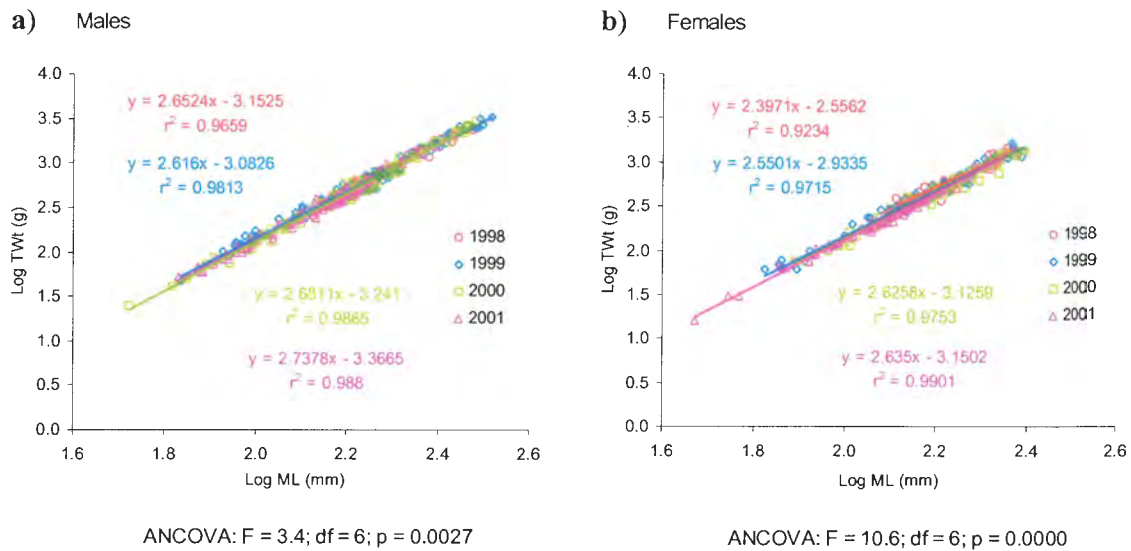


Figure 4.9 Comparison of annual linear length-weight relationships for male (a) and female (b) *S. apama*; ANCOVA results indicated below each graph.

4.4.7 Biomass estimates

The spatial distribution of biomass within the aggregation area varied over the four years of sampling (Fig. 4.11). In 1998, the fishery catch accounted for approximately 50% of the total biomass, despite the open-closed area only being open to fishing for half the season. Fishery catch was negligible in each of 1999 to 2001, due to the main fishery closure. The catch from Block 21 was still incorporated in total estimates in those years as fishing was still observed in the open-open area.

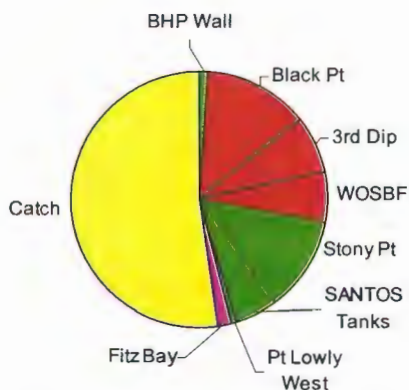
The estimated biomass in the closed-closed area (Fig. 4.11) changed little between seasons, accounting for only 19 to 28% of the total. Only one site, i.e. Stony Point, consistently showed a substantial biomass. Following the closure of the adjacent open-closed area in 1999, biomass increased at Stony Point from 25 t in 1998 to 42 t and remained high in the following two years (Fig. 4.11; Table 4.3).

The estimate of biomass in the open-closed area (Fig. 4.11) increased by 150% from 59 t in 1998 to 167 t in 1999 when fishing was not allowed (Table 4.3). The increase in biomass was consistent across all sites (Fig. 4.11). The spatial distribution of biomass within the area changed little after 1999. One change to note, however, was the variation in biomass at Black Point, where biomass increased from 28 t in 1998 to 69 t in 1999, but was lower at 43 t in 2000 and 47 t in 2001. In contrast, the biomass at all other sites in the open-closed area either remained constant or increased since 1999. Prior to this,

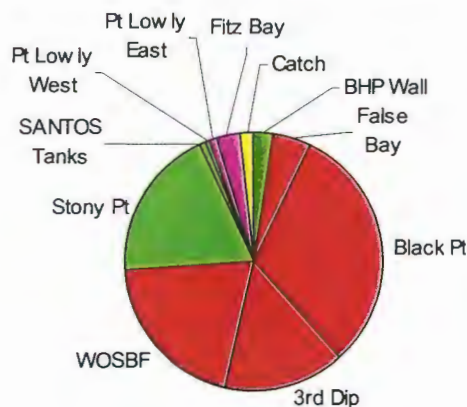
the site recorded the highest densities for the area even when open to fishing, despite generally being the most heavily fished site.

The estimates of biomass for the sites in the open-open area (Fig. 4.11) initially increased in 1999 following the closure of the other areas to fishing, but declined markedly in 2000 and 2001, when they were the only ones left open to fishing. Note the biomass for these sites were included in the totals for the original fished area in Table 4.3.

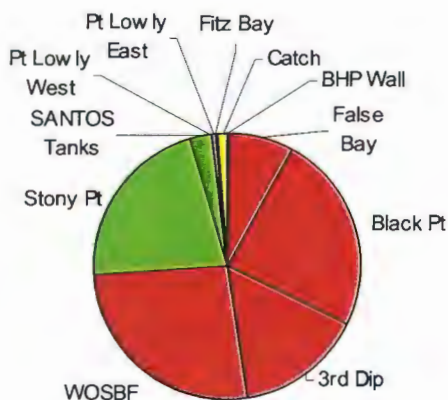
a) 1998 – 207 tonnes



b) 1999 – 222 tonnes



c) 2000 – 181 tonnes



d) 2001 – 184 tonnes

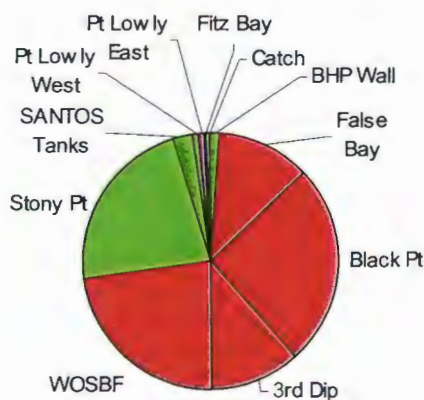


Figure 4.10 Breakdown of the annual estimates of total biomass according to individual sites monitored and amount removed as catch at the end of May in each year (1998 to 2001). Closed-closed area sites are indicated in green, open-closed area sites in red, open-open area sites in pink and the fishery catch in yellow. NB: False Bay and Point Lowly East were not sampled in 1998.

Although sampling the aggregation area did not start until after 1997, the total commercial catch in the area in that year was 235 t. The estimates of total biomass obtained for the whole aggregation area between 1998 and 2001 (Table 4.3) suggest that the biomass was 18% lower in 2000, and remained at that lower level in 2001. Nevertheless, these estimates are bounded by relatively high estimates of uncertainty (95% confidence limits of ± 29 to 44%; 1.96 SD) (Table 4.3).

Table 4.3 Comparison of estimates of biomass and fishery catch for the last four spawning seasons (1998 to 2001).

| Area | Year | | | | |
|--|------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|
| | 1997 | 1998 | 1999 | 2000 | 2001 |
| TOTAL ORIGINAL CLOSED AREA \pm SD | | 39 \pm 10 | 51 \pm 26 | 48 \pm 6 | 51 \pm 11 |
| % error of estimate | | 25% | 51% | 14% | 21% |
| TOTAL ORIGINAL FISHED AREA \pm SD | | 59 \pm 14 | 167 \pm 33 | 134 \pm 40 | 132 \pm 25 |
| % error of estimate | | 24% | 20% | 29% | 19% |
| TOTAL Whole aggregation area \pm SD | | 98 \pm 17 | 218 \pm 42 | 179 \pm 40 | 183 \pm 27 |
| % error of estimate | | 17% | 19% | 22% | 15% |
| CUMULATIVE CATCH (Block 21) Biomass removed from FISHED AREA | 235 | 109 | 4* | 2* | 1* |
| GRAND TOTAL Whole aggregation area | | 207 | 222 | 181 | 184 |

* Amount removed as catch from Block 21 in 1999 to 2001, potentially from sites in the open-open area.

4.4.8 Uncertainty estimates

The formulae used to estimate total abundance and biomass rely on direct multiplication of the average density obtained from count data by the estimate of the stratum-area. Therefore, any biases or error in the methods used to estimate density or stratum-area would translate directly into the estimates of biomass or abundance. However, the catch values used in the construction of the total estimates of biomass (Table 4.3) are fixed with an unknown level of uncertainty and hence not subject to these variations. Therefore, the estimates of biomass in 1999 to 2001 are more labile in relation to errors or biases associated with the methods used than that of 1998, which has a large catch component, and obviously the catch of 1997. As such, if the methods used overestimated either the densities or stratum-area, a more significant reduction in the total biomass in 1999 to 2001 would be masked in the current results.

4.4.9 Statistical analysis - annual data

The statistical analysis of annual trends used the raw count data rather than the annual estimates of abundance or biomass due to the complex error structures inherent in the latter. The analysis compared counts at the single peak sampling time between years (1998 to 2000) for the same combination of areas, sites and habitats as that used for the 1998 data (Appendix 1). The extra sites incorporated in the stratified random sampling design after 1998, i.e. False Bay, BHP Wall and all those in the open-open area, were not included due to their absence from the 1998 data set.

The results indicated no significant difference in distribution of cuttlefish between the open-closed area and the closed-closed area between years ($p = 0.1067$). This was an unexpected result, which at least in 1998 may relate to movement of cuttlefish from the closed-closed area to open-closed area, when the latter was fished. Lower counts were recorded at all sites in the closed-closed area in that year compared to the other two years, even though the area was not fished. Thus, a similar pattern of increase was observed across both areas after fishing ceased, but appeared more pronounced in the open-closed area. There was also no significant difference between years within a site, i.e. a similar distribution pattern between sites was observed over all years. However, there was highly significant variation between sites within years and between the two habitats within sites, which supported the results of the analysis of the 1998 data.

4.4.10 Movement studies

Two types of movement were documented for cuttlefish tagged at the BHP Wall. Between 7 and 15 tagged individuals (mean = 11.3; SE = 2.4; 5% of total tagged; Table 4.4) were resighted in the proximity of the wall up to 6 weeks after tagging, with some individuals resighted more than once. Thus, some individuals including both large and small males, stayed within the wall area. Only one female was resighted at the BHP wall after 6 weeks. There was no significant difference between the sexes in the proportion of tagged individuals that were resighted ($\chi^2 = 1.29$; $p = 0.2559$), probably due to the low number of females that were tagged relative to males.

Five tagged cuttlefish were resighted at the main aggregation area located 15 to 18 km from the BHP Wall (Table 4.5). Three were small males, which were resighted at Black Point and 3rd Dip just 6 to 8 days after tagging. One large male was resighted at Black Point after 4 weeks and a large female at Stony Point after 6 weeks. One small male was recaptured at the Whyalla jetty adjacent to the BHP Wall site by a recreational jig fisher almost 9 weeks after tagging, the longest recorded period at large.

Although the number of re-sightings was low, the tagging study indicated that individual cuttlefish spent up to 6 weeks within the aggregation area, and possibly even longer. Females and large males were also included within those animals sighted 6 weeks after tagging. Therefore, it appears individual cuttlefish were resident in the aggregation area for a large proportion of the spawning season. However, it was also evident that some moved considerable distances within the aggregation area, although it was not possible to determine if there was an overall net direction of movement.

Table 4.4 Results from cuttlefish tagged and resighted at the BHP Wall. The left side of the table shows the number of both sexes tagged on the nominated date. Data on the right show the number resighted on the indicated date.

| Sex | Number tagged | | Number resighted | | | |
|---------------|---------------|---------|------------------|--------------------------|-------------|-------------|
| | 17-18 May | 15 June | 2 Jun 2000 | 15 Jun 2000 | 19 Jun 2000 | 28 Jun 2000 |
| Males | 178 | | 13 | 7 | 10 | 4 |
| Females | 19 | | | | | 1 |
| Males | | 0 | | | | |
| Females | | 35 | | | 5 | |
| Totals | | | 13 | 7 | 15 | 8 |
| | | | | Mean = 11.3 ± 4.4 | | |

Table 4.5 Results for cuttlefish tagged at the BHP Wall and then resighted elsewhere.

| Tag No. | Sex | ML (mm) | Date resighted | Days after tagging | Site |
|---------|-----|---------|----------------|--------------------|---------------------|
| C021 | M | 176 | 24 May 2000 | 6 | Black Point |
| B205 | M | 183 | 24 May 2000 | 7 | Black Point |
| B195 | M | 205 | 25 May 2000 | 8 | 3 rd Dip |
| C054 | M | 262 | 16 Jun 2000 | 29 | Black Point |
| C001 | F | 243 | 27 Jun 2000 | 40 | Stony Point |
| B303 | M | 201 | 18 July 2000 | 61 | Whyalla Jetty |

4.4.11 Abundance and distribution of the non-spawning population

Due to the variation in sampling design used for surveys of the non-spawning NSG population between years it is difficult to compare the distribution of relative abundances of cuttlefish caught with the otter trawl gear used for juvenile snapper surveys. However, some trends are apparent (Fig. 4.12). The numbers caught in April (mean = 1.54 ± 0.14 and 1.61 ± 0.19 per trawl) were generally greater than those in February (mean = 0.46 ± 0.10 and 0.55 ± 0.18 per trawl). However, there was only one year in which both months were sampled for direct comparison (2000), and the coverage of stations was more extensive on both April surveys (122 and 127 in April compared to 79 and 33 in February). Cuttlefish were patchily distributed, such that stations in some areas consistently had high numbers, whereas those in other areas recorded none or a single individual. Areas that had higher cuttlefish numbers were within the channel areas south and south-west of Point Lowly between $33^{\circ}00'S$ and $33^{\circ}15'S$ and within the channel areas east of Middle Bank in the centre of the Gulf between $33^{\circ}15'S$ and $33^{\circ}45'S$. Very few cuttlefish were caught north of Point Lowly, other than in the immediate vicinity of the Point.

At non-spawning times cuttlefish were more sparsely distributed throughout northern Spencer Gulf than in the aggregation area during the spawning season. The mean density of cuttlefish determined from swept-area calculations, was less than 0.036 ± 0.004 cuttlefish. $100m^{-2}$, even when the trawl net was assumed to capture only 50% of the cuttlefish present. The maximum number of cuttlefish caught in a single trawl was 13, which equated to a density of 0.29 cuttlefish. $100m^{-2}$, compared with the maximum density of 85 cuttlefish. $100m^{-2}$ in the aggregation area. The recapture of one small male (185 mm ML) at the main aggregation area on 29 July 2000, 3.5 months after it was tagged near Plank Shoal (Fig 4.12d), approximately 65 km south of the aggregation area, on 13 April 2000 indicated a connection between the wider NSG population and the spawning aggregation population.

4.5 Discussion

The spawning aggregation of *S. apama* in northern Spencer Gulf was highly localised both spatially and temporally. There was a distinct spawning season from May to August, with a consistent peak in numbers at the end of May/early June. Outside this period, densities of cuttlefish in the aggregation area were less than 1 cuttlefish. $100m^{-2}$, indicating that the cuttlefish were not residents but migrated to the area from elsewhere. Over 170,000 animals were estimated at the area each year when fishing did not occur. Due to the small area of reef this resulted in very high densities of up to 85 cuttlefish. $100m^{-2}$ during the peak of the season.

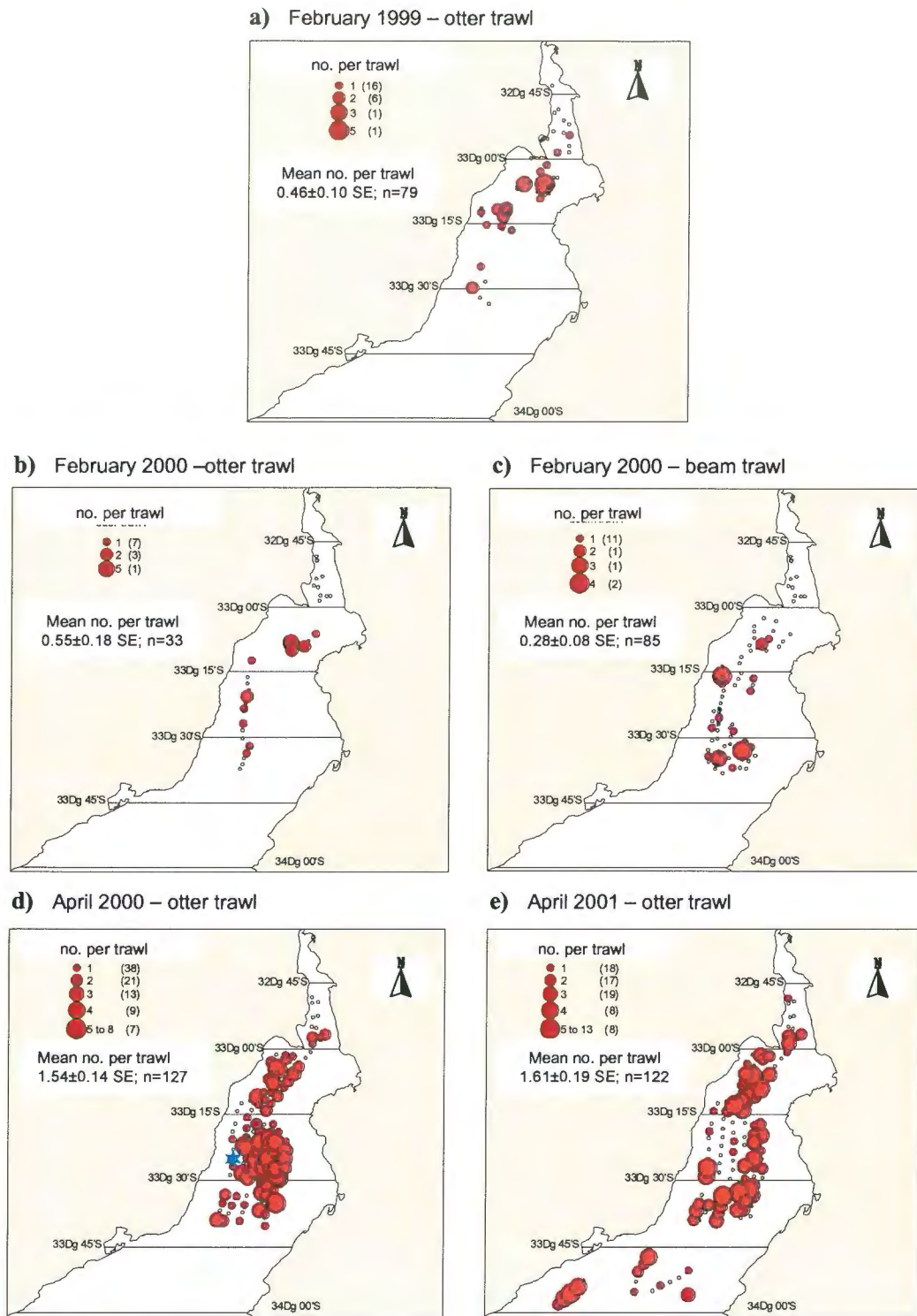


Figure 4.11 Abundance distribution of *S. apama* in northern Spencer Gulf at non-spawning times sampled by juvenile snapper trawling. Open circles indicate stations that were sampled but no cuttlefish were caught. ★ Indicates the tagging location of the tagged cuttlefish that was recaptured at the aggregation area in July 2000.

The spawning aggregation of *S. apama* is unique. Although other *Sepia* species concentrate in inshore waters or bays to spawn, many of which support large commercial fisheries (e.g. *S. officinalis hierredda* off the north-west African coast; Bakhayokho 1991), they generally form pairs or small groups over quite extensive areas rather than localised dense aggregations (Corner and Moore 1980; Hanlon and Messenger 1988; Gutsal 1989; Norman 2000). The densities observed in this study were far higher than any previously reported in the literature for a *Sepia* species. It is also the only aggregation known for *S. apama* that reaches such densities. Elsewhere through the broad distribution of the species, only less concentrated spawning occurs in most areas of rocky reef. For example, Rowlings (1994) reported up to 13 *S. apama* over an area of 500 m² of rocky reef habitat, which equates to a density of 2.67 cuttlefish.100m⁻² at Edithburgh in the Gulf St. Vincent, South Australia.

It is unknown why *S. apama* aggregate in such high densities at the one specific location, but may relate to the need to attach their eggs to hard substrate (Hall 1998; Norman et al. 1999). There is little other rocky habitat in northern Spencer Gulf (Gostin et al. 1984; Edyvane 1999), so it may attract high densities of cuttlefish by default due to its isolated location, with cuttlefish forced to aggregate to have access to egg-laying sites. The Black Point area provides large slabs of broken bedrock that are clearly suitable for egg deposition. The fact that high densities of cuttlefish were also recorded over an artificial rocky habitat in the area (BHP Wall), suggests that habitat limitation may be a factor.

Two migration hypotheses could account for the exceptionally high densities at Black Point. The first would involve individuals "homing" to their original spawning site. If survival of individuals spawned in the aggregation area was higher than elsewhere, then the density may have gradually increased over time. This hypothesis implies migration is directed rather than passive and separate spawning populations may, in general, be reproductively isolated. Tag recovery studies of *S. officinalis* in the English Channel showed evidence of homing, as most individuals returned to spawn at the site where they were hatched (Boucaud-Camou and Boismery 1991).

The alternative hypothesis involves cuttlefish migrating in either a set direction or indiscriminately until an appropriate spawning area is encountered. Under this hypothesis, individuals might either gradually migrate through a number of spawning sites during the spawning season or stop once reef is encountered. The Point Lowly peninsular projects into the Gulf and effectively separates the northern reaches from the wider central section, and thus may intercept migrating cuttlefish. This hypothesis, however, seems inconsistent with why so many individuals reach the aggregation area at the top of the Gulf when many areas of rocky reef further south detain far fewer individuals (pers. obs.).

The geographic region from which cuttlefish migrate and the distances covered by individual cuttlefish to reach the spawning aggregation area are unknown. The one return from tagging in April 2000 indicated that they can migrate from at least 65 km south of the aggregation area. The higher numbers of cuttlefish caught in the juvenile snapper surveys in April compared to February, may represent an influx to the top of the Gulf prior to the spawning season, suggesting a northwards migration. Furthermore, the higher cuttlefish densities in the channels of the Gulf suggest that the cuttlefish may migrate northward within these. Alternatively, the increased numbers of cuttlefish in the NSG samples collected in April may have resulted from the arrival of new recruits from juvenile feeding grounds, that had originated in the previous spawning season. The whereabouts of juveniles subsequent to hatching at the aggregation area also remain unknown. Clearly, the migration of *S. apama* throughout their lives is a topic for further research.

The winter spawning period of *S. apama* was also unusual, and it remains the only species known to have a peak spawning time during winter. Most cuttlefish species migrate inshore to spawn during the spring or summer months (Table 1.1). The reasons for the difference are unknown, but may relate to the large size of *S. apama* and the timing of hatching to coincide with favourable growth conditions. The large eggs take a long time to develop over the cold winter months but juveniles hatch just as water temperature increases in spring. Large swarms of mysids, which are a common food source for many juvenile *Sepia* species (Hanlon and Messenger 1988), also appear around this time over rocky reef areas (pers. obs.). Hence, hatching coincides with an influx of juvenile food and warming temperatures providing for optimal juvenile growth rates.

The influx of animals to the aggregation area occurred relatively quickly and the timing varied by only one to two weeks between years. The factors that control or regulate the timing of the inshore migration are unknown. Light (photoperiod), temperature, water density and salinity have been suggested as environmental factors that influence the migration of other cephalopod species (Hanlon and Messenger 1996). The migration of the common cuttlefish *S. officinalis* in the English Channel was related to temperature, for the migration of juveniles and sub-adults away from the inshore waters in autumn, and internal factors which regulate the processes of genital maturation and reproduction, for the more rapid inshore migration of mature adults in spring for spawning (Boucaud-Camou and Boismery 1991). The variation in intensity and photoperiod of light reaching the optic gland of *S. officinalis* is thought to regulate the onset of sexual maturation and start of spawning through the endocrine system (Richard 1971, cited in Mangold et al 1975).

Once at the spawning aggregation area, cuttlefish were not distributed evenly. Some strata consistently had higher densities than others. In particular, the urchin habitat in all sites in the original fished area consistently had higher densities of cuttlefish, even when open to fishing. This resulted in a higher overall abundance and biomass in the original fished area compared to the closed area, even though the estimated stratum-areas for each were fairly similar. It was assumed that if approximately half the available habitat was closed to fishing, approximately half the population would be protected. But this was obviously not the case.

Furthermore, the tagging study in 2000 clearly demonstrated that cuttlefish were capable of redistributing themselves within the aggregation area and moving from the closed to the fished area. Therefore, the lower biomass and abundance in the closed-closed area in 1998, compared to later years, may have resulted from animals moving from the closed area to replace those removed from the fished area. Fishing along the edge of the closure was high during the 32 days of fishing in 1998 (pers. obs.). Thus, only a small proportion of the spawning biomass was protected by the closure. This highlights the potential pitfalls involved in introducing spatial or temporal closures without understanding the distribution and behaviour of the resource being protected (Kramer and Chapman 1999).

The method used to estimate total abundance and biomass assumed that there was not a constant turnover of animals in the aggregation area. If there was such a turnover, because the cuttlefish had short residence times, the method would grossly underestimate the total number of cuttlefish that visited the area during the spawning season. In such a case, an area-under-the-curve method that accounted for the residence times of individual fish would be necessary, similar to the methods used for spawning aggregations of salmon in Canada (e.g. English et al. 1992; Hilborn et al. 1999). However, there was a distinct peak in numbers around the end of May in all sites and years and the results from tagging indicated that at least some cuttlefish remained within the aggregation area for most of the spawning season. Furthermore, the condition of many cuttlefish declined noticeably through the season, suggesting that they had been present for a long time. The tagging study was limited because it could not verify whether those individuals that were not resighted in the aggregation area had left nor whether those resighted had moved in and out of the area between sightings. Further studies using radio-telemetry technology would help resolve the questions regarding residence times (cf. Zeller 1998).

The underwater visual transect method proved successful for estimating cuttlefish densities. The various biases and limitations of this technique have been previously discussed with respect to estimating the sizes and composition of reef fish populations (e.g. Sale and Sharp 1983). However,

certain ecological and behavioural features of *S. apama* in the aggregation area made this technique particularly suitable for this study and eliminated a number of usual potential biases. The animals were relatively large in comparison with some reef fish, were conspicuous when involved in reproductive behaviours, and tended to ignore the diver or remained stationary. Most were out in the open, as only a small proportion of females and small males hid under rocks, and even then the habitat allowed most potential hiding places to be searched. Potential variation that would eventuate using different observers was avoided by using the same observer for all surveys. Some bias may have resulted from estimating the transect width. Despite its suitability here, the potential application of this method to other coastal cephalopod fisheries may be limited, due to the difficulty in sampling large areas and ensuring adequate coverage. The area to be sampled in this study was relatively small and discrete.

The uncertainties associated with estimating the stratum-areas were quite large (up to 57% of the stratum-area estimate) and more than doubled the uncertainties had only sampling errors been considered. Refining the stratum-area estimates could decrease the level of uncertainty in the biomass and abundance estimates. Alternatively, a larger number of transects could be completed within each stratum, with the number either proportionally or optimally allocated according to stratum size or variance. The initial sampling strategy used to monitor the spawning aggregation was designed to meet a number of different objectives using the one set of data, including comparative analyses. Hence, a simple random stratified sampling design was used. However, to increase precision in estimates of total abundance derived from multiple strata, stratified sampling with optimal or proportional allocation of sampling units is often more appropriate (Andrew and Mapstone 1987).

Fishing in the aggregation area caused an obvious within-season decline in the level of total abundance and biomass remaining in the area. In 1999, when fishing did not occur in the aggregation area there was a vast increase (by 150%) in the abundance and biomass in the previously fished area. However, the results were less conclusive with respect to the possible long-term effects on the spawning population caused by the large catches removed in 1996 to 1998. Monitoring in the area did not commence until 1998. Therefore, the only information available to indicate the level of biomass before 1998 was the commercial catch. These two data sets do not provide estimates of biomass that are readily comparable as they were derived in different ways. Furthermore, the commercial catch prior to 1997 is unlikely to reflect biomass levels, as there was little effort in the fishery before then. Therefore, there is no knowledge of what the virgin biomass levels were prior to and including 1997.

The conservative interpretation of the data available, even considering the high uncertainty in the biomass estimates, is that there has been a decrease in the population biomass between 1997 and 2001.

The estimates of biomass in 2000 and 2001 represent only 77-78% of the total catch in 1997, and obviously the biomass present in 1997 would have exceeded the catch. Whether this decrease represents the direct result of fishing or a natural fluctuation in population size due to variable recruitment remains to be determined. There are very few documented cases in the literature of declines in cephalopod populations as a result of fishing, due to the difficulty in separating the relative effects of natural variation and potential declines from fishing (Rodhouse 2001). Even the apparently definitive case of the declines in *Todarodes pacificus* in Japanese waters have become doubtful in the light of the recent rapid recovery of population abundances to previous levels (Sakurai 2000).

5 Sex and size composition

K.C. Hall

5.1 Introduction

The three characteristics commonly used to describe individuals within a population are sex, size and age. The relative frequencies of these determine the population composition. Many life history traits, which may be averaged over an entire population to describe general patterns, often vary significantly with one or more of the above variables. For example, individual fecundity or growth rates may be sex-, size- or age-specific. Therefore, the composition of a population and its variation over time will influence the estimation and interpretation of population characteristics and processes such as total fecundity. Furthermore, the population at any given time or location is related to the original composition of recruits, and subsequent changes caused by the life history traits of individuals (e.g. life cycle, growth rates and life span) and population processes (e.g. patterns of migration or mortality). Therefore, an analysis of population composition may provide a valuable insight into the general life history traits and population processes affecting it.

In most multicellular animals that undergo sexual reproduction, there are two separate gamete-producing morphs or sexes, with sex usually determined prior to birth or hatching. There are many different phenotypic or genetic mechanisms that control sex determination within a species (White 1973; Mittoch 1996; Werren and Beukenboom 1998), and ultimately the sex ratio at birth. Environmental or physiological control over sex determination, common in many reptiles and insects, may result in large fluctuations in initial sex ratio (Mittoch 1996). Alternatively, genetic sex determination via chromosomal control, that is common in vertebrates, is generally thought to produce an initial sex ratio close to unity due to the constraints of the Mendelian processes of meiosis and individual-based frequency dependent selection (Fisher 1930; Sheldon 1998). However, recent sex ratio studies have indicated that even in these cases unequal sex ratios at birth may arise through differential sex allocation of parental investment pre- or post-fertilisation (Godfray and Werren 1996; Sheldon 1998). The sex determination mechanisms for cephalopods are currently unknown, but sex differentiation of the gonad is usually present prior to hatching (Mangold 1987) and initial sex ratios approximating unity are generally assumed for most species.

Many other factors may (further) bias the sex ratio toward one sex over time, including: (1) differential survivorship or longevity between the sexes; (2) sexually selective removal by fishing operations; and (3) asynchronous spatial or temporal distribution of sexes. Sex ratios influence the potential reproductive output of a population, which is usually related to the number of females rather than males. Females generally produce relatively few large expensive gametes, whereas males produce numerous small inexpensive ones. Therefore, one male is usually capable of fertilising the eggs of many females. This means that the potential number of offspring is limited by the number of adult females.

The size of an individual is usually easier to measure than its age, which can often be difficult or impossible to determine. Therefore, in many studies the size composition of a population is determined and then age and growth are inferred. The size and age composition of a population can be strongly influenced by the life history strategy of a species. Most cephalopods have an estimated life span of less than 1 year and a single spawning period near the end of their life cycle. Combined with a short discrete annual spawning period, this life history strategy would be expected to produce a population consisting of a single year class of similarly-sized individuals (Boyle and Boletzky 1996). Obviously, the situation is more complex if the spawning season is protracted and encompasses a wide range of water temperatures, or if spawning occurs year-round with multiple peaks. In these instances, a broader size distribution with multiple size and age classes would be expected (Forsythe 1993). *Sepia apama* in the northern Spencer Gulf has a relatively short discrete spawning period (Chapter 4), and is thought to be semelparous (Lu 1998b); therefore, a life span of less than 12 months would be expected to produce a narrow size distribution with one distinct size mode. The size and sex composition of *S. apama* at the aggregation area and in the wider northern Spencer Gulf were assessed in the context of these expected characteristics.

5.2 Aims

The specific aims of this Chapter were:

- (1) to determine the sex and size composition of the spawning aggregation population and the wider NSG population;
- (2) to use the sex composition data to determine the proportion of the spawning biomass contributing to egg production each year; and
- (3) to interpret the size composition data in terms of the current life history model of *S. apama*.

5.3 Methods

The methods used to sample the aggregation area and NSG populations were outlined in Chapter 3. For the aggregation area, length and sex ratio data from the underwater visual transects were used, as these had larger sample sizes and were considered more representative of the population than samples collected using the hand net while snorkelling. Sex was determined underwater with 100% accuracy. Lengths were estimated to within ± 19.5 mm (Chapter 4), so corrected lengths were grouped according to broad 5 mm size intervals to reflect this level of accuracy. All NSG samples collected outside the aggregation area were obtained using trawls, and the individuals were later measured in the laboratory to within ± 1 mm. The broad size intervals of 5 mm were also used for these data for consistency. The size distributions and sex ratios of the spawning aggregation were compared to those throughout NSG at non-spawning times. Evidence to support a link between the wider NSG samples and the aggregation samples was provided by a single cuttlefish that was tagged at Plank Shoal in April 2000 and recaptured at Black Point in July 2000 (Chapter 4).

A single sample of the commercial fishery catch was obtained from one fish processing plant in Adelaide on 27 May 1999. The catch had been packed in 50 L bins and was transported fresh overnight from Whyalla. Two randomly selected bins were sampled. Each cuttlefish was sexed externally by the presence or absence of the sperm receptacle in the buccal region and the dorsal mantle length was measured to within ± 1 mm. As the main aggregation area was closed to fishing during 1999, a jig sample from the Black Point site was taken at the end of each field-sampling trip (Chapter 3).

Chi-squared (χ^2) tests with Yates correction for continuity (i.e. $df = 1$) were used to test the null hypothesis that sample sex ratios did not differ from unity (Zar 1999). Samples within years were pooled and heterogeneity χ^2 analyses used to test the null hypothesis that all samples were from a homogenous population, i.e. that sex ratios did not differ across samples. As recommended by Zar (1999), χ^2 values without Yates correction were used for these latter tests. A 2x2 contingency table and χ^2 with Yates correction were used to test whether the sex ratio of the commercial catch sample in May 1998 was different to that recorded on transects at the aggregation area at around the same time.

5.4 Results

5.4.1 Sex ratios

The sex ratios of the spawning population were significantly different from unity at all sampling times, and at both sites (Table 5.1). Similar results were obtained for both sites, even though one was in the open-closed area that was fished in 1998 and the other was in the closed-closed area. The males outnumbered females in all cases, with pooled ratios of from 3.6:1 to 6.3:1 (Table 5.1). The sex ratios varied significantly throughout each season (significant heterogeneity χ^2 tests; Table 5.1), becoming less biased towards males at the end of the season ($\chi^2 < 20$; $p = 0.0001-0.0216$; Table 5.1). The sex ratio at the start of the season in 1999 was highly biased toward males (17.5M:1F), which suggested males moved into the aggregation area earlier than females. However, only on one occasion was the density of cuttlefish sufficient at the start of the season to obtain a sex ratio estimate, and even then the sample size was small ($n = 37$), so the consistency of this trend across years could not be verified.

Some females were hidden under rocks while laying eggs in tight crevices, and hence were not readily visible on transects on first inspection. However, at the aggregation area there were few females that were not accompanied by a male (Chapter 9), and the presence of a female under a rock was usually evident by a stationary male at the entrance. Careful searching of rocks near such males usually revealed the presence of a female. On some occasions a female could not be located near a stationary male, despite him persistently returning to the same spot after disturbance. Such instances were recorded as a potentially hidden female. Reanalysis of the sex-ratio data this time including counts of potentially hidden females, still resulted in highly male-biased sex ratios (Table 5.1).

In contrast, the sex ratios of NSG samples collected at non-spawning times were closer to unity (Table 5.2). Only two samples collected in February and April 2000, had ratios significantly biased toward males, but even these were less biased than those of the spawning population.

The biased sex ratios of the spawning population have a considerable influence on the fraction of the spawning biomass that could potentially contribute to egg production in each year. In 2001, females constituted just 30 t of the total of 184 t, thus representing just 16% of the total biomass (Fig. 5.1).

Table 5.1 Estimated sex ratios (M:F) for two sites at the aggregation area, Black Point in the open-closed area and Stony Point in closed-closed area, for each sampling time during four spawning seasons (1998 to 2001). Chi-squared (χ^2) with Yates correction and probability (p) values indicate results from tests of H_0 : sample sex ratios not different from 1:1. Heterogeneity χ^2 and p values indicate results from tests of H_0 : all samples from a homogenous population. Dashes indicate where sample sizes were too small to be included (< 30 individuals) and M:F+F? refers to ratios with potentially-hidden females near stationary males added to observed females.

| Date | Black Point | | | | | Stony Point | | | | |
|------------------------|----------------|------------------|------------|---------------|---------------|----------------|------------------|------------|---------------|---------------|
| | M : F | M : F +F? | n | χ^2 | p | M : F | M : F +F? | N | χ^2 | p |
| 30/4/1998 | - | | | | | - | | | | |
| 14/5/1998 | 3.8 : 1 | | 182 | 60.6 | 0.0000 | 6.4 : 1 | | 89 | 46.0 | 0.0000 |
| 1/6/1998 | 4.2 : 1 | | 141 | 52.5 | 0.0000 | 6.0 : 1 | | 134 | 66.4 | 0.0000 |
| 28/6/1998 | 8.1 : 1 | | 82 | 48.4 | 0.0000 | 3.2 : 1 | | 127 | 34.3 | 0.0000 |
| 31/7/1998 | - | | | | | 2.6 : 1 | | 31 | 5.3 | 0.0216 |
| Pooled 1998 | 4.5 : 1 | | 405 | 161.8 | 0.0000 | 4.4 : 1 | | 381 | 151.2 | 0.0000 |
| Heterogeneity χ^2 | | | | 2.3 (df=2) | 0.1307 | | | | 4.4 (df=3) | 0.0369 |
| 1/5/1999 | 17.5 : 1 | (8.8 : 1) | 37 | 27.7 | 0.0000 | - | | | | |
| 11/5/1999 | 3.0 : 1 | (2.7 : 1) | 255 | 64.3 | 0.0000 | - | | | | |
| 26/5/1999 | 3.9 : 1 | (3.6 : 1) | 297 | 101.9 | 0.0000 | 5.6 : 1 | (4.8 : 1) | 201 | 98.4 | 0.0000 |
| 29/6/1999 | 3.6 : 1 | (3.2 : 1) | 120 | 37.4 | 0.0000 | 3.8 : 1 | (3.8 : 1) | 130 | 43.3 | 0.0000 |
| 27/7/1999 | 3.0 : 1 | (2.7 : 1) | 107 | 25.3 | 0.0000 | 2.1 : 1 | (2.0 : 1) | 71 | 8.1 | 0.0044 |
| Pooled 1999 | 3.6 : 1 | (3.2 : 1) | 816 | 255.9 | 0.0000 | 4.0 : 1 | (3.7 : 1) | 406 | 145.4 | 0.0000 |
| Heterogeneity χ^2 | | | | 5.5 (df=4) | 0.0187 | | | | 6.3 (df=2) | 0.0118 |
| 3/5/2000 | - | | | | | - | | | | |
| 16/5/2000 | 4.7 : 1 | (4.0 : 1) | 113 | 45.9 | 0.0000 | - | | | | |
| 2/6/2000 | 3.1 : 1 | (3.0 : 1) | 243 | 63.3 | 0.0000 | 5.6 : 1 | (5.2 : 1) | 244 | 117.1 | 0.0000 |
| 14/6/2000 | 4.0 : 1 | (3.4 : 1) | 111 | 39.2 | 0.0000 | 3.6 : 1 | (3.6 : 1) | 228 | 70.7 | 0.0000 |
| 26/6/2000 | 4.9 : 1 | (4.9 : 1) | 88 | 36.9 | 0.0000 | 4.7 : 1 | (4.6 : 1) | 244 | 101.0 | 0.0000 |
| 25/7/2000 | 5.8 : 1 | (4.8 : 1) | 169 | 82.4 | 0.0000 | 7.5 : 1 | (6.7 : 1) | 145 | 83.4 | 0.0000 |
| 22/8/2000 | - | | | | | 2.8 : 1 | (2.8 : 1) | 73 | 15.8 | 0.0001 |
| Pooled 2000 | 4.1 : 1 | (3.7 : 1) | 724 | 268.6 | 0.0000 | 4.6 : 1 | (4.5 : 1) | 934 | 386.7 | 0.0000 |
| Heterogeneity χ^2 | | | | 4.1 (df=4) | 0.0434 | | | | 6.4 (df=4) | 0.0117 |
| 7/6/2001 | 5.4 : 1 | (3.8 : 1) | 223 | 103.6 | 0.0000 | 6.3 : 1 | (4.7 : 1) | 240 | 124.7 | 0.0000 |

Table 5.2 Sex ratios (M:F) of samples collected from the Gulf population at non-spawning times.

| Year | Date | Sample type | M : F | n | χ^2 | p |
|------|------------|---------------|---------|-----|----------|---------|
| 1998 | 15/11/1998 | Prawn Trawl | 0.9 : 1 | 31 | 0.0 | 1.0000 |
| 1999 | 2/2/1999 | Snapper Trawl | 0.8 : 1 | 34 | 0.3 | 0.6069 |
| | 14/4/1999 | Prawn Trawl | 0.9 : 1 | 84 | 0.1 | 0.7434 |
| | 7/11/1999 | Prawn Trawl | 1 : 1 | 4 | 0.3 | 0.6171 |
| 2000 | 20/2/2000 | Snapper Trawl | 2 : 1 | 42 | 4.0 | 0.0449* |
| | 5/4/2000 | Prawn Trawl | 1.1 : 1 | 82 | 0.0 | 0.9121 |
| | 13/4/2000 | Snapper Trawl | 1.7 : 1 | 190 | 13.7 | 0.0002* |
| 2001 | 4/4/2001 | Snapper Trawl | 1.1 : 1 | 197 | 0.5 | 0.4762 |

* Significant at the $\alpha = 0.05$ significance level.

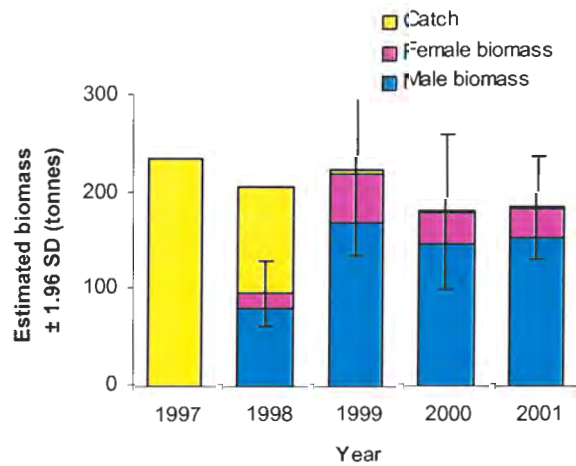


Figure 5.1 Annual estimated total biomass in the aggregation area, divided into proportions accounted for by males and females and the commercial fishery catch.

The sex ratio of the commercial catch sample taken in May 1998, was less biased toward males than those recorded on transects at approximately the same time (significant 2x2 contingency χ^2 test; Table 5.3). This suggested the jigging methods used by the commercial fishery may have been selective toward females. However, the sex ratios of samples obtained from the spawning aggregation by jigging at various times throughout the 1999 season, reflected a similar trend to those of the transects (Table 5.1), with a highly biased sex ratio toward males at the start of the season and less biased later in the season (significant homogeneity χ^2 test; Table 5.3).

Table 5.3 Sex ratios (M:F) of a single sample from the commercial catch in 1998 and four jig samples collected from the aggregation area during 1999.

| Year | Date | Sample type | M : F | n | χ^2 | p |
|------|--------------------|------------------|----------|----------------|--------------------|---------------------|
| 1998 | 27/5/1998 | Commercial Catch | 2.0 : 1 | 256 | 14.25 [♠] | 0.0002 [♠] |
| | 1/6/1998 | Transects | 4.2 : 1 | 141 | | |
| 1999 | 3/5/1999 | Jig Sample | 30.0 : 1 | 31 | 25.3 | 0.0000 |
| | 21/5/1999 | Jig Sample | 8.8 : 1 | 137 | 85.1 | 0.0000 |
| | 2/7/1999 | Jig Sample | 2.3 : 1 | 39 | 5.0 | 0.0250 |
| | 29/7/1999 | Jig Sample | 3.6 : 1 | 60 | 18.2 | 0.0000 |
| | Pooled 1998 | | | 5.7 : 1 | 267 | 129.6 |
| | Heterogeneity | | | | 7.9 (df=3) | 0.0049 |

[♠] 2 x2 contingency table χ^2 test comparing ratios between commercial catch and transects in 1998.

5.4.2 Size distributions

Modal progression analysis of the length frequency data could not be done for a number of reasons: (1) the frequency of sampling varied and was low relative to the longevity of the species; (2) some sample sizes were very small; and (3) different methods were used to sample the aggregation area and the wider NSG area. Nevertheless, a qualitative consideration of the data indicates several features relevant to the life cycle of the species.

The size distributions of *S. apama* recorded on underwater transects at Black Point over the last four years and those taken from various locations around NSG during the subsequent summer months are presented by sex in Figs. 5.2 and 5.3. A wide range of size classes of males was evident in all years in both the spawning population (range = 130 to 365 mm ML) and NSG samples (range = 53 to 285 mm ML). The size distribution of females was narrower than for males in the aggregation area (range = 140 to 270 mm mL), but still showed considerable variation in the NSG samples (range = 47 to 225 mm ML). The large males (i.e. up to 365 mm ML) were much larger than the females (270 mm ML), but only accounted for up to 20% of the total number of males, which further declined as the season progressed (Table 5.4). The remaining males were approximately the same size as the females.

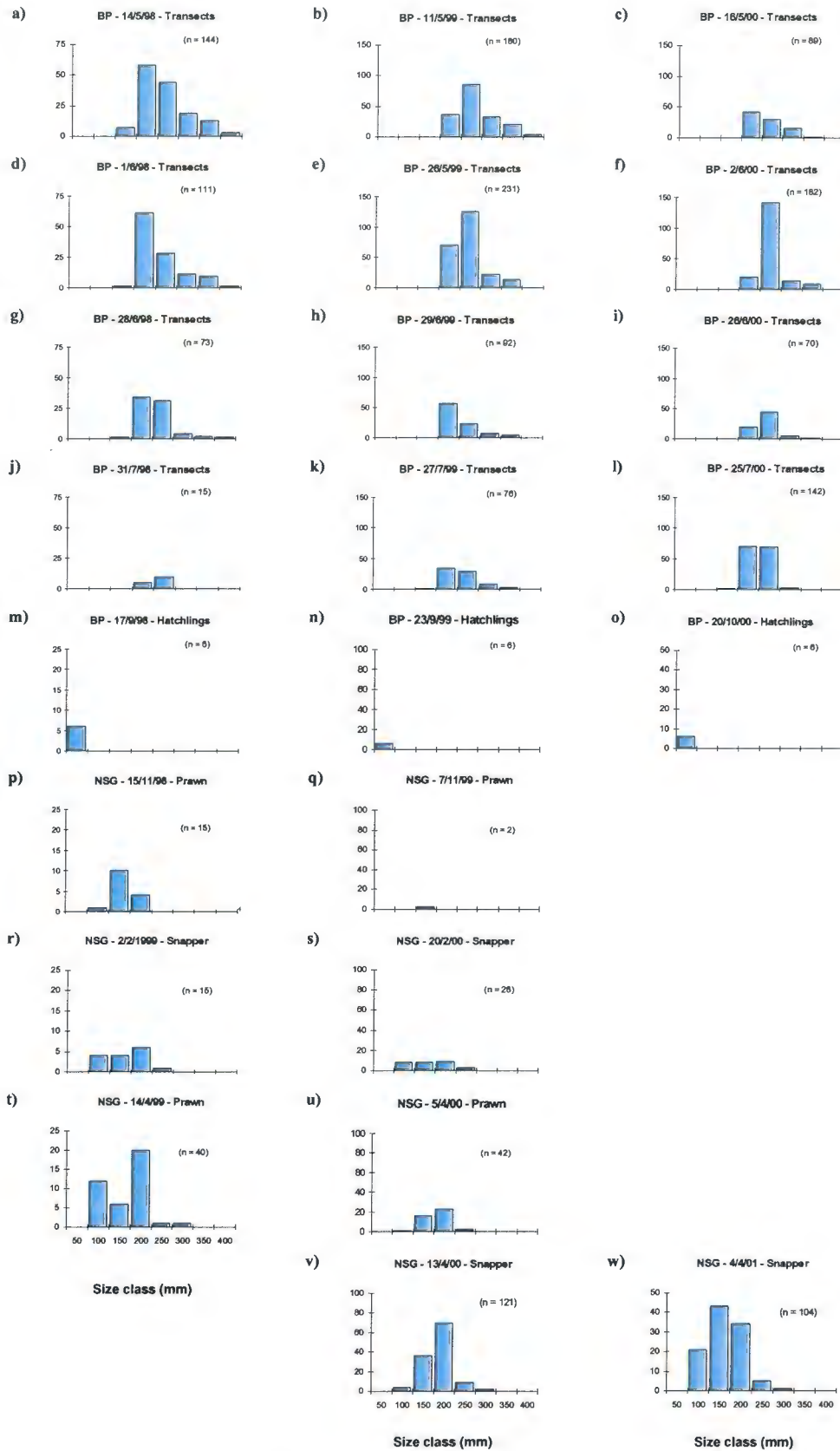


Figure 5.2 Length frequency histograms of male *S. apama* observed on transects at Black Point (BP) (a-l); collected from northern Spencer Gulf (NSG) by prawn trawlers (p-q) and (t-u) and juvenile snapper trawling (r-s) and (v-w); and hatchlings collected from BP (m-o) (May 1999 to April 2001).

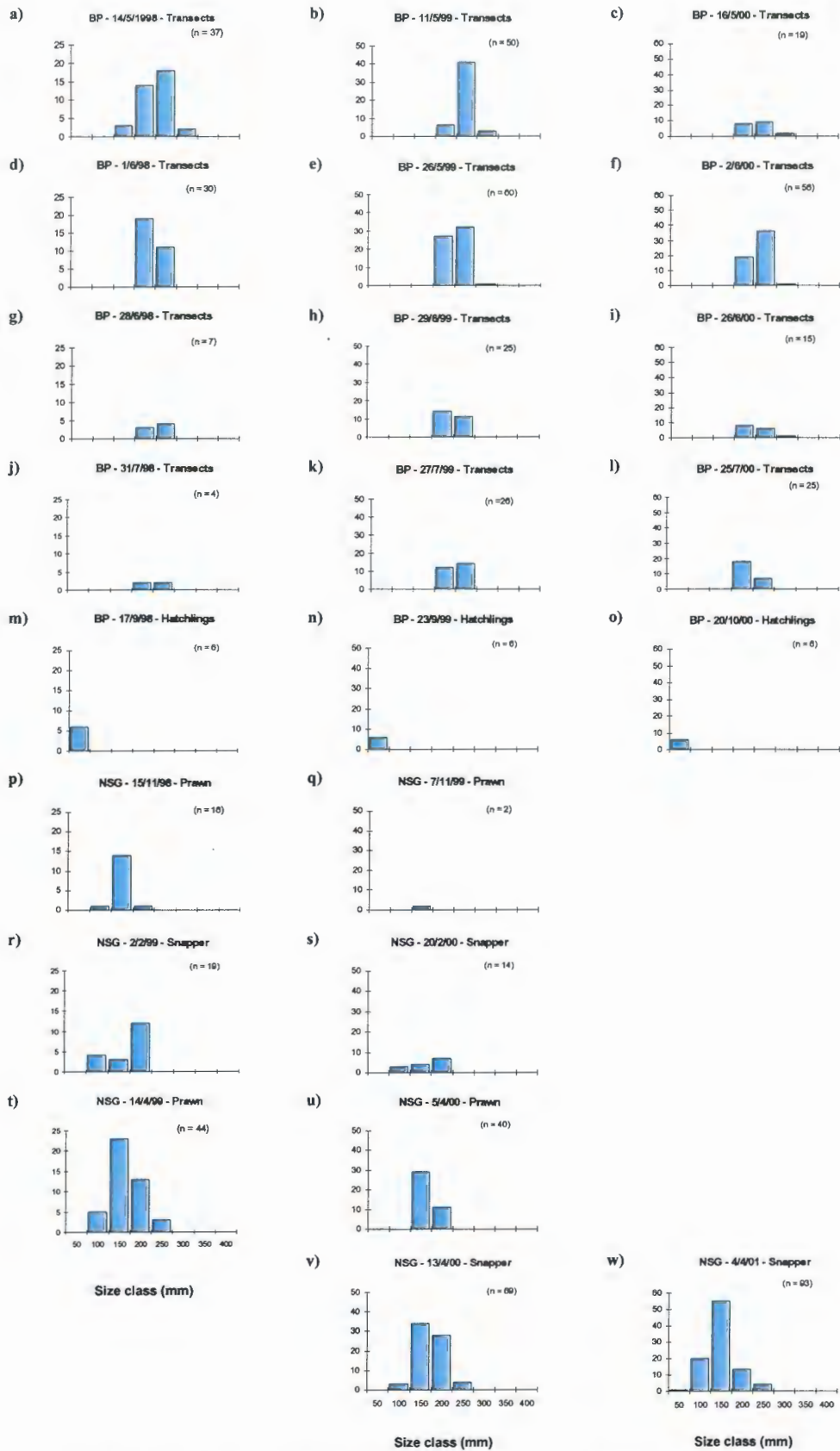


Figure 5.3 Length frequency histograms of female *S. apama* observed on transects at Black Point (BP) (a-l); collected from northern Spencer Gulf (NSG) by prawn trawlers (p-q) and (t-u) and juvenile snapper trawling (r-s) and (v-w); and hatchlings collected from BP (m-o) (May 1999 to April 2001).

Table 5.4 Percentage of male *S. apama* recorded on transects at Black Point with estimated ML larger than females (i.e. > 270 mm ML), at various times during 1998 to 2000.

| Year | Date | % Males > 270 mm ML | n (Males) |
|------|-----------|---------------------|-----------|
| 1998 | 14/5/1998 | 17.4 | 144 |
| | 1/6/1998 | 14.4 | 111 |
| | 28/6/1998 | 4.1 | 73 |
| | 31/7/1998 | 0 | 15 |
| 1999 | 11/5/1999 | 22.8 | 180 |
| | 26/5/1999 | 16.0 | 231 |
| | 29/6/1999 | 9.8 | 92 |
| | 27/7/1999 | 5.3 | 76 |
| 2000 | 16/5/2000 | 18.0 | 89 |
| | 2/6/2000 | 16.5 | 182 |
| | 26/6/2000 | 4.3 | 70 |
| | 25/7/2000 | 0.1 | 142 |

In September and October each year, after all adults had left the aggregation area, hatchlings emerged at a size of around 10 mm ML (Fig. 5.2*m-o* and Fig. 5.3*m-o*). Note hatchlings were not sexed so the same unsexed samples from each year were used in both male and female length frequency figures. Then in November, there were only small adult-sized cuttlefish of one distinct size mode (range = 80 to 160 mm ML) in the NSG samples (Figs. 5.2*p-q* and Fig. 5.3*p-q*). Whereas, in February and April, some smaller cuttlefish were also present (range = 50 to 100 mm ML), in addition to many larger individuals.

The size composition of males in the commercial catch in May 1998 (Fig. 5.4*a*), were primarily in the 150 to 200 mm ML size class. Alternatively, the female size distribution was similar to that recorded using transects in the aggregation area (Fig. 5.3*b*). The size distributions from jigging samples taken at various times throughout the 1999 spawning season, showed a similar trend to those of the transects for both sexes. Initially, early in May a high percentage of large individuals were represented. Alternatively, later in May, as well as in all subsequent samples, the smaller size classes dominated.

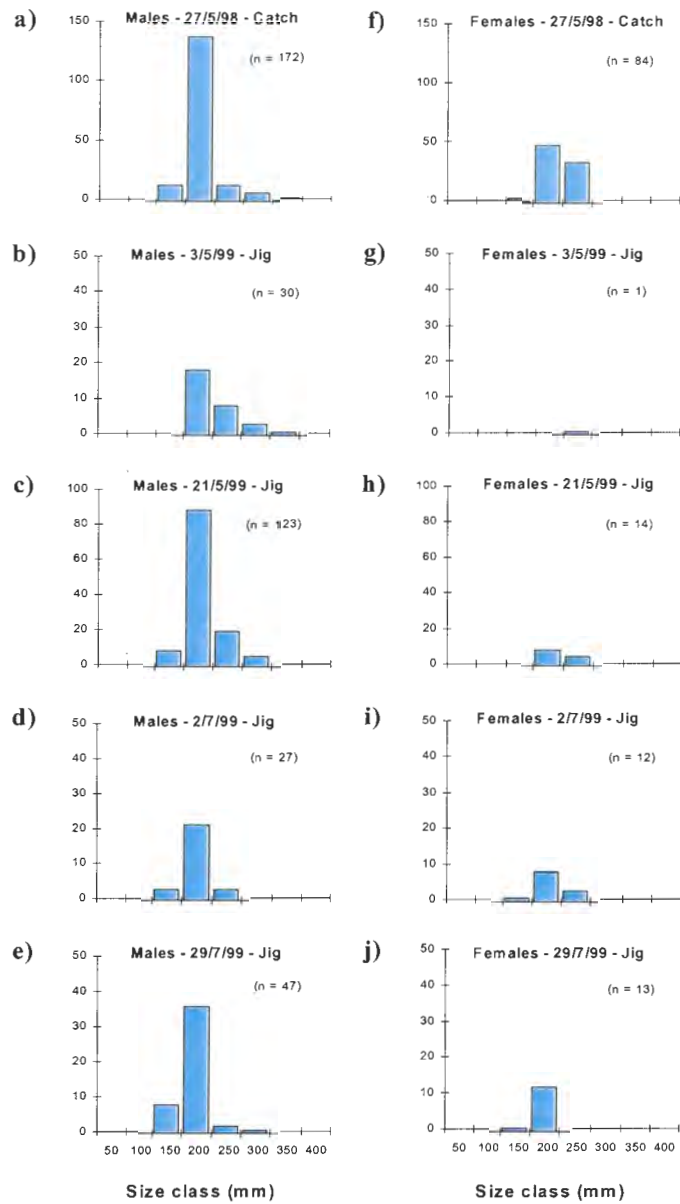


Figure 5.4 Length frequency histograms of male (a-e) and female (f-j) *S. apama* sampled from the commercial catch in May 1998 and the aggregation area during 1999 using jigging methods.

5.5 Discussion

Male-biased sex ratios were consistently recorded in the aggregation area, compared with the wider NSG, where ratios were generally close to unity. The uneven sex ratios in population samples may

result for a number of different reasons. A discussion of the plausibility of each with respect to the results obtained in this study is provided below.

(1) *Unequal sex ratio at time of hatching.* The sex ratios of the NSG samples were close to unity suggesting that sex ratios at the time of hatching may be equal. Although, a connection between the NSG and aggregation populations was provided through the migration of one tagged individual to the aggregation area, this does not exclude the possibility that many individuals in the NSG may be from another spawning population. If this were so, biased sex ratios at the time of hatching might dominate in the aggregation area but not be reflected in the overall NSG population. However, Richard and Lemaire (1975) found that pieces of undifferentiated gonads from *S. officinalis* embryos, kept *in vitro* in a non-hormonal medium, auto-differentiated into testicular or ovarian tissue at a ratio of 1:1. These results suggest that sex determination in *Sepia* may be under genetic rather than hormonal control, resulting in a sex ratio of 1:1 at the time of hatching (Richard and Lemaire 1975).

(2) *Differential survivorship or longevity between the sexes.* This may occur at any stage prior to, during or after recruitment. However, this is unlikely to be the cause of the biased sex ratios in the spawning population since the sex ratios at the non-spawning times approximate 1:1.

(3) *Bias in sampling methodology toward the inclusion of one sex over the other.* Some females were hidden under rocks while laying eggs in tight crevices and were not readily visible on transects. The counts for cryptic fish species are underestimated by underwater visual transect methods (Willis 2001). However, female cuttlefish were rarely unaccompanied at the aggregation area, due to the bias in numbers towards males. Hence, hidden females were usually indicated by a stationary male at the entrance. Reanalysis of these sex-ratio data including the counts of the possible cryptic ones, still resulted in highly male-biased sex ratios. Analysis of the reproductive behaviour at the aggregation area (Chapter 9), revealed that some small males were also cryptic, suggesting the possibility that a small percentage of males were also missed in the counts. Finally, male-biased sex ratios were also evident in the samples obtained by hand line and squid jig.

(4) *Modification of the population sex composition by previous exploitation.* Just as sampling methods may be biased toward one sex, so too may fishing methods have a higher probability of catching one sex over another. The only commercial fishing method used in the aggregation area was hand line and squid jig, whose efficacy depends on the feeding behaviour of the cuttlefish. Therefore, if there is differential feeding behaviour between the sexes, or with respect to reproductive behaviour (which in turn varies between the sexes), one sex may have a higher probability of capture than another (Wirtz and Morato 2001). Schoener (1971) suggested that females of most fish species are "energy

maximisers" and thus spend more time foraging than males as their fitness is principally determined by the net energy acquired. Alternatively, males increase their reproductive success by other activities, such as mate guarding or competing for access to females, which detract from feeding time.

Previous studies suggest that squid jigging can be selective toward certain components of a population (Lipinski 1994). However, there was no evidence of this in this study. In addition, observations of the response of individuals to squid jigs in the water column, suggested that small males not actively involved in reproductive behaviours were more likely to attack a squid jig than females that were actively egg-laying or large males that were defending a female. However, the responses of females that were moving about the area and not engaged in egg-laying were not tested. Sauer (1995) found a similar result for jig fishing on *Loligo vulgaris reynaudii* off the South African coast, where males not accompanying females were most likely to approach the squid jig. It is also unknown whether the effects of selective fishing on population composition in previous years can be accumulated or carried over to subsequent years for cephalopod populations, given the short life span of most species and lack of persistence in biomass from one generation to the next.

(5) *Different spatial or temporal distribution of sexes.* Unequal sex ratios in a given area may result from the preferential use of the area by one sex or asynchronous timing in the use of the area by the two sexes, such as the asynchronous spawning of one sex at a spawning location (Emlen and Oring 1977). If female *S. apama* matured at different times, such that their arrival at the aggregation area was staggered, and then only remained for a short time relative to males, only a proportion of the total number of females would be present at any one time. Whereas, if males all matured and migrated to the spawning area at approximately the same time each year and remained for the duration of the spawning season, virtually all males would be present at all times. Tagging work completed in the aggregation area in 2000 (Chapter 4) suggested that at least some females remain in the area for up to 6 weeks during the spawning season, however, this did not rule out the possibility that many others may have left and been replaced by different females or that they may be repeatedly moving in and out of the area.

In conclusion, the proximity to unity of the sex ratios of the wider NSG samples suggests that differential spatial distribution of the two sexes remains the most likely factor to account for the highly biased sex ratios on the spawning grounds. It appears that more males migrate to the spawning aggregation area than females. Clearly, further directed research is needed to account for the unequal sex ratios. Nevertheless, the impact on the spawning biomass is obviously important to the

management of the fishery as the biased sex ratios mean that less than a third of the estimated total biomass in the aggregation area contributes to egg production in each year.

Another peculiar feature of the composition of the spawning population was the broad size distribution of males compared to females. Males of all sizes were mature and present throughout the spawning season, suggesting that more than one age class may reproduce at the aggregation area. Furthermore, the existence of the small pre-adults in November (after the end of the spawning season when all adults are thought to have died) and immature juveniles in February and April (when all individuals should be approaching maturity for the spawning season about to start), suggests that not all *S. apama* present in the Gulf belong to the same age class.

S. apama have been described as an annually-spawning semelparous species (Lu 1998b), which means that because of the discrete spawning season the population should consist of a single cohort of similarly-sized individuals (Boyle et al. 1995), especially given the discrete spawning season. The existence of different size classes within a cohort of squid has often been explained by: (1) variable growth rates that result from a high degree of physiological plasticity to different environmental conditions (Collins et al. 1995a); or (2) by the presence of micro-cohorts within a single year class caused by multiple hatch dates over a protracted spawning season or multiple peaks in year-round spawning (Hatfield 1996, 2000); or (3) the migration or mixing of different stocks which spawn and/or grow in different areas (Boyle et al. 1995). Back calculation of hatching dates from statolith growth ring analyses suggest that many squid species, such as *L. pealei*, that were previously thought to spawn at specific times of the year, may actually spawn throughout the year (Macy 1995; Brodziak and Macy 1995; Macy and Brodziak 2001).

Several size classes of males co-exist in the spawning aggregations of many *Loligo* species (Hanlon 1998). The different sized males commonly use different tactics to compete for access to females. The larger ones generally fight for, pair with and defend females, whereas, the smaller males tend to use sneak mating tactics (Hanlon 1996; Hanlon et al. 1997; Sauer et al. 1997). These different-sized males were previously thought to be of different year classes based on length-frequency analysis (Summer 1971; Mesnil 1977). However, statolith growth ring analysis in most cases indicated that the different size classes of males hatched within the same twelve month period and that all had a life span of less than one year (Rodhouse and Hatfield 1990).

These results are harder to rationalise in the case of a definite uniseasonal spawner, such as *L. forbesi* (Collins et al. 1999). There are two size modes at maturity for both sexes at most locations, but particularly for males which showed much greater variation in size than females (Boyle et al. 1995;

Collins et al. 1995b). It was originally thought that the larger animals were late-hatching juveniles that were unable to spawn in their first winter and returned early the following winter as larger spawners. Yet, statolith analysis indicated that both sizes of mature animals were of similar age with a maximum life span of slightly over a year (Collins et al. 1995a). No single explanation could account for the opposing results (Boyle et al. 1995; Collins et al. 1999).

Given the short, well-defined spawning season of *S. apama* (Chapters 4 and 9), the existence of different size classes in the spawning population suggests the possible presence of multiple year classes, particularly for males. This would suggest that *S. apama* has a life span of more than one year. This could occur if the species were not semelparous, and males survived to spawn over several consecutive spawning seasons as they became progressively larger. Admittedly, such a life history tactic would be very unusual for a cephalopod species (Mangold 1987; Hanlon and Messenger 1996). Alternatively, male *S. apama* may be semelparous, but have alternative life cycles, which serve as alternative reproductive tactics, as described for some salmon species (Gross 1985). All males are semelparous, but some opt to return at a younger age and smaller size, whilst others delay maturity until they are older and larger before returning to spawn (Fleming 1996; Foote et al. 1997). Longer life spans and the possibility of alternating life cycles of different lengths have been proposed for a number of other cuttlefish species including *S. officinalis* (Boletzky 1983). However, to date verifying the ages of cuttlefish through analysis of statoliths have proved inconclusive (Raya et al. 1994; Bettencourt and Guerra 2001). Le Goff et al. (1998) managed to separate *S. officinalis* from southern Brittany into two age classes based on the seasonal variation in chamber widths in cuttlebones collected at regular intervals. The establishment of an accurate method of age estimation for *S. apama* would help resolve whether or not the different size classes in the spawning population represent different year classes.

6 Age estimation

K.C. Hall

6.1 Introduction

For cephalopod species age-based population parameters such as growth rate and life span have usually been estimated indirectly using length-based methods, such as modal progression analysis of length frequency data (Voss 1983). However, direct ageing methods and the culture of known-age individuals in captivity have raised concern over the applicability and accuracy of length-based methods (Caddy 1991; Jackson et al. 2000). The interpretation of length modes for cephalopod species may be complex for a number of reasons: (1) the protracted spawning periods of some species may produce several "micro-cohorts" within a single year class and be incorrectly interpreted as separate year-classes (Pierce and Guerra 1994); (2) there may be substantial variation in growth rates within a single cohort because of physiological plasticity, such that similar-sized individuals have different ages and vice versa (Lipinski 1998; Jackson et al. 2000); and (3) many species are migratory such that the movement of individuals into or out of a given area may mix cohorts and confound patterns of growth (Caddy 1991; Hatfield and Rodhouse 1994). Thus, indirect length-based methods are only applicable where one or several short well-defined spawning events occur and there is little change in population structure due to migration (Arkhipkin 1991).

It is preferable to develop a direct method of age estimation. This commonly involves interpreting periodic growth increments in hard tissues over the lifetime of the animal (Campana 2001). Four criteria must be fulfilled for successful age estimation (Beamish and McFarland 1983): (1) that increments are sufficiently clear to facilitate precise interpretation; (2) that increments can be correlated with a regular and determinable time scale; (3) that the formation of increments continues at a measurable rate throughout life; and (4) that increments are permanent and not resorbed during remobilisation of hard tissue. Such increments in fish otoliths have been effectively used for this purpose for many years (Panella 1971; Campana and Neilson 1985) and techniques to optimise viewing and interpreting increments with accuracy and precision are well established (Campana 2001).

Attempts to develop direct ageing techniques for cephalopod species are relatively recent. Regular growth increments have been identified in the statoliths, gladius, cuttlebone, beak and eye lenses of many species (Rodhouse and Hatfield 1990). Statoliths are functionally analogous to fish otoliths and

have thus been the focus of most attention to date with promising results. Like otoliths, they are composed of calcium carbonate in the form of aragonite prisms in an organic matrix (Rodhouse and Hatfield 1990) and apparently function as sense organs for detection of gravity and changes in acceleration (Radtke 1983). They are located in two adjacent cavities, the statocysts, within the cartilaginous skull posterior to the brain (Clarke 1978). Growth increments have been viewed in thin sections prepared by grinding both sides of the statolith usually in the concave antero-lateral plane (Jereb et al. 1991). A growth increment consists of 2 rings, one light (rich in calcium carbonate laid down during the night) and one dark (rich in organic matter laid down during the day) (Lipinski et al. 1991; Bettencourt and Guerra 2000). For various squid species validation studies using known-age individuals reared in captivity or the incorporation of a chemical time marker in statoliths have provided evidence for the daily formation of increments (Jackson 1994). Estimates of life span based on studies of statolith microstructure have generally been less than one year, and are usually much shorter than estimates obtained from length-based methods (Jackson et al. 2000).

Aging cuttlefish from statoliths has been less successful. Growth increments have proven difficult to distinguish due to the irregular and concentric deposition of the aragonite crystals, which result in a strong radial appearance, and the lower percentage of organic matter, which results in weak dark rings (Bettencourt and Guerra 2000). Raya et al. (1994) established a method to view the increments in the statoliths of *Sepia officinalis hierreda* from the north-western African coast; however, fine-scale increments were only visible for a small number of specimens and the periodicity of formation of the broader, more distinct increments was unresolved. Bettencourt and Guerra (2001) aged *S. officinalis* reared in captivity at two different temperatures using statoliths. The deposition of daily increments was validated for individuals as old as 240 days but the age of older specimens was underestimated due to the poor resolution of increments in the outer regions of the statolith. Furthermore, a large proportion of the statoliths (31 to 77%) could not be aged due to poor resolution of increments.

Most attempts to age cuttlefish have concentrated on the cuttlebone. This structure functions as a dorsal backbone providing both support and buoyancy control (Fig. 6.1). It consists of a thin, hard, calcified, dorsal shield and a ventral porous phragmocene comprised of numerous narrow chambers, delineated by chitinous septa (Bandel and Boletzky 1979). The cuttlefish controls its buoyancy by moving gas or liquid into or out of the chambers as required (Denton and Gilpin-Brown 1961). As the cuttlefish grows, further septa are laid down at the anterior end (Fig. 6.1). Early studies concluded that the periodicity of chamber formation was daily (Choe 1963; Packard 1972), however, recent studies found it was related to growth rate rather than chronological age (Richard 1969; Boletzky 1974a; Ré and Narciso 1994; Bettencourt and Guerra 2001). The growth rate of cephalopods is strongly influenced by

temperature and food availability and thus subject to seasonal fluctuations. The width of individual chambers also vary with growth rate (Hewitt and Stait 1988), which allowed *S. officinalis* from Southern Brittany to be separated into two age groups based on the seasonal differences in chamber widths (Le Goff et al. 1998). Analysis of patterns within the cuttlebone microstructure also separated two different stocks of *S. esculenta* in Korean waters (Kim and Hong 1991).

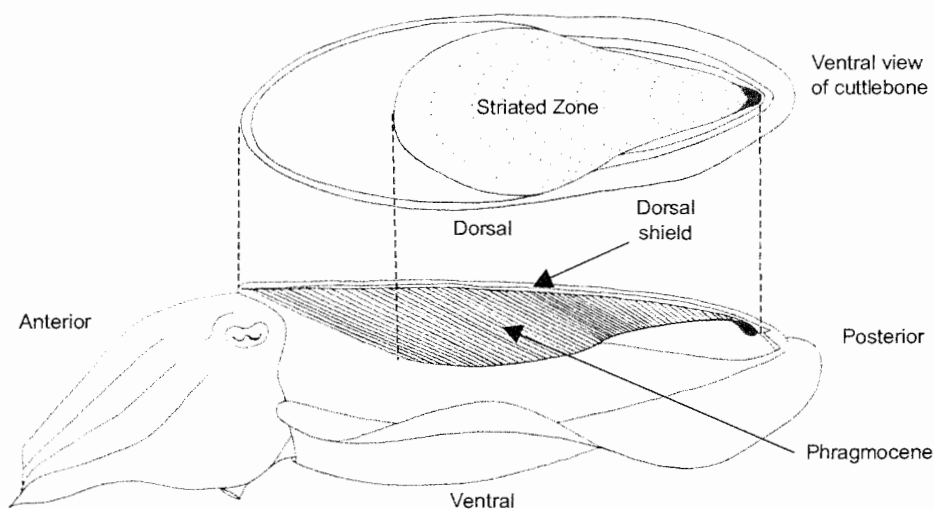


Figure 6.1 Diagram illustrating the location and orientation of the cuttlebone within the dorsal mantle of cuttlefish.

Few attempts have been made to age cephalopods from their crystalline eye lenses, which closely resemble those of vertebrates (Sivak 1991). They grow continuously throughout life by the addition of concentric layers of fibre cells to their outer surface (Friend 1967). Based on this, there have been numerous attempts to relate eye lens wet weight, dry weight or volume, to age for various mammals (Lord 1959; Dudzinski and Mykytowycz 1961; Kolenosky and Miller 1962) and fish (Carlton and Jackson 1968; Burkett and Jackson 1971; Crivelli 1980), but results have proven inconclusive for older age groups due to significant overlap. Douglas (1987) first explored the possibility of using eye lens diameter instead of weight and found the average lens diameter of cultured, known-age brown trout, *Salmo trutta*, increased with age with no overlap between age groups. Lens diameter varied little within the one age group despite the variation in fish length. This simple technique has not yet been tested with cephalopods. Bettencourt (2000) examined the internal microstructure of the crystalline eye lenses of *S. officinalis*, but no relationship with age or growth was determined (referred to in Bettencourt and Guerra 2001).

Similarly, there have been few attempts to age cephalopods using the chitinous beaks (mandibles), since Clarke (1965) first noticed growth increments on the outer surface of the lower beaks of the Antarctic squid, *Moroteuthis ingens*. Growth increments were also visible in the rostral area of sagittal sections of the beak of the octopus, *Octopus vulgaris* (Raya and Hernández-González 1998), although the periodicity of their formation was not determined.

6.2 Aims

The length frequency data for *S. apama* from the spawning population at the aggregation area and the wider Spencer Gulf population suggests the possible presence of multiple year classes (Chapter 5). Given the uncertainty in determining age from length-based methods for cephalopod species, the aim of this chapter was to assess the possibility of a direct method of age determination for *S. apama*. Firstly, the four hard structures were examined to identify the presence of any visually discernible, regular growth increments within the microstructure. From this, an ageing technique was developed that could assign individuals to an age class. The technique was applied to a large number of individuals to determine the age structure of the spawning population in the main aggregation area and the wider NSG population.

6.3 Methods

The details of samples collected were provided in Chapter 3. The cuttlebone, statoliths, eye lenses and beak were extracted from each adult and hatchling as part of the standard laboratory processing of samples. The dorsal mantle length (ML) and total body weight (TWt) were recorded for all specimens prior to dissection.

A pilot study was done to trial preparation methods to expose any internal microstructure of the four hard structures. Ten individuals across a broad size range from hatchlings to adults of both sexes were considered (Table 6.1). This pilot study indicated that only the cuttlebone showed promise as a structure for direct age estimation (refer Results section).

Table 6.1 Details of *S. apama* hatchlings from the aggregation area and individuals from the April 1999 NSG sample used for the pilot study to examine the internal microstructure of the four hard structures.

| ID No. | ML (mm) | TWt (g) | Sex |
|--------|---------|---------|-----------|
| H10 | 13.4 | 0.64 | hatchling |
| H11 | 12.2 | 0.53 | hatchling |
| NSG100 | 72 | 70 | F |
| NSG82 | 100 | 152 | M |
| NSG76 | 140 | 337 | F |
| NSG78 | 137 | 303 | M |
| NSG89 | 183 | 582 | F |
| NSG88 | 183 | 616 | M |
| NSG107 | 233 | 1533 | F |
| NSG108 | 230 | 1503 | M |

Table 6.2 Summary of samples used for age analysis using cuttlebones.

| Date | Sample Location | Sample Type | Number M : F | Hatchlings ML range (mm) | Males ML range (mm) | Females ML range (mm) |
|--------------------------|-----------------|----------------------------|----------------------|--------------------------|---------------------|-----------------------|
| 19 May 1998 | Aggregation | Hand Net | 17 : 13 | | 147 - 280 | 135 - 233 |
| 4 Aug 1998 | Aggregation | Hand Net | 14 : 10 | | 140 - 275 | 137 - 210 |
| 17 Sep 1998 | Aggregation | Hatchlings | 10 | 12.3 - 13.6 | | |
| 15 Nov 1998 | NSG | Prawn Trawl | 15 : 16 | | 88 - 170 | 100 - 155 |
| 2 Feb 1999 | NSG | Snapper Trawl | 6 : 4 | | 69 - 107 | 74 - 97 |
| 14 Apr 1999 | NSG | Prawn Trawl | 22 : 21 | | 85 - 265 | 67 - 233 |
| 28 May 1999 | Aggregation | Hand Net | 23 : 13 | | 133 - 330 | 163 - 247 |
| 29 Jul 1999 | Aggregation | Hand Net | 19 : 11 | | 152 - 300 | 147 - 215 |
| 23 Sep 1999 | Aggregation | Hatchlings | 6 | 12.4 - 14.2 | | |
| 7 Nov 1999 | NSG | Prawn Trawl | 2 : 2 | | 140 - 150 | 103 - 140 |
| 20 Feb 2000 | NSG | Snapper Trawl | 28 : 14 | | 53 - 236 | 92 - 192 |
| 5 Apr 2000 [#] | NSG | Prawn Trawl [#] | 15 : 12 [#] | | 124 - 235 | 117 - 196 |
| 13 Apr 2000 [#] | NSG | Snapper Trawl [#] | 11 : 8 [#] | | 78 - 205 | 78 - 160 |
| 19 May 2000 | Aggregation | Hand Net | 14 : 15 | | 170 - 305 | 172 - 248 |
| 3 Aug 2000 | Aggregation | Hand Net | 15 : 19 | | 153 - 302 | 138 - 189 |
| 20 Oct 2000 | Aggregation | Hatchlings | 12 | 10.7 - 13.7 | | |
| 4 Apr 2001 | NSG | Snapper Trawl | 52 : 51 | | 68 - 273 | 47 - 225 |
| TOTAL | | | 253 : 207 | 10.7 - 14.2 | 53 - 330 | 47 - 248 |

[#] April 2000 samples were pooled with a mean date of 8 April for all analysis

A more comprehensive sub-sampling strategy was then undertaken for the cuttlebone analysis (Table 6.2). A total of 460 bones from 207 females and 253 males as well as 28 bones from hatchlings were analysed. These were collected from the aggregation area and NSG population from 1998 to 2001 (Table 6.2).

6.3.1 Statoliths

The head was removed from the mantle via an incision on the dorsal side, exposing the posterior cartilage of the skull, through which the solid, white statoliths could usually be seen. They were removed, rinsed in distilled water, air dried at room temperature, and stored in labelled bags.

A thin section of each statolith was then prepared. Firstly, the statolith was mounted on a microscope slide using heat-sensitive quick-drying Crystal Bond. Several different orientations were tried (Fig. 6.2): (1) anterior (concave) side facing down, with lateral dome, rostrum and wing all in contact with the slide; (2) posterior (convex) surface facing down, with posterior side of lateral dome and dorsal dome in contact with the slide and rostrum elevated; (3) lateral side facing down, with lateral dome and rostrum in contact with the slide and medial wing and dorsal dome pointed upwards; (4) dorsal surface facing down, with dorsal dome and lateral dome in contact with the slide and rostrum pointed upwards; and (5) the dorsal dome angled over the edge of the slide such that the lateral dome was sectioned longitudinally by the edge of the slide. The statolith nomenclature established by Clarke (1978) was followed throughout

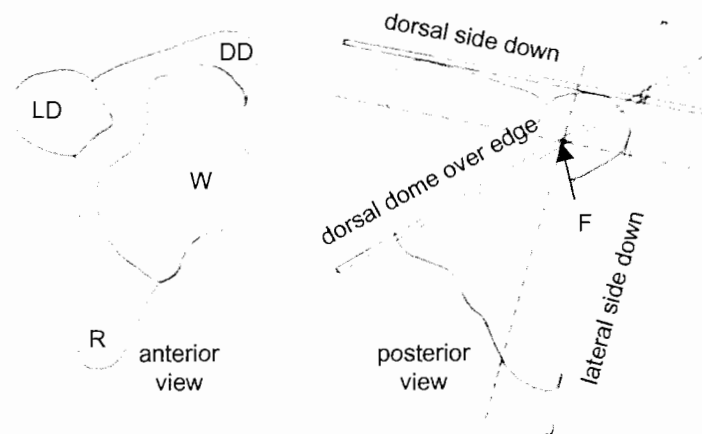


Figure 6.2 Diagram of the anterior and posterior view of the right statolith detailing the 4 main parts - lateral dome (LD), dorsal dome (DD), rostrum (R) and wing (W). Three mounting positions in relation to the microscope slide and resulting plane of section through the focal point (F) are also indicated.

The exposed side was ground by hand using a series of progressively finer silicium carborundum paper (30, 9 and 3 μm grades) until the focal point was reached. Progress of grinding was monitored under a dissecting microscope. Once the focal point was reached the crystal bond was remelted and the statolith was turned over, re-mounted and ground on the opposite side until a thin section was obtained. Sections were examined with image analysis equipment involving a compound microscope fitted with a video camera and computer monitor. Oil immersion at 400x magnification was required to see the fine increments.

6.3.2 Eye lenses

The eye lenses were extracted by an incision through the cornea, followed by careful severing of the ciliary musculature surrounding the lens. The fresh lens was weighed (EWt) and the largest diameter (ED) measured, before being preserved in 10% buffered formaldehyde in seawater. It was not possible to measure the lenses from frozen specimens due to deterioration.

The lenses were prepared for microscopic examination using standard histological techniques. The mid-transverse or longitudinal portion of the lens was dehydrated in a graded series of ethanol solutions, cleared with histoclear and embedded in paraffin wax. Sections of 7 μm thickness, were cut with a microtome, mounted on slides and stained with either Mayer's haematoxylin and eosin or Masson's trichrome stain (Smith and Bruton 1977).

6.3.3 Beaks

The upper and lower beaks were removed from the relaxed buccal musculature, rinsed in water and stored in 70% ethanol to prevent desiccation prior to analysis. The medial and lateral surfaces of the lateral wall of both beaks were examined under a dissecting microscope at high magnification for regular growth features. To expose the medial surface the beak was sliced in half with a diamond-tipped gem saw. The lower beak was then set in clear-casting resin from which a medial longitudinal section of 400 μm was cut with a diamond-tipped gem saw through the mid-line of the rostrum and hood. This was mounted on a microscope slide covered with immersion oil and examined under a compound microscope.

6.3.4 Cuttlebones

Cuttlebones were extracted via a longitudinal incision along the mid-line of the dorsal mantle. The total bone length (BL) was measured, before it was rinsed and set aside to dry at room temperature.

To reveal the internal microstructure of the cuttlebone, the soft ventral phragmocene of the dry bone was sliced along the longitudinal axis with a scalpel, until the surface of the hypostracum (ventral surface of the dorsal shield) was reached. The phragmocene was carefully cut away and the remainder scraped clear of the surface of the hypostracum on one half of the bone (Fig. 6.3a). The dorsal shield was very thin and fragile, and on occasion fractured during preparation; however, most could be reassembled sufficiently for analysis (Fig. 6.3c). A growth increment was considered to be the width of a complete chamber, that is the distance between two consecutive septa. The remnants of the removed septa left a distinct line on the surface of the hypostracum (Fig. 6.3b).

Growth increments within the hatchling bones could be seen without preparation through the dorsal surface of the whole cuttlebone under a dissecting microscope with transmitted light. Digital images of the whole bones were taken and increment widths measured from the images.

Digital images were taken along the length of the hypostracum from the posterior forked region to the anterior rim with an image analysis system (Chapter 3). Incident light directed at an angle onto the bone was the best form of illumination. Increments were counted (Inc No) and their individual widths (IncWi) measured to the nearest 0.01 mm from the saved images.

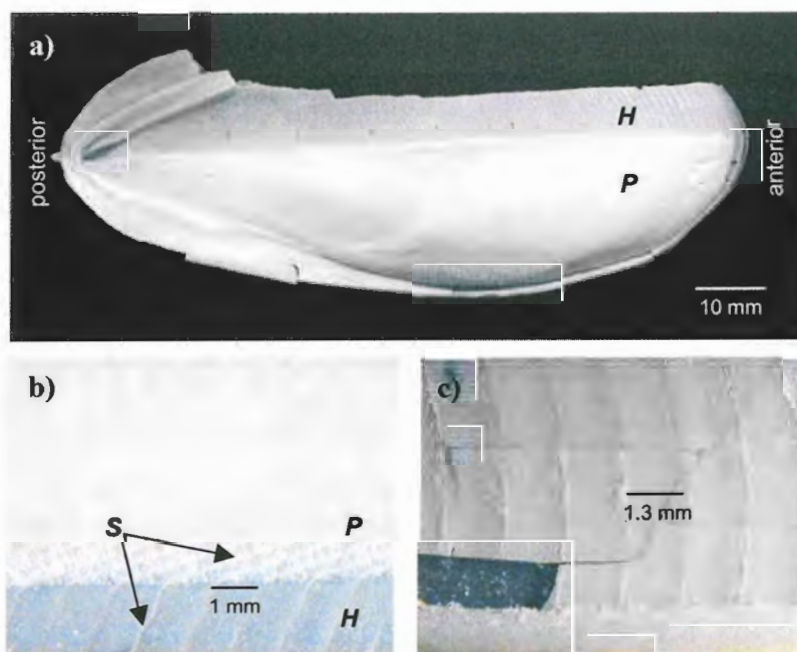


Figure 6.3 (a) Preparation of a cuttlebone to view the internal increment structure on the ventral surface of the dorsal shield; (b) Junction between the internal chambers of the phragmocene (*P*) and the surface of the hypostracum (*H*) showing the pattern formed by the remnants of the septa (*S*) on the hypostracum after the phragmocene was removed; (c) Reassembled dorsal shield after fracturing during preparation.

The increments along each bone were numbered from the anterior end (region of most recently formed increments) to the posterior end. This allowed the patterns of IncWi of different bones of the same sample date to be plotted on one graph, aligned from the date of capture. Three consistent types of patterns of IncWi were observed, and the bones of each sample were divided into three "bone types" accordingly. For each combination of bone type and sex within a sample, the mean IncWi of every increment was calculated and plotted against increment number from the anterior end, to provide an average pattern of IncWi. Since the pre-hatch increments of different individuals were rarely aligned exactly, mean IncWi was only calculated until the first hatching mark of a bone was reached. The mean IncWi of the last 10 anterior increments (AvIncWi) (excluding the very last increment) was determined for each individual as an indication of the growth rate just prior to capture. The very last increment was not included in case of partial formation at the time of capture.

The mean BL, Inc No and AvIncWi were calculated for each bone type and sex combination in each sample. No statistical between-sample date comparisons were attempted due to the unbalanced distribution of bone types amongst samples and the small size of some samples. Therefore, only for the 6 samples of adequate size, a 2x2 factorial multivariate analysis of variance (MANOVA) was used to test for differences between the two independent variables of bone type and sex, for the three attributes of BL, IncNo and AvIncWi as dependent variables. Wilk's criterion was used in the presentation of results, but all four criteria tested produced similar results. For most samples only two of the three bone types were present in sufficient numbers for statistical comparison, and only in the large April 2001 sample were all three bone types tested simultaneously. Sample sizes within combination cells were usually unequal. To investigate the impact of each main effect and interaction on the individual variables, a 2x2 univariate ANOVA was completed post-hoc on each variable at each sample date, using Bonferroni adjusted alpha values of 0.015 to guard against inflated Type I errors (Tabachnick and Fidell 2001).

A discriminant function analysis (DFA) was also completed on the whole data set for each sex (149 males and 122 females) to assess whether group membership within the three bone type groups could be reliably predicted using the 3 variables, regardless of sample date. The multivariate statistical assumptions of homoscedascity of variances, linearity and normality were evaluated by visual assessment of residual plots, bivariate scatterplots, frequency histograms and normal probability plots of appropriately grouped data. Both BL and Inc No required logarithmic transformation to correct for heteroscedasticity of variances (Zar 1999). As both MANOVA and DFA tests are particularly susceptible to the influence of outliers, the Mahalanobis distance of each case from the overall group

centroid was checked to ensure it did not exceed the critical χ^2 value of 16.3 (df = 3; $\alpha = 0.001$; as per Tabachnick and Fidell 2001). No outliers were identified based on this criterion.

6.3.5 Adult age validation experiments

Adult cuttlefish were maintained in aquaria in 1998 and 1999 for age validation experiments. Different collection locations and experimental protocols were used in the two years. In the first year, cuttlefish were collected from Myponga Reef, Gulf St. Vincent. The proximity and shorter transport time to the SAASC aquarium facility was considered preferable to collecting animals from the NSG, and attendant increased transport stress. Five cuttlefish were collected on 3 April (4 females and 1 male), four on 5 May (2 female and 2 males) and five on 3 June (1 female and 4 males) - with hand lines and squid jigs. Cuttlefish were transferred to aerated 70 L insulated containers, with less than 3 cuttlefish per container, for the 1 h journey to the aquarium facility. Cuttlefish were maintained in the outdoor aquarium facility at SAASC (Chapter 3). Since the experiment was simultaneously serving as a spawning experiment (Chapter 8) water temperatures were left at ambient in all tanks, lest a change in water temperature should interfere with egg-laying. One male-female pair was placed in each tank with spawning substrate (Chapter 8).

In May 1999, i.e. at the start of the spawning season, adults were collected from the spawning aggregation area in northern Spencer Gulf. Four males and 9 females were collected with a hand net while snorkelling. The cuttlefish were transported as in 1998 with 3 cuttlefish per 70 L container, however, the journey to the aquarium facility was much longer (6 h) requiring a complete exchange of seawater at the mid way point. In this year, the cuttlefish were maintained in the indoor aquarium facility at SAASC (Chapter 3). Four temperature treatments were used: (1) constant 20°C; (2) constant 16°C; (3) constant 12°C; and (4) variable ambient (Amb) water temperature, with three tanks per treatment. As per the requirements of the spawning experiment (Chapter 8), one female was randomly allocated to each tank, resulting in 3 females per treatment, but only one male was allocated to each treatment and rotated between tanks.

Cuttlefish were acclimated for 4 weeks to allow for egg-laying (Chapter 8) before they were measured, sexed and injected with calcein (2,4-bis-[N,N'-di(carbomethyl)-aminomethyl]-fluorescein: @Sigma C-0875). This is a fluorescent chemical that binds to calcium and is incorporated into calcium carbonate structures such as the cuttlebone and statoliths of cuttlefish. Calcein was chosen in favour of other fluorochromes because it generally produces more intense bands and is less toxic at appropriate doses than other fluorescent dyes such as tetracycline (Wilson et al. 1987; Monaghan 1993; Gelsleichter et

al. 1997; though see Brooks et al. 1994). Furthermore, calcein has been successfully incorporated into molluscan shells, such as for the black-lip abalone *Haliotis rubra* (Day et al. 1995).

A stock solution of 300 mg calcein in 6 ml sterile saline solution (0.9% sodium chloride) was prepared with sodium bicarbonate to buffer the solution to a pH of 7.3 to increase the solubility of the calcein. The cuttlefish were removed from the tank and their mantle length measured to within 1 mm. The weight of the cuttlefish was estimated using the length-weight relationship for each sex (Chapter 5) to calculate the appropriate quantity of stock solution to inject to achieve a 25 mg kg⁻¹ of body weight dose of calcein. This equated to 0.4 mL of stock solution for a 200 g female, whilst all doses were between 0.3 to 0.6 mL. The needle was inserted into the ventral mantle muscle just below the lateral fin with care taken to ensure the tip of the needle remained within the muscle and did not penetrate the mantle cavity. Cuttlefish were not fed for 24 h prior to injection to prevent potential complications caused by the presence of food in the stomach.

Adults were fed daily with live fish when available: either small striped perch, *Pelates octolineatus*, or yellow-eye mullet, *Aldrichetta forsteri*; or frozen pilchards, *Sardinops sagax*, or western king prawns, *Penaeus latisulcatus*. All cuttlefish were held until they died naturally or were killed due to poor condition. Most were dissected fresh. Cuttlebones were removed, air-dried at room temperature and stored in darkness until aged. A fibre-optic light source fitted with a narrow bandwidth interference filter (λ_0 central 485 nm; 10 nm band width; EdmundTM) in conjunction with a dissecting microscope was used to illuminate and examine the bones for a calcein band.

6.4 Results

6.4.1 Statoliths

The statoliths of hatchlings, juveniles and adults of *S. apama* were all similar in shape (Fig. 6.4). The four principal parts previously described for squid and *Sepia* statoliths were evident - a very distinct bulbous lateral dome, angular dorsal dome, long rounded rostrum, and broad wing, with soft opaque area of attachment (Clarke 1978).

Growth increments in statoliths of *S. apama* were very difficult to see. The two mounting positions, which gave most consistent results were: (1) the lateral side facing down, providing good sections of the lateral dome and rostrum; and (2) the dorsal surface facing down, resulting in good sections of the lateral dome and to a less extent the dorsal dome. However, the clearest resolution of fine increments was obtained in sections of the lateral dome prepared with the dorsal dome originally protruding over

the edge of the slide. The success depended on accurate initial placement of the statolith on the edge of the slide and three mountings of the statolith were usually required to produce the finished product.

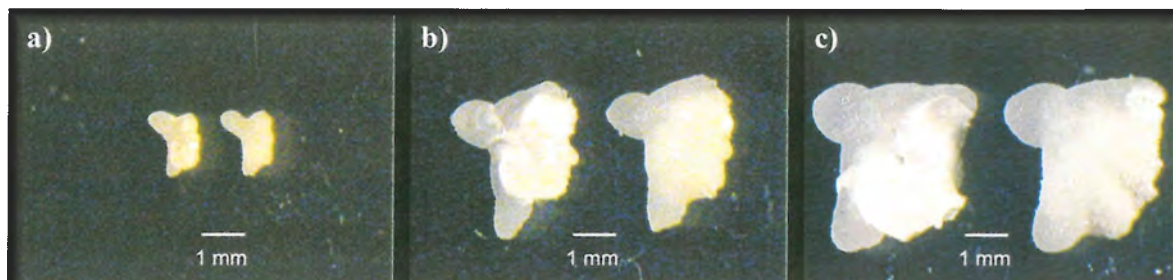


Figure 6.4 Statoliths of different sized *S. apama*. (a) A 13.4 mm ML hatchling from the aggregation area; (b) a 100 mm ML juvenile from the northern Spencer Gulf; (c) 230 mm ML mature male from the aggregation area.

The nucleus and broad increments composed of light and dark zones could be seen in most sections. Finer incremental structure was only visible in small patches of some sections and usually over different focal planes. Therefore, resolution and enumeration of increments was not possible across the breadth of the statolith especially near the margin.

6.4.2 Eye lenses

The mean weight and mean diameter of the paired fresh eye lenses showed strong positive linear relationships with mantle length (Fig. 6.5), indicating that eye lenses increase in size as body size increases. The linear regressions obtained were similar between the two sexes for each variable. The negative intercept of the linear regression between lens weight and mantle length (Fig. 6.5a) indicates that the relationship was only valid for mantle lengths of larger than 100 mm.

The lens was almost spherical in shape with two distinct halves separated by a septum connected to the ciliary musculature that held the lens in place. The internal microstructure of the lens was clearly visible in the histological sections examined with light microscopy (Fig. 6.6). Staining with Masson's trichrome stain produced more pronounced definition of fibre cell membranes than haematoxylin and eosin staining. Individual fibre cells formed distinct rings toward the margins of the lens (Fig. 6.6b) but became wider and less obvious toward the centre (Fig. 6.6c), such that the middle section, which corresponded to the embryonic nucleus, formed an amorphous mass with no discrete cell membranes visible. Thus, total enumeration of rings across the radius of the lens was not possible. Furthermore, the

fibre cells were very narrow (less than 3 μm) and thus very numerous across even a small juvenile lens (greater than 1,500) rendering counts impractical.

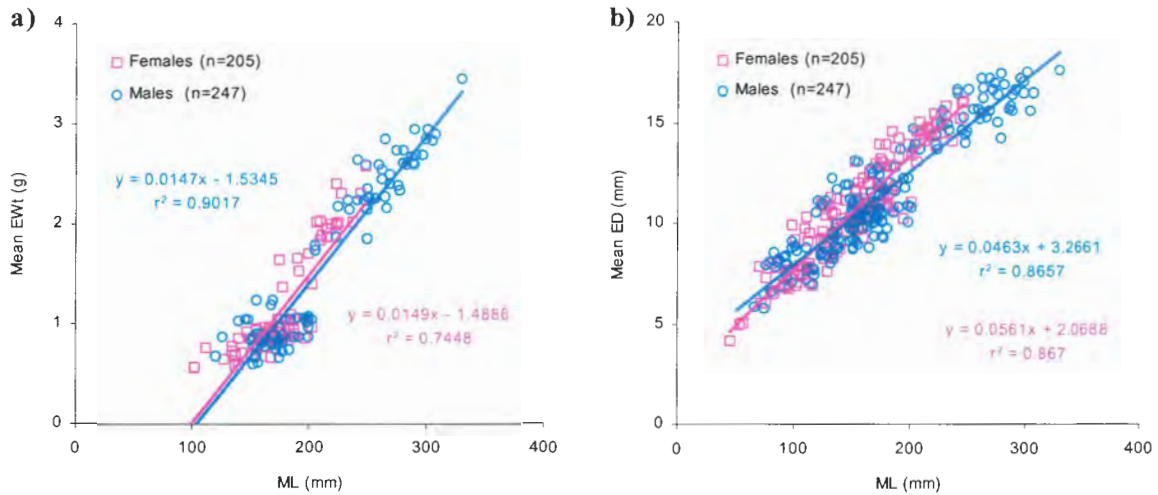


Figure 6.5 Relationship between the mean EWT (a) and mean ED (b) of the paired eye lenses with respect to ML for specimens analysed between 1998 and 2001 (Table 1). Results from regression analysis are also indicated.

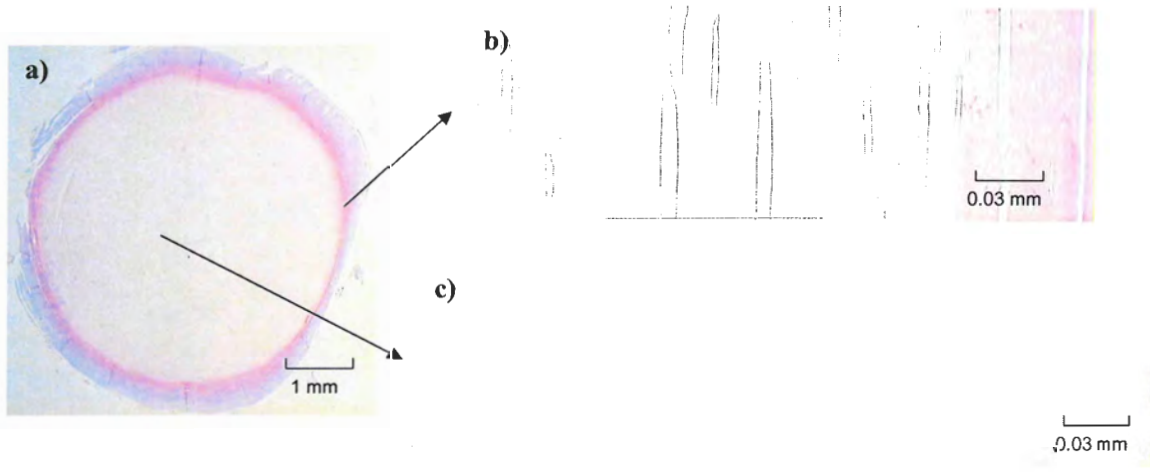


Figure 6.6 (a) Longitudinal histological section of the posterior half of the eye lens of a juvenile *S. apama*. (H&E, 60x). (b) Fibre cells in the marginal region of the lens section with clearly defined membranes forming rings (H&E, 250x). (c) Less distinct and wider fibre cells toward the centre of the lens (H&E, 250x).

6.4.3 Beaks

The upper and lower beaks were bilaterally symmetrical with three main parts, the solid opaque rostrum, hood and paired lateral walls (Fig. 6.7). Regular fine invaginations were evident on the medial surface of the lateral wall of the upper beaks (Fig. 6.7c), which were visible under a dissecting microscope with incident light reflected off the uneven surface. However, no increments were discernible in the region of the rostrum, precluding enumeration of the increments over the full range of the structure. No regular growth increments could be identified within the medial sections of the lower beak. The sections were very opaque and possibly too thick for optimal microscopic analysis.

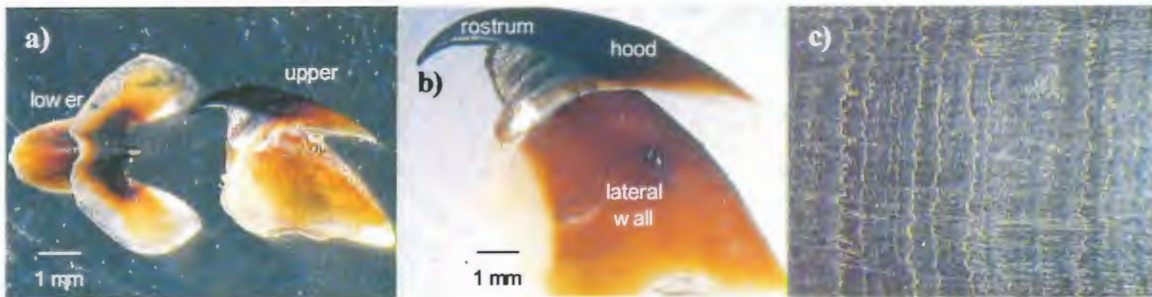


Figure 6.7 (a) Lower and upper beaks of *S. apama*. (b) Upper beak indicating the main parts of the beak. (c) Close-up of the inside surface of the lateral wall showing growth increments.

6.4.4 Cuttlebones

There was a strong linear relationship between BL and ML for both sexes (males: $r^2=0.9911$, $n=247$; females $r^2=0.9918$, $n=205$) (Fig. 6.8). This relationship was significantly different from isometry in both cases (paired t-test (2-tailed); males: $t = -26.4$, $df = 246$, $p = 0.0000$; females: $t = -29.8$, $df = 204$, $p = 0.0000$) with the difference between the two variables increasing in larger individuals.

Growth increments, as delineated by the remnants of removed septa, were highly visible on the surface of the hypostracum and were easily measured and counted. Few bones, only those that were very deformed or injured, were rejected from analysis of the internal microstructure ($n= 10$; 2.2% of bones examined). In those bones, there was a high degree of calcification on the dorsal shield in the region of the injury and increments were obscured and could not be resolved confidently. For all other bones, growth increments could be discerned along the entire length of the bone, including the pre-hatch region in the fork at the posterior end.

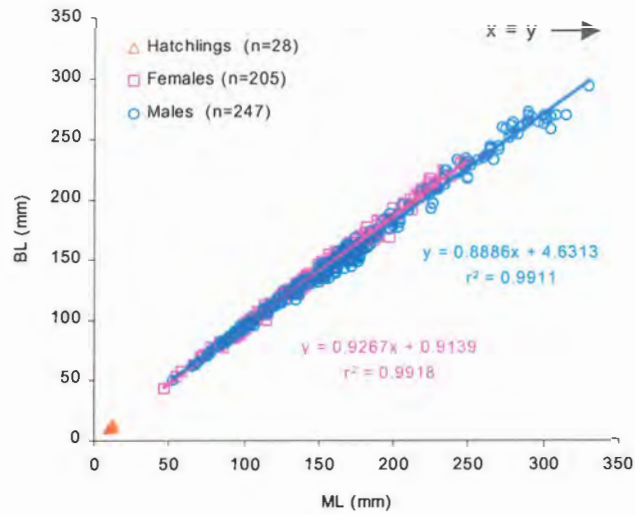


Figure 6.8 Relationship between BL and ML of *S. apama*. Specimens were collected between May 1998 and April 2001. Linear regressions and corresponding r^2 values are also indicated.

The IncWi of increments varied substantially over the length of a single bone, from regions of narrow increments of less than 0.5 mm to regions of wide increments of over 1.5 mm (Fig. 6.9a-b). There were a number of consistent patterns in the variation of IncWi over the length of bones taken from cuttlefish collected at different times of the year. Representative examples of these are shown for one twelve month period in Fig. 6.10.

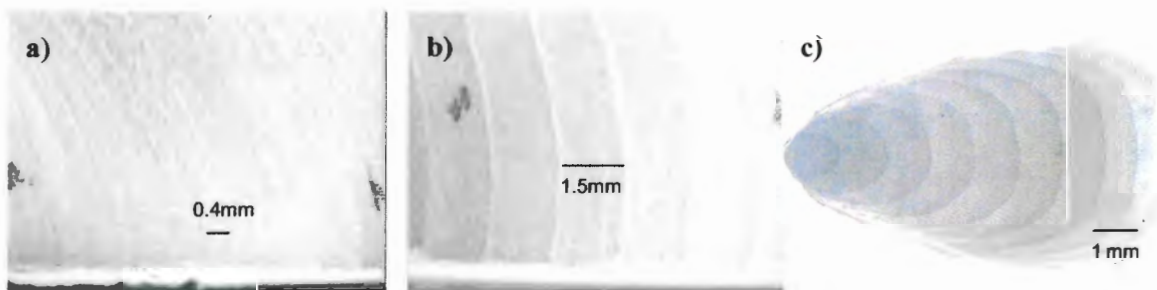


Figure 6.9 (a) An example of the narrow increments and (b) wide increments found in a single bone; Note, these two pictures were taken from different regions of the same bone at the same magnification. (c) The cuttlebone of a juvenile at time of hatching with fully formed internal structure.

Hatchlings emerged from egg capsules in September and October, with fully formed cuttlebones of between 10-13 mm BL and 10-13 Inc No (Fig. 6.9c and Fig. 6.10a). The increments became progressively narrower toward the anterior end of the hatchling bone, which corresponded to

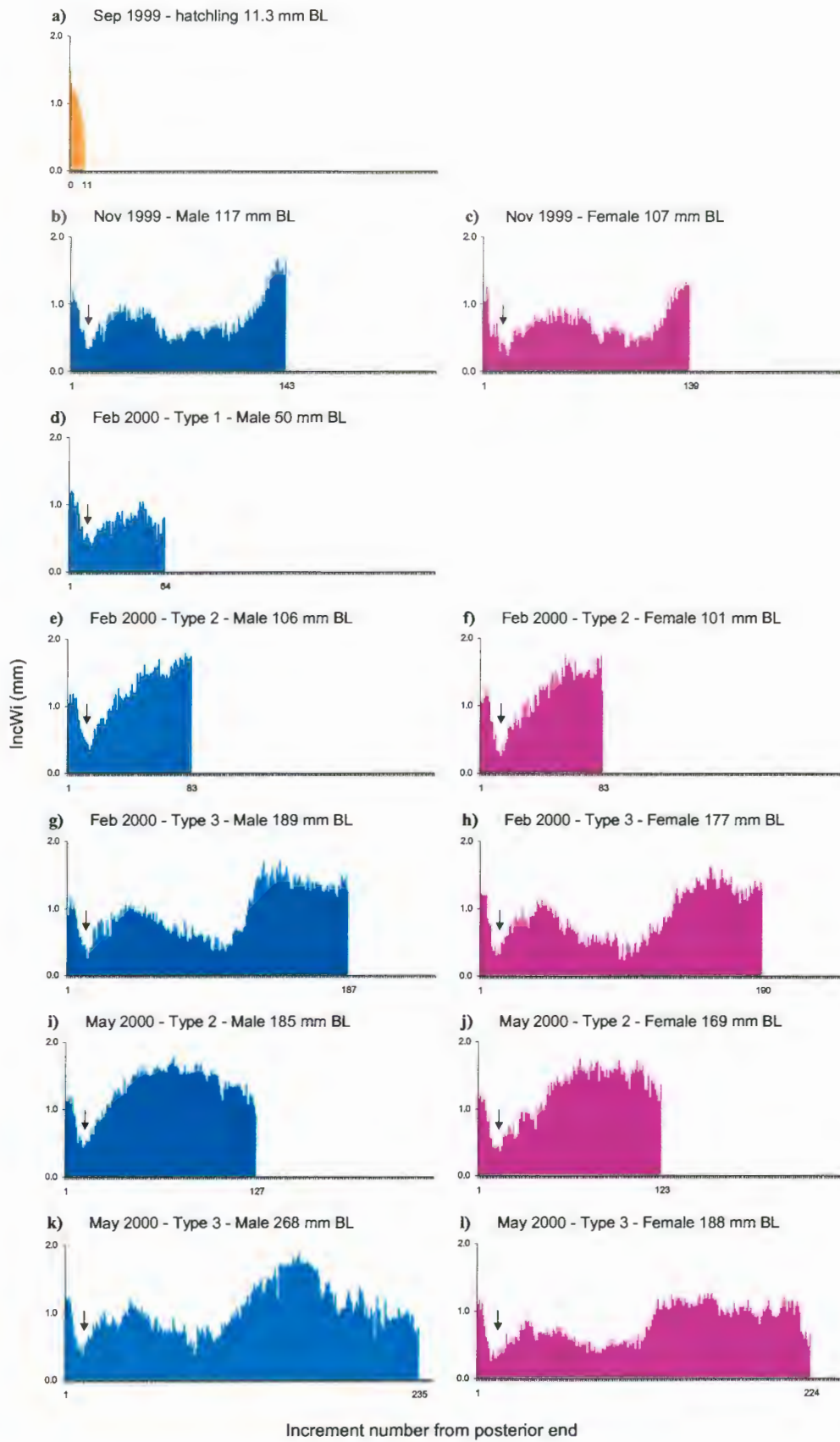


Figure 6.10 Representative examples of the different IncWi patterns of the cuttlebones of *S. apama* collected at various times throughout the year.

increments formed just prior to hatching. Two months later in November, small adults of both sexes in NSG samples all contained bones of between 100-120 mm BL with only one distinctive pattern of IncWi apparent (Fig. 6.10*b-c*). The posterior end of the bone represented the pre-hatch increments, which formed a similar pattern to those found in the hatchling bones. This was followed by a short section of very narrow increments, interpreted as the “hatching mark” (indicated with arrows in Fig. 6.10). This section was interpreted as the minimal growth period of juveniles as they made the transfer from endogenous feeding on yolk reserves to exogenous feeding.

After the “hatching mark”, IncWi initially increased to form a section of wider increments (Fig. 6.10*b-c*). Assuming all individuals hatched at the same time in spring following the winter spawning season, this section was interpreted as corresponding to the increased growth rate associated with the rise in water temperature as summer approached. This was followed by a second section of narrow IncWi, which was interpreted as corresponding to decreased growth rates associated with lower water temperature during winter. This was followed by another section of rapidly increasing IncWi, until the animals were captured in November. This final section was interpreted as increased growth rates associated with the start of a second summer, suggesting that the animals were over 12 months old at the time of capture. This interpretation and those that follow rely on the assumption that all cuttlefish in northern Spencer Gulf hatch in spring from September to October and that IncWi is positively correlated with water temperature, such that wide increments are deposited during summer and narrow increments in winter. These assumptions will be discussed further in a following section.

In NSG samples taken in February and April, there were three distinct pattern types of bones present in the population (Fig. 6.10*d-h*; only those for February are shown). Type 1 was found in the smallest individuals, which had bones of around 50-60 mm BL. The increment pattern showed a single section of increased IncWi following hatching, with a slight decrease just prior to capture (Fig. 6.10*d*). The assumed hatching period places these cuttlefish at around 4-6 months old at the time of capture. The Type 2 pattern was found in larger individuals with bones of around 90-100mm BL (Fig. 6.10*e-f*). IncWi increased rapidly after hatching with no subsequent decline before capture. These individuals were interpreted as the same age as the smaller animals, i.e. hatched during the same season, but had grown faster and reached a larger size in the same period. Type 3 was found in markedly larger individuals with bones of around 170-200 mm BL (Fig. 6.10*g-h*). The increment pattern showed two sections of increased IncWi after hatching, interpreted as corresponding to two periods of increased summer growth. These animals were interpreted as being around 16-18 months old at the time of capture. This bone type appeared to be an extension of those found in November.

All cuttlefish sampled at the aggregation site in May, regardless of sex or size, had bones with either of two distinct patterns of IncWi. The first type (Fig. 6.10*i-j*) had only one period of increased IncWi after hatching and appeared to be an extension of the Type 2 pattern found in the February samples (Fig. 6.10*e-f*). The smaller males and females had bones of this type and based on the assumed hatch date would have been around 6-8 months old at the start of the spawning season in May.

Within the second type (Fig. 6.10*k-l*) two regions of wide IncWi after hatching were evident and the pattern appeared to be an extension of the Type 3 pattern found in the February samples (Fig. 6.10*g-h*). This type of bone was found in the larger adults of both sexes on the spawning grounds. These animals were considered to be 18-20 months old at the start of May and would reach a maximum age of just over 2 years by the end of the spawning season.

All 460 bones examined from the samples from May 1998 to April 2001, conformed to one of the three types of IncWi pattern. Bones were subjectively assigned a bone type based on graphical assessment of the IncWi pattern. Within a single sample, the individual patterns of all bones of the one type were relatively consistent (Fig. 6.11*a-b*). Although quite large variation between individual patterns was evident in certain sections (labelled LV in Fig. 6.11*a*), little variation was usually found in regions of either increasing or decreasing IncWi (Fig. 6.11*a*). The coincidental nature of these events suggests that the timing was controlled by an external condition that uniformly affected all individuals. It also implies that the non-alignment of the different pre-hatch sections of the individual patterns (Fig. 6.11*a*) might represent differences in hatch date.

Variation in the IncWi pattern between individuals also resulted from prior injuries to the cuttlebone (Fig. 6.11*b*). These were apparent as either a calcified aberration on the dorsal shield or phragmocene or indicated by a black line on the striated zone of the phragmocene, as described for *S. officinalis* (Boletzky and Overath 1991). Most injuries had been repaired to some extent by calcification over the injured portion. Immediately following an injury, a short period of reduced IncWi was usually observed (Fig. 6.11*b*) causing increased variation in mean pattern of IncWi at that point. The incidence of prior injury to cuttlebones was quite high, with injuries observed for 12.6% (n = 58) of all bones examined. Nevertheless, mean patterns of IncWi for a given type, sex and sample date usually resulted in relatively small error bars over most of the pattern (Fig. 6.11*c*).

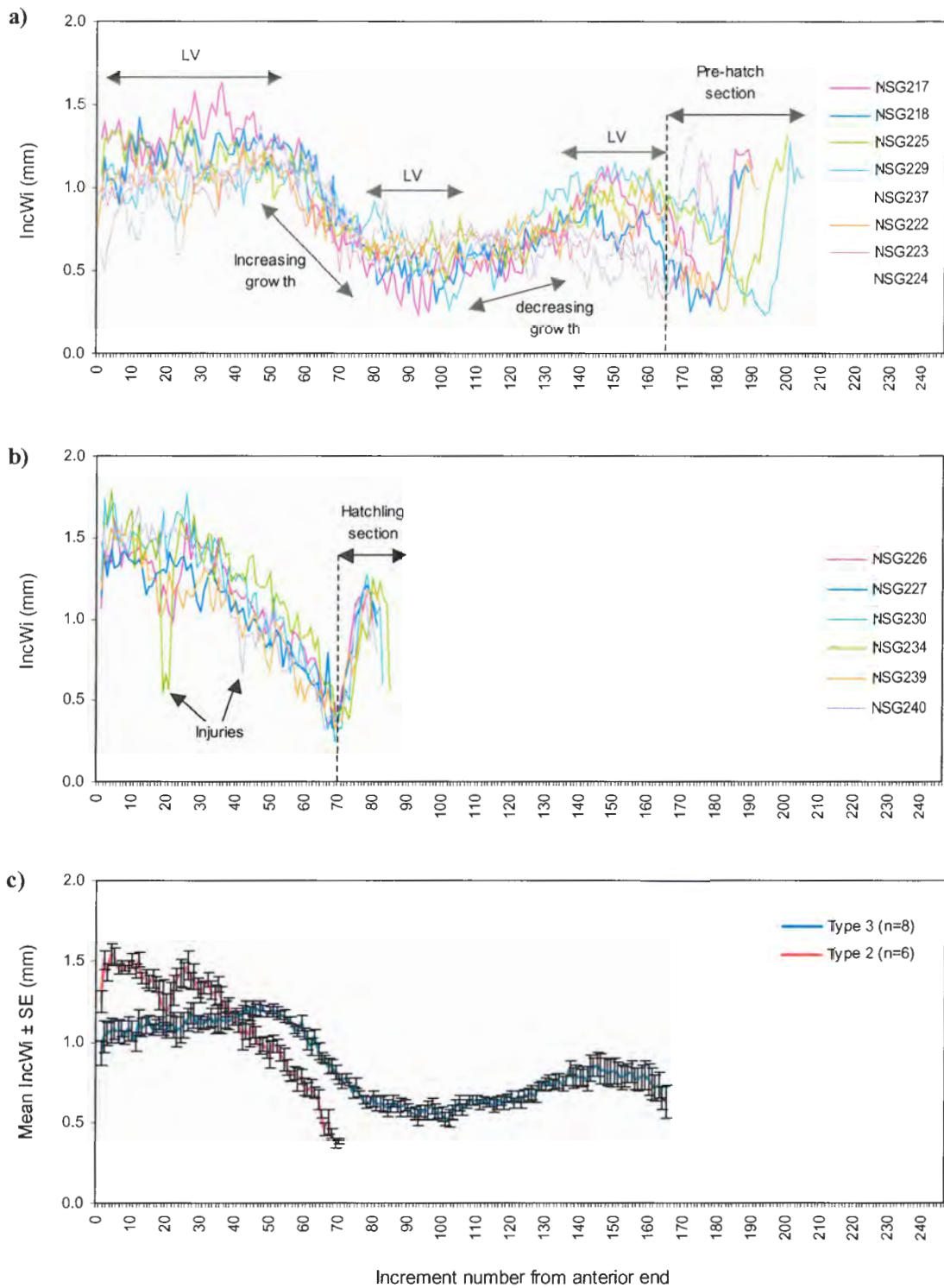


Figure 6.11 Individual IncWi patterns of bones from female *S. apama* of Type 3 (a) and Type 2 (b) bones, sampled from the NSG on February 2001. (c) Mean IncWi patterns averaged for each bone type with standard error bars indicating level of variation between individuals. LV indicates sections with relatively large variation between individual patterns.

Mean IncWi patterns for all sample dates for males and females separately are compiled in Fig. 6.12-14. The patterns were consistent across all years for samples collected on similar dates. For example, the patterns of Type 2 and 3 bones in May 1998 (Fig. 6.12*a-b*), May 1999 (Fig. 6.13*a-b*) and May 2000 (Fig. 6.14*a-b*) are all very similar. There was little difference in the patterns of Type 2 and 3 bones between the May and August samples in any given year.

Not all bone types were present in every sample. For example, in November only Type 3 bones were collected. Although there were low numbers of Type 2 and Type 3 bones in NSG samples from February 1999 and April 2000, these were an artefact of the sampling methods as all large individuals were removed from the samples for genetics and tagging studies, respectively.

The assignment of bone type by measuring the IncWi of all increments was time-consuming and laborious. Therefore, three other attributes that were easier to measure, i.e. BL, IncNo and AvIncWi, were tested for variation related to bone type, sex or sample date. Bivariate scatterplots of all combinations of the three variables indicated potential grouping of data points with respect to bone type (Fig. 6.15*a-c*) but little variation between the two sexes (Fig. 6.15*d-f*). There was a significant correlation between BL and IncNo (correlation coefficient = 0.8836) when data were pooled for all sample dates (Fig. 6.15*a,d*).

The mean BL (Fig. 6.16*a*) of each bone type showed a similar pattern of variation across all sample dates. Type 3 bones were the largest in all samples, followed by Type 2 and then Type 1 bones. In contrast, there was little variation in mean BL between the two sexes for any given bone type in most samples, with the obvious exceptions of the Type 3 bones in samples collected in May and August each year (Fig. 6.16*a*). In these, the males were usually much larger than the females. Likewise, within the Type 2 bones in the April 2001 sample, males were also larger than females. There was a general increase in the mean BL of all bone types from November to May of the following year. Similarly the mean Inc No of each bone type increased over the same time period (Fig. 6.16*b*).

The difference in mean Inc No between Type 2 and Type 3 bones was very pronounced in all samples (Fig. 6.16*b*), where Type 3 bones had approximately twice the number of increments as Type 2 bones. In contrast, there was little difference between the mean IncNo of Type 1 and Type 2 bones. There was also little difference between the two sexes for any given bone type.

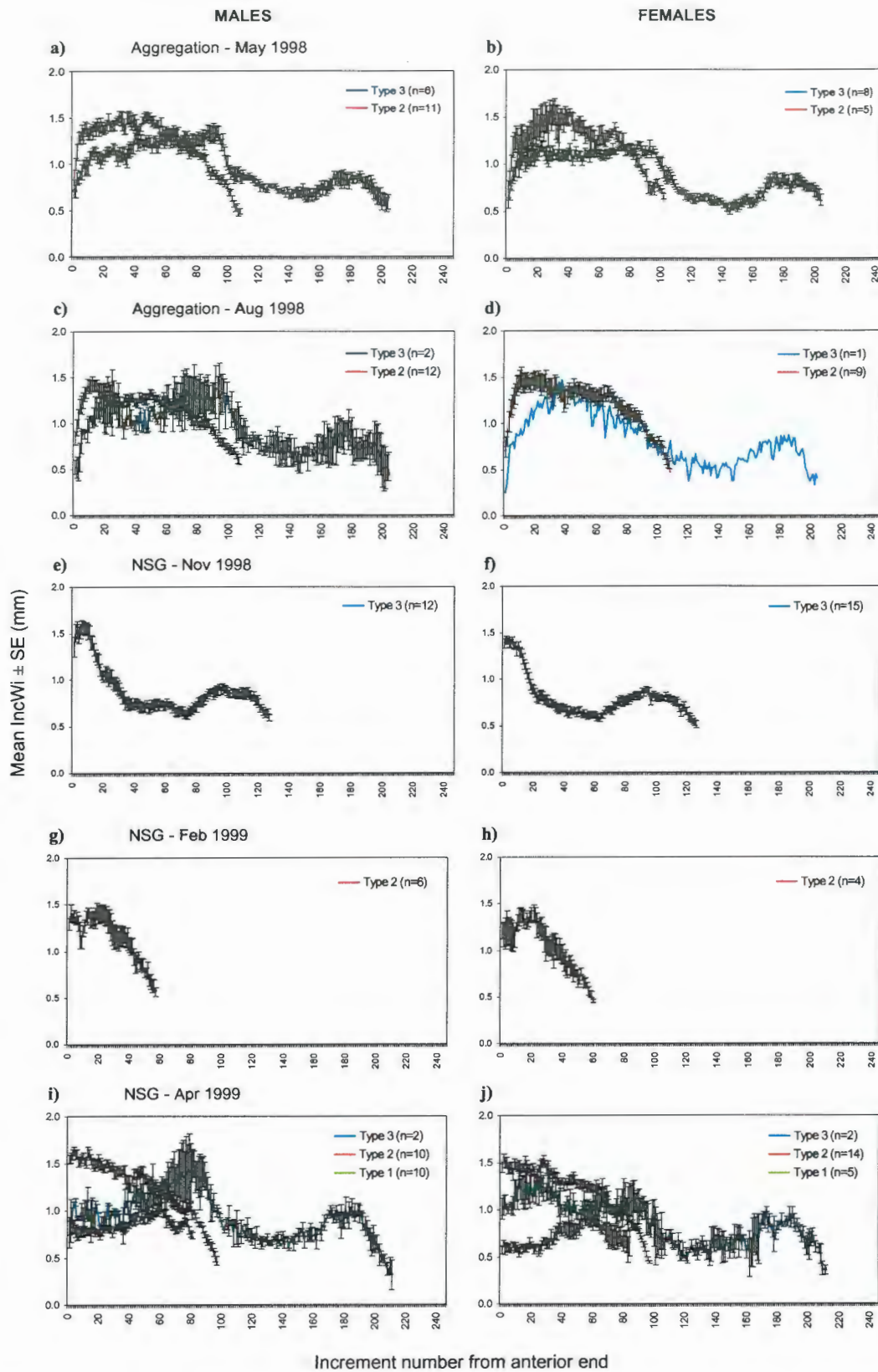


Figure 6.12 Mean IncWi patterns of each bone type in samples from the aggregation are (a-d) and NSG (e-j) collected from May 1998 to April 1999. Standard error bars indicate the level of variation between individual patterns.

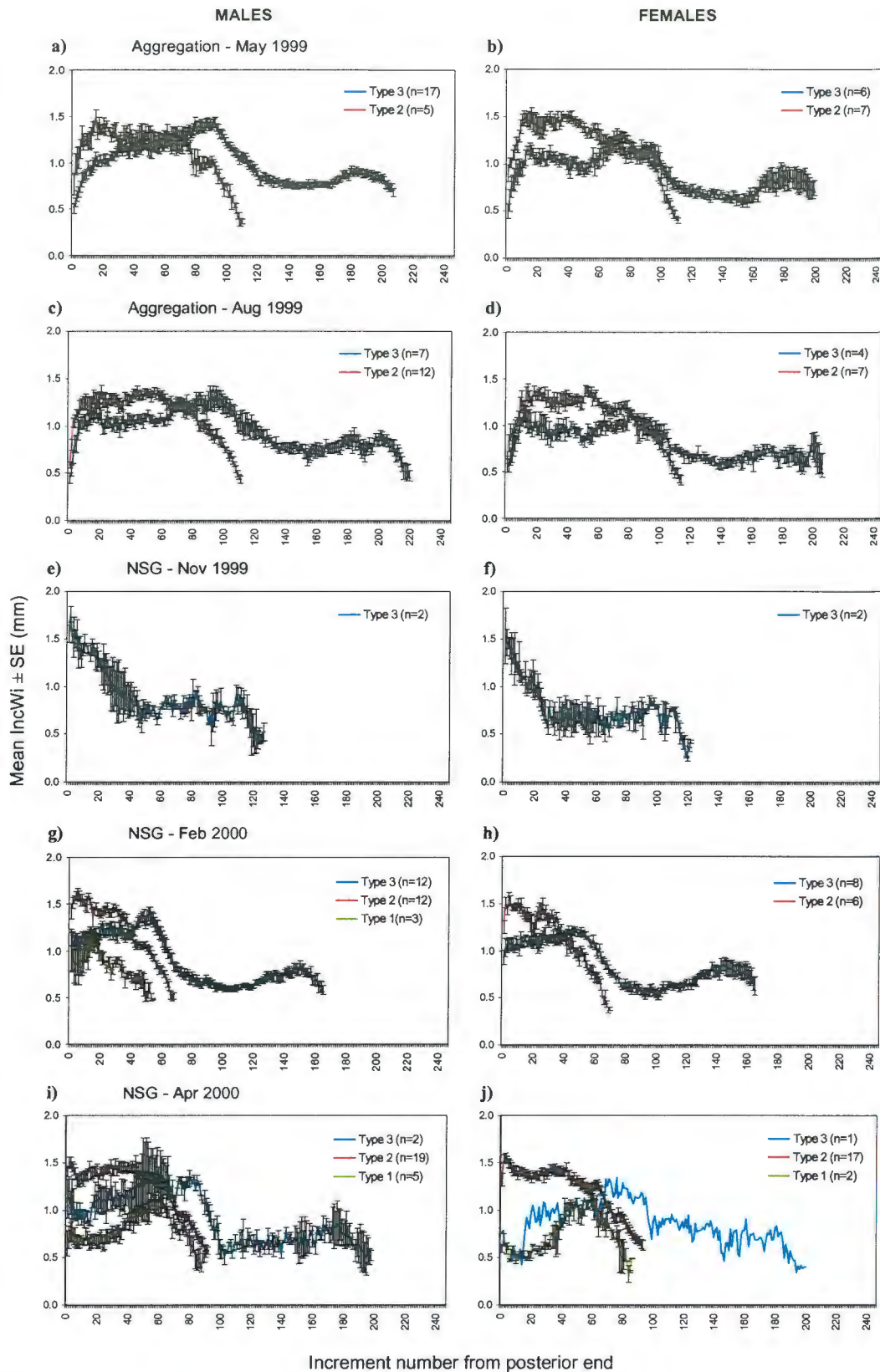


Figure 6.13 Mean IncWi patterns of each bone type in samples from the aggregation are (a-d) and NSG (e-j) collected from May 1999 to April 2000. Standard error bars indicate the level of variation between individual patterns.

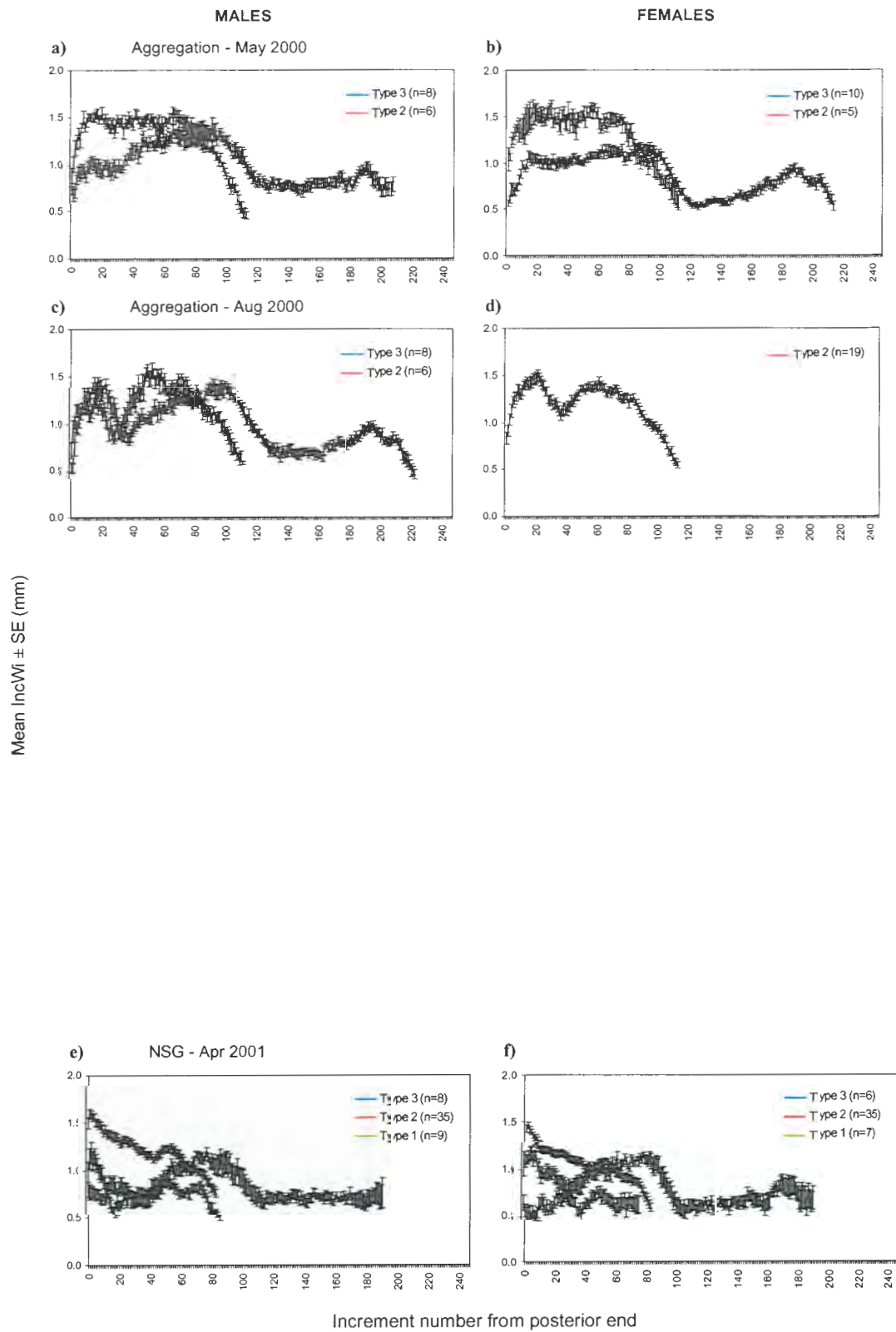


Figure 6.14 Mean IncWi patterns of each bone type in samples from the aggregation are (a-d) and NSG (e-f) collected from May 2000 to April 2001. Standard error bars indicate the level of variation between individual patterns.

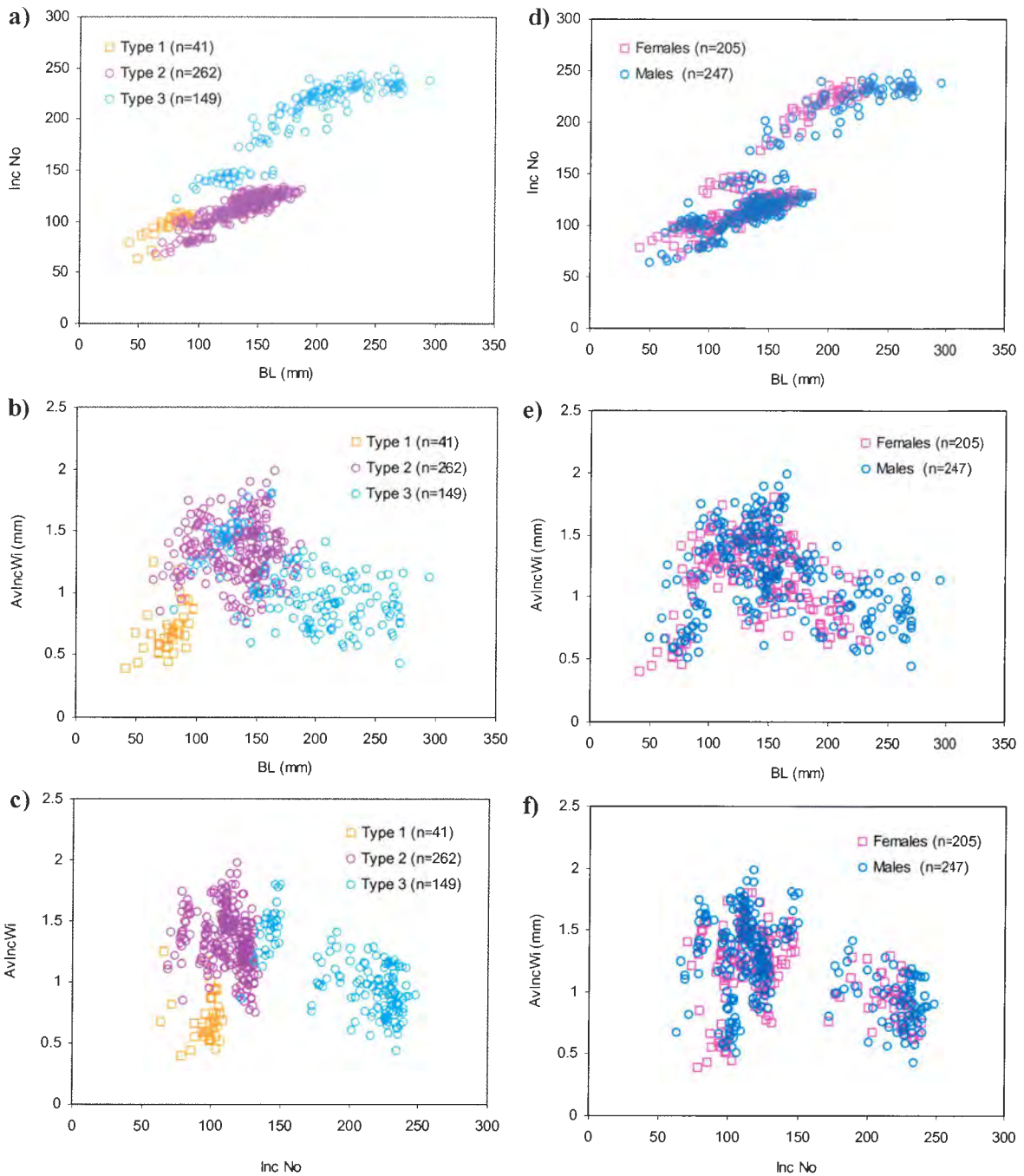


Figure 6.15 Bivariate scatterplots of the three cuttlebone attributes BL, Inc No and AvIncWi grouped according to bone type (a-c) and sex (e-f), for all samples pooled.

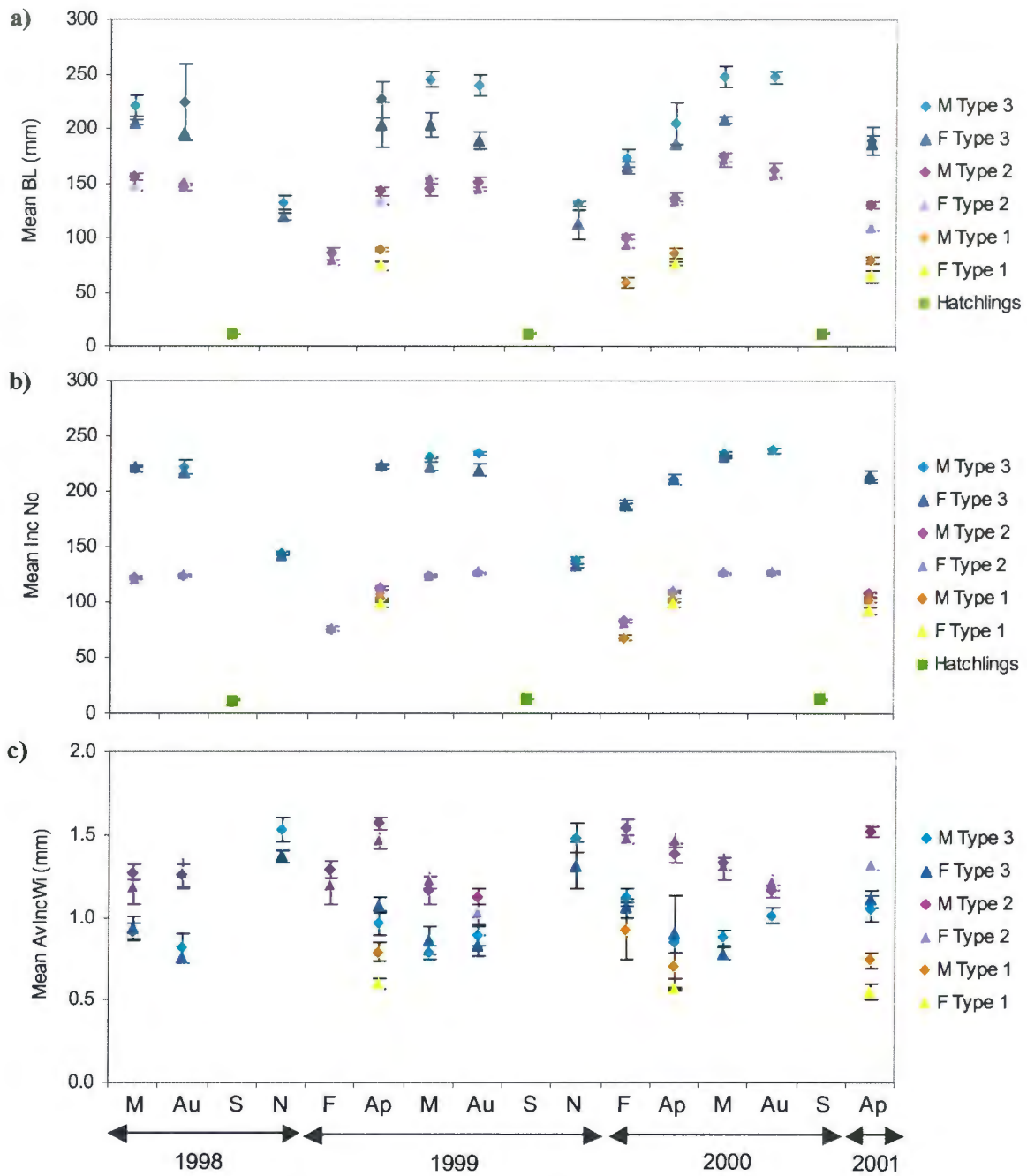


Figure 6.16 Mean BL (a), Inc No (b) and AvIncWi (c) of each bone type and sex combination in different samples from May 1998 to April 2001.

The trends in the variation of mean BL and Inc No suggest that animals with Type 2 and Type 1 bones may be of similar age (same mean Inc No) but that Type 2 individuals reach a larger mean BL for the same time period, probably through higher growth rates. This hypothesis was supported by the trends

in the variation of mean AvIncWi between bone types (Fig. 6.16c). This attribute was assumed to reflect the growth rate of the individual just prior to capture. Type 2 bones had a much higher mean AvIncWi than Type 1 bones in all samples, suggesting a higher growth rate than Type 1 bones sampled at the same time. This was consistent across all sample dates. Mean AvIncWi was also high for Type 3 bones in November, but subsequently declined through February to May. Once again there was little variation in the attribute between the two sexes for any given bone type, particularly relative to the degree of difference between the different bone types.

The results from the statistical analyses were in concordance with the interpretation of graphical results. No between-sample statistical comparisons were possible as not all bone types were represented in every sample or sample sizes were insufficient to analyse for all bone types. Therefore, only within-sample comparisons between bone types and sexes were completed on samples with adequate replication for each combination. The results of the combined MANOVA and individual ANOVA tests for all three attributes are presented in Tables 6.3 and 6.4, respectively. The use of multiple dependent variables and the significant correlation between BL and Inc No meant that multivariate statistical methods were more appropriate in the first instance, and ANOVA's were only used for post hoc investigation of significant main effects or interactions on individual attributes.

In all MANOVA and ANOVA tests there was a highly significant difference ($p = <0.0001-0.0003$) between bone types for all three attributes. This was particularly evident in the extremely high F ratios obtained from ANOVA tests on Inc No (Table 6.4). These statistical results supported the division of the samples into three discrete bone types, which had significantly different characteristics of BL, Inc No and AvIncWi.

In 4 of the 6 samples analysed, a significant difference between the two sexes was also determined for all three attributes combined, based on the results of the MANOVA tests (Table 6.3), and in two of the samples there was also a significant interaction between bone type and sex. These significant interactions suggest that the variation in the attributes with respect to sex depended upon which bone type was under consideration and vice versa. Closer examination of the individual attributes via separate ANOVA tests, indicated that the variation between the sexes was greatest for mean BL and to a lesser extent Inc No, and only significant in one sample (April 1999) for AvIncWi (Table 6.4). In all other samples AvIncWi only showed significant differences between bone types.

Table 6.3 Results of multivariate analysis of variance (MANOVA) test between the different pattern types and sexes using the three variables BL, IncNo and AvIncWi simultaneously. Different samples were tested separately.

| Sample date | n | Sources of variance | Wilks' Λ | F | df | P>F |
|-------------|-----------------|-------------------------|------------------|--------|-------|----------|
| May 1998 | 30 | Type (2,3) | 0.1521 | 69.67 | 2,25 | <0.0001* |
| | | Sex | 0.8138 | 2.86 | 2,25 | 0.0761 |
| | | Type*Sex | 0.9400 | 0.80 | 2,25 | 0.4612 |
| Apr 1999 | 39 [#] | Type (1,2) [#] | 0.1030 | 148.00 | 2,34 | <0.0001* |
| | | Sex | 0.7255 | 6.43 | 2,34 | 0.0043* |
| | | Type*Sex | 0.9391 | 1.10 | 2,34 | 0.3435 |
| May 1999 | 35 | Type (2,3) | 0.2206 | 53.00 | 2,30 | <0.0001* |
| | | Sex | 0.9231 | 1.25 | 2,30 | 0.3009 |
| | | Type*Sex | 0.7519 | 4.95 | 2,30 | 0.0139* |
| Feb 2000 | 38 ^o | Type (2,3) ^o | 0.1267 | 113.74 | 2,33 | <0.0001* |
| | | Sex | 0.8734 | 2.39 | 2,33 | 0.0257* |
| | | Type*Sex | 0.9986 | 0.02 | 2,33 | 0.1643 |
| May 2000 | 29 | Type (2,3) | 0.0673 | 166.42 | 2,24 | <0.0001* |
| | | Sex | 0.6159 | 7.48 | 2,24 | 0.0030* |
| | | Type*Sex | 0.7791 | 3.40 | 2,24 | 0.0500* |
| Apr 2001 | 100 | Type (1,2,3) | 0.1866 | 61.14 | 4,186 | <0.0001* |
| | | Sex | 0.9112 | 4.53 | 2,93 | 0.0132* |
| | | Type*Sex | 0.9452 | 1.33 | 4,186 | 0.2604 |

[#] Type 3 bones (n=3) removed from sample for analysis due to small sample size;

^o Type 1 bones (n=3) removed from sample for analysis due to small sample size;

* significant at the $\alpha = 0.05$ significance level.

The DFA resulted in the description of two discriminant functions with a combined χ^2 value of 543.99 (df = 6; p = 0.000). After removal of the first discriminant function there was still a strong association between the groups and predictors ($\chi^2 = 164.65$; df = 2; p = 0.000). The two functions accounted for 85.7% and 14.3%, respectively, of the between-group variability for males and 89.1% and 10.9%, respectively, for females. The analysis resulted in 100% correct classification of both males and females according to bone type groups when prior probabilities for each group were weighted according to sample sizes.

For both sexes, the first discriminant function maximally separated Type 3 bones from the other two groups, while the second function discriminated Type 1 bones from Type 2 bones (Fig. 6.17). The loading matrix of correlations between predictors and discriminant functions (Table 6.5) indicate that the best predictor for distinguishing Type 3 bones from the other two types was logIncNo (Function 1), whilst AvIncWi and logBL were the best predictors for separating between Type 1 and Type 2 bones (Function 2).

Table 6.4 Results of two-way univariate analysis of variance (ANOVA) test between the different pattern types and sexes for variables of BL, IncNo and AvIncWi. Different samples were tested separately.

| Sample date | n | Sources of Variance | df | Log BL | | Log Inc No | | AvIncWi | |
|-------------|-----------------|-------------------------|----|--------|----------|------------|----------|---------|----------|
| | | | | F | P>F | P>F | P>F | P>F | P>F |
| May 1998 | 30 | Type (2,3) | 1 | 147.29 | <0.0001* | 6301.62 | <0.0001* | 21.14 | <0.0001* |
| | | Sex | 1 | 4.71 | 0.0392 | 0.00 | 0.9859 | 0.18 | 0.6708 |
| | | Type*Sex | 1 | 0.12 | 0.7360 | 1.86 | 0.1842 | 0.72 | 0.4024 |
| Apr 1999 | 39 [#] | Type (1,2) [#] | 1 | 322.00 | <0.0001* | 104.86 | <0.0001* | 223.24 | <0.0001* |
| | | Sex | 1 | 16.23 | 0.0003* | 7.02 | 0.0120* | 7.14 | 0.0114* |
| | | Type*Sex | 1 | 3.93 | 0.0553 | 4.23 | 0.0472 | 0.63 | 0.4339 |
| May 1999 | 35 | Type (2,3) | 1 | 91.57 | <0.0001* | 3297.62 | <0.0001* | 29.22 | <0.0001* |
| | | Sex | 1 | 1.66 | 0.2068 | 1.79 | 0.1906 | 0.92 | 0.3454 |
| | | Type*Sex | 1 | 10.65 | 0.0027* | 4.22 | 0.0484 | 0.01 | 0.9056 |
| Feb 2000 | 38 [‡] | Type (2,3) [‡] | 1 | 207.71 | <0.0001* | 2700.77 | <0.0001* | 62.28 | <0.0001* |
| | | Sex | 1 | 2.25 | 0.1429 | 0.02 | 0.8780 | 1.35 | 0.2537 |
| | | Type*Sex | 1 | 0.13 | 0.7181 | 0.49 | 0.4901 | 0.00 | 0.9811 |
| May 2000 | 29 | Type (2,3) | 1 | 81.92 | <0.0001* | 3600.71 | <0.0001* | 98.49 | <0.0001* |
| | | Sex | 1 | 10.01 | 0.0039* | 0.00 | 0.9860 | 1.45 | 0.2397 |
| | | Type*Sex | 1 | 6.33 | 0.0187 | 1.21 | 0.2824 | 0.55 | 0.4655 |
| Apr 2001 | 100 | Type (1,2,3) | 2 | 139.65 | <0.0001* | 629.11 | <0.0001* | 121.13 | <0.0001* |
| | | Sex | 1 | 11.02 | 0.0013* | 7.14 | 0.0089* | 5.44 | 0.0218 |
| | | Type*Sex | 2 | 1.95 | 0.1483 | 2.65 | 0.0758 | 2.93 | 0.0582 |

[#] Type 3 bones (n = 3) removed from sample for analysis due to small sample size;

[‡] Type 1 bones (n = 3) removed from sample for analysis due to small sample size;

* significant at the $\alpha = 0.05$ significance level; total $\alpha = 0.044$.

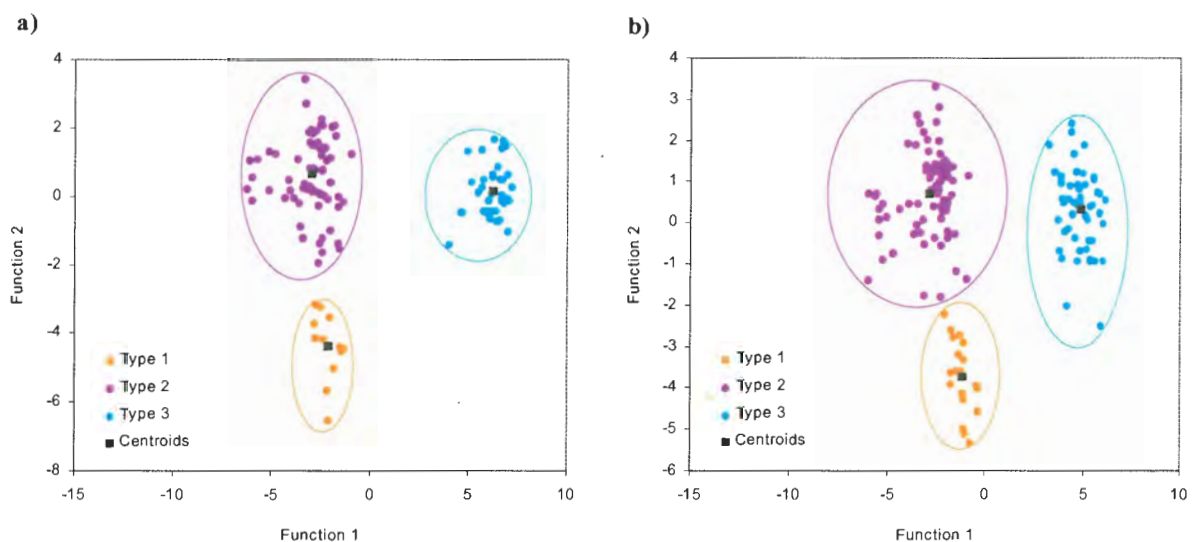


Figure 6.17 Scatterplots of first two discriminant function scores for male (a) and female (b) *S. apama* derived using three the cuttlebone variables log IncNo, log BL and AvIncWi. Group centroids for each bone type group are also indicated.

Table 6.5 Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions.

| Sex | Variable | Function 1 | Function 2 |
|---------|-----------|------------|------------|
| Males | Log IncNo | 0.796* | 0.415 |
| | Log BL | 0.369 | 0.711* |
| | AvIncWi | -0.293 | 0.630* |
| Females | Log IncNo | 0.726* | 0.369 |
| | AvIncWi | -0.193 | 0.796* |
| | Log BL | 0.302 | 0.668* |

* Largest absolute correlation between each variable and any discriminant function.

To assess the periodicity of increment formation, the difference in mean Inc No of each bone type between successive sample dates was divided by the number of elapsed days in the time interval (Table 6.6). A visual representation of these "growth periods" is provided in Fig. 6.18, where the mean IncNo at each successive sample date was superimposed on the final IncWi patterns observed in Type 2 and Type 3 bones at the aggregation area. As the exact hatch date could not be determined from analysis of the cuttlebone microstructure a range of possible values for hatch dates between 1 September and 31 October were used to calculate the rates for the first growth period. These initial rates of increment formation were higher for Type 2 bones (1.6-2.4 days per increment) than Type 1 bones (2.0-3.0 days per increment). During the second growth period, however, from February to April, this was reversed and Type 1 bones had a very rapid rate of increment formation (1.4 days per increment), but were narrow in comparison to those from Type 2 bones (Fig. 6.18).

Data for Type 1 bones were combined with that of Type 3 bones to investigate the potential connection between the two. The similarity in the IncWi pattern of the early part of the Type 3 bone with that of the Type 1 bones, suggests that the Type 1 bones grow and become Type 3 bones in the subsequent year. No Type 1 bones were found in the aggregation area samples during winter but quite advanced Type 3 bones were found in November not long after the hatching season. If these were the Type 1 bones from April, a much slower rate of increment deposition must occur during the time period (almost 6 days per increment) (Table 6.6). For all growth periods after November, the rate of increment formation for Type 3 bones was much higher at around 2 days per increment.

The implications of these findings in relation to the possible age composition of the NSG population are explored in Fig. 6.19, which shows the size distribution of individuals of the April 2001 NSG sample, and Fig. 6.20 for the aggregation area population in May 2000.

Table 6.6 Mean Inc No of Type 2 and Type 3 bones at successive sample dates, differences in the number of days between sample dates and the mean Inc No over that time period, and corresponding number of days per increment. Type 1 bones also included in the Type 3 analysis.

| Bone type | Sample date | Mean Inc No | No days difference | Inc No difference | No days/Inc |
|-------------------------------|-----------------------------|-------------|------------------------|-------------------|------------------------|
| Hatchling Type 2 | 1 Sep - 31 Oct [#] | 11 | | | |
| | 20 Feb | 82 | 172 - 112 [#] | 71 | 2.4 - 1.6 [#] |
| | 8 Apr | 110 | 47 | 28 | 1.7 |
| | 19 May | 128 | 35 | 18 | 2.3 |
| Hatchling Type 1 Type 3 | 1 Sep - 31 Oct [#] | 11 | | | |
| | 20 Feb | 67 | 172 - 112 [#] | 56 | 3.1 - 2.0 [#] |
| | 8 Apr | 101 | 47 | 34 | 1.4 |
| | 7 Nov | 137 | 213 | 36 | 5.9 |
| | 20 Feb | 186 | 105 | 49 | 2.1 |
| | 8 Apr | 211 | 47 | 25 | 1.9 |
| | 19 May | 233 | 41 | 22 | 1.9 |

[#] potential range of values created by the variation in hatch date from either early (1 Sep) or late (31 Oct) in the hatching period.

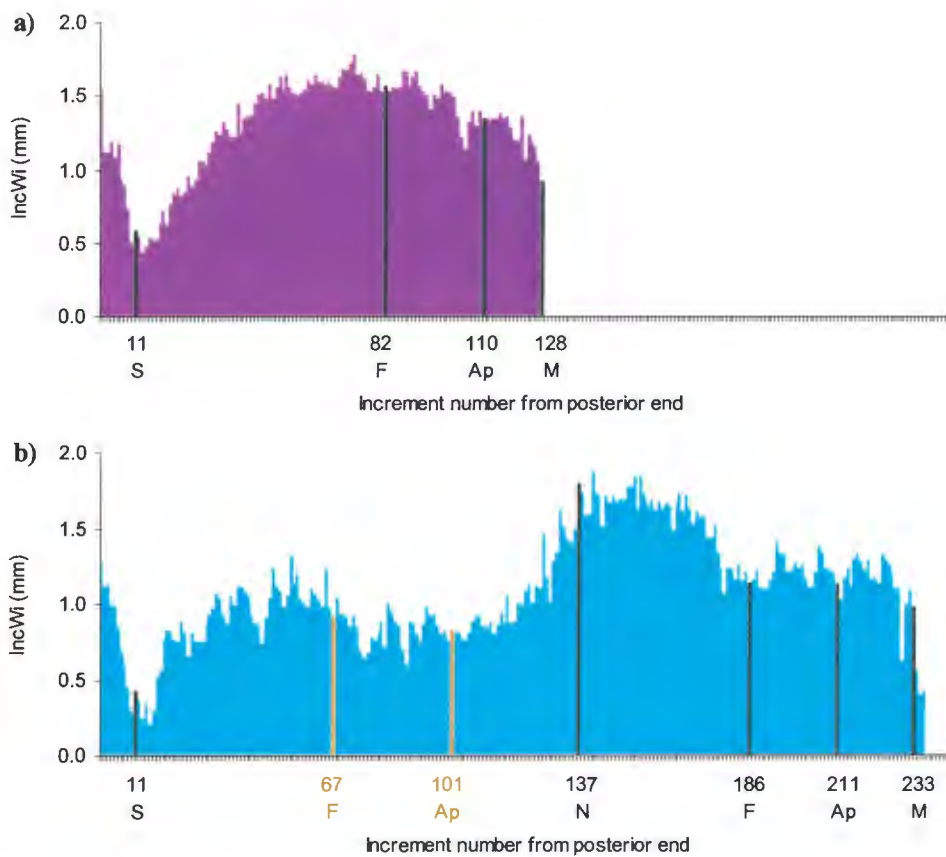


Figure 6.18 Two types of IncWi pattern in cuttlebones of adults from the aggregation area in May; Type 2 (a) and Type 3 (b). The mean Inc No of each at successive sample dates is superimposed on the graphs to provide a time reference. Type 1 means are also indicated in orange on the Type 3 bone pattern for comparison. Letters indicate months: S = September, F = February, Ap = April and M = May.

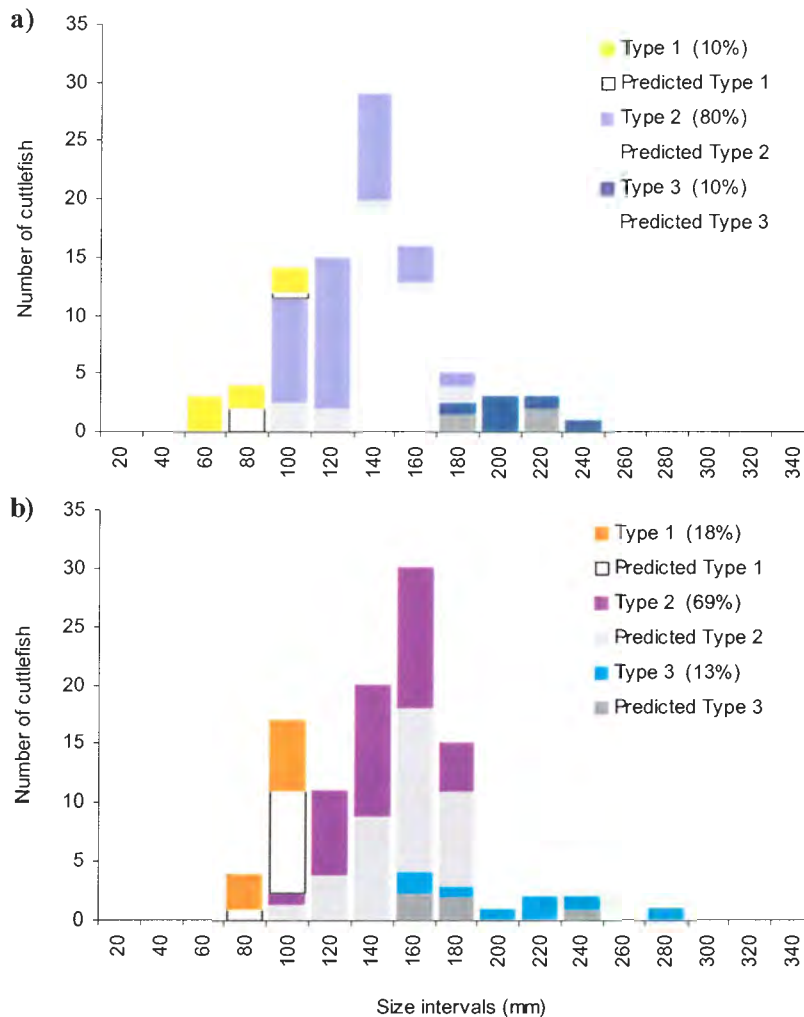


Figure 6.19 Length frequency histograms of female (a) and male (b) *S. apama* in the NSG sample collected in April 2001, with the proportion of different bone types in each size interval indicated. Bone types of non-aged individuals were predicted based on the frequencies of analysed bone types in each size class.

A wide size distribution of both sexes was evident in the NSG sample from April 2001. Although the female distribution appeared unimodal the individuals were divisible into three groups according to bone type (Fig. 6.19a). The Type 2 group accounted for 80% of the individuals, whilst Type 1 and Type 3 groups accounted for only 10% each. The male size distribution showed at least 3 size modes, which were concurrent with the division of the individuals into three groups according to bone type (Fig. 6.19b). For males the Type 2 group accounted for only 69% of the total due to a larger percentage (18%) of Type 1 males.

At the spawning aggregation area in May 2000 the females conformed to a unimodal size distribution with a mode of 220 mm ML (Fig. 6.20a). These were divisible into two bone types, i.e. Type 2 and Type 3, accounting for 34% and 66% of the total respectively. The size distribution of the males was skewed to the right, also had a mode of 220 mm ML but had a small number of large individuals as well (Fig. 6.20b). Males were also divisible into two bone types, with Type 2 individuals contributing 58% and Type 3 contributing 42% of individuals. Based on the size distributions alone a single year class of females and a larger proportion of young males was predicted.

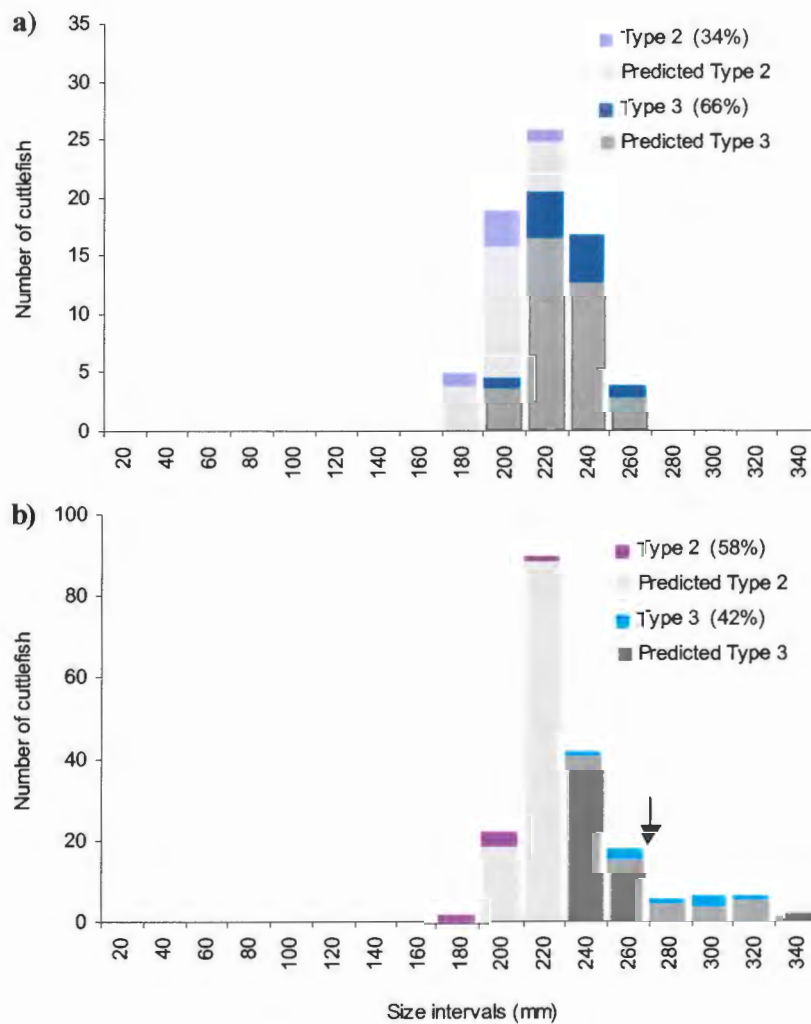


Figure 6.20 Length frequency histograms of female (a) and male (b) *S. apama* in the aggregation sample collected in May 2000, with the proportion of different bone types in each size interval indicated. Bone types of non-aged individuals on transects were predicted based on the frequencies of analysed bone types in each size class. The arrow in (b) indicates the original division of small and large males based on the size distribution alone.

6.4.5 Adult age validation experiments

The injection of calcein successfully marked the adult cuttlebones. The day after injection the normally white parts of the cuttlefish were coloured fluorescent green, indicating that the dye had spread throughout the body. The dye was taken up by many of the chambers already present in the cuttlebone so a distinct band that corresponded to the time of marking was not evident. Because of this complication for the purposes of this study, growth after injection was taken to include all recent non-stained increments and the last prominently stained one. The number of increments deposited in the cuttlebone following marking was very small relative to the number of days after marking, and all increments were very narrow (< 1mm) (Table 6.7). There was no apparent relationship between temperature treatment and the number or width of increments in 1999.

Table 6.7 Number of increments deposited in the adult bone after injection with calcein, in relation to the number of days elapsed, sex and water temperature.

| Year | Temp | ID No. | Sex | ML (mm) | No. days after marking | Total IncNo | Bone Type | IncNo after marking | AvIncWi (mm) |
|------|------|--------|-----|---------|------------------------|-------------|-----------|---------------------|--------------|
| 1998 | Amb | T1A | F | 205 | 35 | NA | NA | 1 | |
| | Amb | T1B | M | 210 | 111 | NA | NA | 4 | |
| | Amb | T2A | F | 205 | 2 | NA | NA | 0 | |
| | Amb | T2B | M | 243 | 65 | NA | NA | 2 | |
| | Amb | T3A | F | 213 | 28 | NA | NA | 1 | |
| | Amb | T3B | M | 232 | 30 | NA | NA | 2 | |
| | Amb | T4B | M | 230 | 39 | NA | NA | 2 | |
| | Amb | T5B | M | 190 | 2 | NA | NA | 0 | |
| | Amb | T6B | M | 220 | 13 | NA | NA | 1 | |
| | Amb | T7B | M | 260 | 30 | NA | NA | 2 | |
| 1999 | 16 | T4A | F | 200 | 46 | 229 | 3 | 1 | 0.39 |
| | 16 | T5A | F | 215 | 64 | 213 | 3 | 1 | 0.60 |
| | 16 | T5B | M | 205 | 63 | 205 | 3 | 4 | 0.63 ± 0.11 |
| | 16 | T6B | M | 310 | 41 | 234 | 3 | 3 | 0.81 ± 0.19 |
| | 20 | T7A | F | 195 | 4 | 229 | 3 | 0 | |
| | 20 | T8A | F | 195 | 11 | - | - | 0 | |
| | 20 | T8B | M | 200 | 34 | 204 | 3 | 1 | 0.34 |
| | 20 | T9A | F | 173 | 4 | - | - | 0 | |
| | Amb | T10A | F | 210 | 31 | 221 | 3 | 1 | 0.26 |
| | Amb | T11A | F | 210 | 31 | 227 | 3 | 1 | 0.56 |
| | Amb | T12A | F | 205 | 30 | 227 | 3 | 1 | 0.53 |
| | Amb | T12B | M | 230 | 95 | 219 | 3 | 2 | 0.45 ± 0.07 |

NA: cuttlebones in 1998 were not processed for age estimation as the individuals were taken from the GSV and the general trends relating growth increment patterns to age classes have not been established for that Gulf.

The minimal growth of the adult cuttlebones after marking most likely related to the poor nutritional condition of the captive animals. Most fed for only 2 months in aquaria before losing interest in food or showing difficulty in capturing prey. Once normal feeding ceased the individuals gradually became more buoyant until they were floating constantly on the surface. This has previously been described as symptomatic of starvation in cuttlefish (Oestmann et al. 1997). Death usually occurred soon after or the individual was terminated. Overall, the results indicated that during periods of minimal growth rate the periodicity of increment formation in the adult cuttlebone was very slow and increments deposited were very narrow.

6.5 Discussion

The analysis of regular growth increments in the cuttlebones of *S. apama* demonstrated a definite polymorphism in the growth patterns of both sexes of cuttlefish in the northern Spencer Gulf. Three distinct bone types were identified based on the patterns of increments widths over the length of the bones. All bones conformed to one of the three types, and the patterns were consistent between the three years of sampling. The three bone types differed significantly in BL, Inc No and AvIncWi just prior to capture.

Type 1 bones were only found in the smallest individuals of the February and April NSG samples. The bones were typified by a single section of increased IncWi from hatching to February, followed by a decline in IncWi to April. They had a similar Inc No as Type 2 bones but a much shorter BL and smaller AvIncWi at the time of capture. Type 1 bones were not found in any individuals collected from the spawning aggregation area.

Type 2 bones were found in all samples except those collected in November. They were the most abundant type, and were characterised by a rapid increase in IncWi from hatching until April, forming one extended section of very wide increments, with a slight decline in AvIncWi in bones collected in May. These bones had the highest AvIncWi just prior to capture in all samples, suggesting higher growth rates. Type 2 bones were found in all small mature cuttlefish of both sexes from the aggregation area.

Type 3 bones were apparent in NSG samples in November following the winter spawning season. The bones were typified by an initial increase in IncWi similar to Type 1 bones, followed by a section of narrower increments and then a second section of much wider IncWi. The BL and IncNo increased from November until the following May, at which time they were found in all large mature cuttlefish

of both sexes at the aggregation area. The mean Inc No of these bones was approximately double that of Type 2 bones collected at the same time.

This polymorphism of growth patterns in the cuttlebones of *S. apama* suggests the existence of two alternative life cycles. The first, represented by the Type 2 bone pattern, involves rapid growth during the juvenile phase over the first summer, such that maturity is reached within 7-8 months and the animal returns to the aggregation area to spawn in the first spawning season as a small individual. The second, represented by the Type 1 and Type 3 bone patterns, involves much slower growth during the juvenile phase over the first summer, and animals defer maturity until the following year when they are much larger. Therefore, these animals return to spawn in their second year as large individuals. This implies the age composition of the population consists of two year classes of both sexes in the NSG population between February and April and at the aggregation area between May and August. This explanation adequately accounts for the wide size distribution recorded for males, but was a surprising result for females, which had a narrower unimodal size distribution.

Based on the different early growth patterns of the cuttlebone microstructure of each year class, neither appears to return to the spawning aggregation in the following year to spawn a second time. There were no Type 2 bones found with a second region of wide increments, that would be indicative of smaller animals returning to spawn again the following year; and there were no bones with three sections of wide increments, which would be expected if the larger animals survived beyond the end of the spawning season. All fully developed Type 2 and Type 3 bones disappeared from samples following the end of the spawning season in August, with a new cohort of Type 3 bones found in November. Whether this means all animals from the aggregation area die after spawning or move elsewhere is not known, but they do not reappear at the aggregation area, nor are they found more widely in the northern Spencer Gulf during the subsequent summer. These observations point to *S. apama* being semelparous irrespective of life cycle type or sex.

The above interpretation of results relies on a number of assumptions: (1) that the width of increments in the cuttlebones are related to growth rate, which is in turn related to seasonal fluctuations in water temperature; (2) that Type 1 bones found in NSG samples collected during February and April, undergo very slow growth over the winter to become the Type 3 bones found in November; (3) that all individuals in the northern Gulf are spawned during the winter (May to August) and hatch during the spring (September to October); and (4) that the consecutive samples from the wild populations are not confounded by the migration of different cohorts into or out of the area. The feasibility of each of these assumptions in relation to the biology of *S. apama* and that of other *Sepia* species is considered below.

Relationship between increment width and growth rate

Marginal increment analysis of the variation in AvIncWi of bones from different sampling dates, provided evidence that width of increments is related to water temperature. Bones collected during the warmer months of November and February had much wider AvIncWi than those collected during the cooler months of April, May and August. A similar result was found for cuttlebones of *S. officinalis* collected from the English Channel (Le Goff et al. 1998; Hewitt and Stait 1998) that had been collected at regular monthly intervals over two years. For the latter species there was also noted two characteristic patterns in the distribution of increment widths along the length of the bone, and distinct zones of narrow increments, which corresponded to winter periods.

Unfortunately, to date experimental studies that have investigated the effects of water temperature on cuttlebone growth of *Sepia* reared in captivity have been primarily concerned with the rate of increment formation, and have only made casual reference to the variation in increment width. Nevertheless, all have observed "narrow tightly spaced" increments deposited in cuttlebones at colder temperatures (e.g. Richard 1969). This essential assumption requires experimental verification for *S. apama* to ensure confidence in the current interpretation of wild population results, and will be addressed in Chapter 7 through the rearing of juvenile *S. apama* in aquaria under a variety of temperature and feeding regimes.

Relationship between Type 1 and Type 3 bones

The similarity between the early pattern of Type 3 and Type 1 bones suggests that they constitute the same life cycle but 12 months apart. This interpretation forms the basis of the predicted age composition of 2 year classes. For this to be so, the rate of increment formation must decrease concurrently with a decrease in IncWi. This has been verified for *S. officinalis* that were experimentally reared in captivity at a number of different water temperatures (Richard 1969; Ré and Narciso 1994; Bettencourt and Guerra 2001). Richard (1969) found the rate of increment formation decreased with decreasing temperature. At 25°C one increment was deposited every 1.6 days, which decreased at each successive drop in temperature to one increment every 2.6 days at 20°C, 4.3 days at 15°C and 5.4 days at 13°C. There was also a decrease in the periodicity of increment formation from 1 every 3 days in summer to 1 every 12 days during the winter for wild populations of *S. officinalis* (Hewitt and Stait 1994). Water temperatures in northern Spencer Gulf decrease to 11-12°C during mid winter; therefore the predicted rate of 5.8 days per increment necessary for Type 1 bones in April to become Type 3 bones in November is considered likely. The rate of increment formation in the cuttlebones of juvenile

S. apama was also investigated in aquarium experiments in relation to water temperature and food availability and will be considered in Chapter 7.

Factors other than variation in water temperature have also been related to changes in either the rate of formation or width of increments in cuttlebones of other *Sepia* species. Malnutrition resulted in the deposition of narrow increments at a slower rate in the cuttlebones of *S. officinalis* reared under suboptimal feeding regimes in captivity (Boletzky 1974a). Certainly the adults maintained in aquaria in this study showed very low rates of increment deposition in their cuttlebones in response to starvation. However, these individuals were most likely near the end of their lives, which may have caused cuttlebone growth to virtually cease. Most individuals died by the end of August, which coincided with the disappearance of wild animals from the spawning aggregation area from where they were caught. At other stages of the life cycle, periods of poor nutrition may not have as drastic an effect on cuttlebone growth. Nevertheless, fluctuations in food availability not coincidental with seasonal variation in water temperature may confound the interpretation of patterns of cuttlebone increments.

Migration has also been suggested as a factor that may decrease the rate of increment formation in the cuttlebone irrespective of water temperature. Natsukari et al. (1991) provided evidence for a decrease in the rate of increment formation in the cuttlebones of *S. esculenta* due to migration from a semi-closed inlet to offshore waters, despite insignificant differences in summer temperatures between the two areas. However, as with most studies of wild populations, the potential complication of successive sampling of different cohorts could not be ruled out as an explanation for the differences observed.

Spawning and hatching times

An alternative hypothesis for the polymorphism in bone types is that the Type 3 bones from November derive from individuals that are spawned and hatched elsewhere at a different time, presumably during the summer or autumn, and are thus aged less than 12 months. Multiple spawning periods or hatch dates have been used as an explanation for the variation in growth patterns observed for many squid species, even in the absence of evidence for multiple spawning periods (Macy 1995; Brodziak and Macy 1996; Macy and Brodziak 2001). There is no evidence to support this for *S. apama*. Only winter spawning has ever been recorded across the species' distribution. Most individuals collected from northern Spencer Gulf during the summer months were immature or maturing (Chapter 8). All eggs monitored in situ at the aggregation area (Chapter 7) hatched between September and October, a relatively short hatching period. Thus, all evidence to date supports the hypothesis that all individuals in northern Spencer Gulf are spawned during winter and hatch in spring.

Appropriate evidence is not available to make similar conclusions for all other areas of the Gulf or State waters. Therefore, it is possible that spawning may occur at other times of the year elsewhere, and the resulting individuals may migrate into the northern Gulf and contribute to the overall composition of the NSG population. If this was the case the polymorphism in patterns of growth increments result from the mixing of different stocks rather than year classes. However, the consistency in patterns for each bone type at each sampling time, suggest that the animals experience similar environmental conditions. This implies that migration would need to be synchronised en masse to produce such consistency. Microchemical techniques applied to either the statoliths or cuttlebones, that track the changes in chemical composition of pre-hatch or juvenile portions to adult sections, or compared between the different bone types, may provide information on stock structure and help elucidate potential migration patterns of the different life cycle types (Campana 1999).

Overall, the possibility that the Type 3 bones found in November are less than 12 months old cannot be ruled out. However, the current evidence suggests that all the individuals are hatched at the same time, and that the variation in bone structure is produced by individuals experiencing different life cycles, rather than the mixing of different micro-cohorts or stocks. Thus the most parsimonious view at present is that the population of *S. apama* in the northern Spencer Gulf consists of 2 distinct year classes.

7 Egg development, juvenile growth and age validation

K.C. Hall

7.1 Introduction

Most cephalopod species have a short life cycle with only one spawning event following maturity. Thus the juvenile or immature phase accounts for a large proportion of the life cycle and the growth rates throughout this period influence many life history characteristics, such as the size and age at sexual maturity, the potential reproductive output and life span (Van Heukelem 1979). Furthermore, the growth pattern is usually exponential during the juvenile phase, such that even small variations in growth rate may translate into large differences in adult characteristics (Forsythe and Van Heukelem 1987; Forsythe 1993).

The energy available for growth is dictated by the energy budget of an individual (O'Dor and Wells 1987; Wells and Clarke 1996), which can be represented by the following equation:

$$E_{\text{ingested}} = E_{\text{metabolism}} + E_{\text{growth}} + E_{\text{excreted}}$$

The upper limit to this energy budget is set by the amount of energy ingested, and growth rate is primarily determined by the balance between ingestion and metabolic rate (Van der Veer et al. 1994). These two rate processes are influenced by many abiotic and biotic factors, of which food availability, water temperature and body size are generally considered the most important (Forsythe and Van Heukelem 1987).

Water temperature has a significant influence on most rate processes that affect the body of ectotherms, such as fish and cephalopods. Increases in temperature, within the normal thermal tolerance range of a species, lead to a rapid increase in the rate of ingestion and concurrent gradual rise in the rate of metabolism (Willmer et al. 2000) (Fig. 7.1). Thus, in situations of unlimited food availability, increases in temperature generally result in an increased rate of growth. However, as water temperatures approach the upper limit of the tolerance range of a species or in situations of limited food availability the response is more complicated, and the rate of ingestion may not increase sufficiently to compensate for the increase in metabolic rate which ultimately results in a decline in the rate of growth.

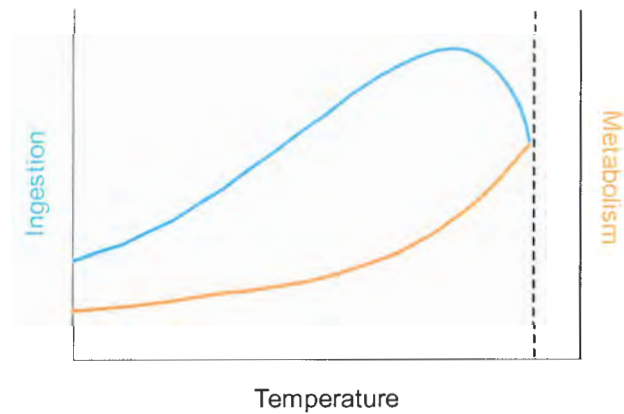


Figure 7.1 The influence of temperature on the rates of ingestion and metabolism in fish. The vertical line indicates the upper thermal tolerance for the species. (Redrawn from Willmer et al. 2000).

Prey abundance and water temperature vary both spatially and temporally in the marine environment even over small scales (Van der Veer et al. 1994). Many cephalopod species have extended spawning periods, such that egg development occurs over a wide range of temperatures and individual hatchlings develop at different rates and hatch over a period of time. Thus different batches of hatchlings may be exposed to vastly different temperature and food regimes, leading to high variation in juvenile growth rates (Forsythe 1993). Furthermore, as growth rate also varies with body size, considerable variation in the initial size of hatchlings may propagate into even greater variation in growth rates later on.

Cephalopods produce relatively large eggs compared with other similar-sized invertebrates, which undergo lengthy direct development into hatchlings that closely resemble miniature adults (Boletzky 1974b). No true larval forms are known, although many squid and octopus eggs undergo continuous development to produce planktonic hatchlings (Boletzky 1974b). Such species tend to produce relatively small eggs with little yolk. In comparison, most cuttlefish species produce large eggs with a high yolk content, which undergo extra growth during the late developmental stages to produce well-developed benthic hatchlings. The duration of embryonic development is negatively related to temperature in all species (Bouchaud 1991). *Sepia officinalis* development time dropped from 150 days at 12°C to 33 days at 24°C (Bouchaud and Daguzan 1989). The efficiency of yolk utilisation is also influenced by temperature with more efficient yolk consumption at low temperatures resulting in larger hatchlings. The size of *S. officinalis* hatchlings developed at 15°C was twice that of those developed at 24°C (Bouchaud 1991).

The lemon-shaped eggs of *S. apama* are the largest known decapod mollusc eggs, and they undergo a long period of development (Cronin 2000). They are found attached to the underside of crevices, rocks or overhangs in the subtidal reef areas of temperate Australia. The eggs are laid during early winter and left unattended during the 3-5 month developmental period. When eggs were experimentally incubated in captivity at 12°C, which is equivalent to the coldest temperature in the field, the developmental period was 160 days (Cronin and Seymour 2000). Since *S. apama* eggs are deposited at the aggregation area over a period of 4 months (May to August) and the eggs have a long developmental period, I hypothesise that eggs laid on different dates will have different development times, that hatching will occur over a range of dates and hatchlings will vary in size. Thus, successive hatchlings would be exposed to different water temperatures and food regimes, resulting in different juvenile growth rates.

7.2 Aims

Analysis of the internal microstructure of the cuttlebones of adult *S. apama* indicated the existence of two different life cycle types in northern Spencer Gulf, which appear to have different juvenile growth rates (Chapter 6). The aims of this Chapter primarily relate to the validation of the aging technique used and the investigation of possible mechanisms that facilitate the development of two different life cycle pathways. A combination of field monitoring and experimental techniques were used to address the following specific aims:

- (1) to determine the variation in water temperature and abundance of *S. apama* eggs at the aggregation area in relation to the timing of egg development and hatching in the wild and the potential survival rates of eggs and hatchlings;
- (2) to investigate the relative influence of original egg size, maternal size or age class and water temperature on egg development, hatchling size and subsequent juvenile growth rates;
- (3) to investigate the relative influence of initial hatchling size, water temperature and food availability on the periodicity and width of growth increments deposited in the cuttlebones of juveniles; and
- (4) to determine any correlations between the attributes of the pre-hatch portion of cuttlebones from juveniles reared under different conditions in captivity and the pre-hatch portion of cuttlebones from adults of the two different life cycles, which may indicate which juveniles are most likely to adopt each life cycle type.

7.3 Methods

7.3.1 Egg development in situ

S. apama eggs were monitored at Black Point from March to November 2000. Three plots of 10x10 m, approximately 300 m apart and within the 3-5 m depth range were selected in March 2000 prior to the arrival of cuttlefish. This avoided any bias relating to the presence of eggs or cuttlefish. However, the plots were purposefully located with the urchin zone, where the highest densities of cuttlefish were observed on transects. Within each plot, 6 rock slabs of similar size (mean underside surface area = $0.29 \text{ m}^2 \pm 0.02 \text{ SE}$; $n = 18$) were selected and individually labelled. Each rock was replaced in its original position, although with one side slightly raised to minimise damage to eggs when it was lifted and lowered for egg counting.

The rocks were checked and eggs counted and staged on each sampling occasion (Chapter 3). A broad scale of egg development that could be applied in situ underwater was developed, based on the external appearance of the egg capsule, size of the egg and visibility and appearance of embryo within (see results for more detail). In addition, an image of each egg rock was recorded with an underwater digital video camera for analysis back in the laboratory. For rocks with high densities of eggs, multiple close ups were used to ensure a clear resolution of all eggs. From the images individual egg batches could be identified, counted and their development followed through successive images. These were cross-referenced with the counts and stages recorded underwater.

Ambient water temperature was monitored at Black Point from May 1999 to November 2000 with submersible inductive temperature data loggers (Dataflow Systems Pty. Ltd.). The loggers were secured approximately 20cm above the substrate in the central plot and changed every 3-6 months to recharge batteries. An independent measure of water temperature was taken during each exchange using a mercury thermometer to validate recorded temperatures. The loggers were programmed to record a temperature every hour, averaged from readings taken every 10 min. After collection, the data were downloaded and raw counts (voltage) translated to degrees Celsius using individual logger calibration information.

7.3.2 Egg development experiment

Eggs of *S. apama* were collected with SCUBA from the aggregation area on 3 June, 17 June and 3 August 2000. Females continuously ovipositing in a single location were identified via the distinctive head and arm postures associated with egg-laying (described in Chapter 9) and the location of deposition isolated. The identified rock was carefully turned over to expose the eggs attached to the underside. Capsules of fresh eggs remain soft and transparent for approximately 1 h after deposition and then become progressively more opaque and white. By collecting only fresh eggs, their deposition time was known to within the hour. Each egg was removed by gently prising the basal ring from the surface of the rock using an abalone iron. The eggs from each female were kept separate in individually labelled, fine-mesh bags and placed inside a weighted collection bucket underwater.

The mantle length of the female was estimated underwater and classified as either small (< 200 mm ML) or large (>200 mm ML). On the second and third collection trips the female was also captured with a hand net and individually stored. This allowed the size of collected eggs to be related to the size and age class (Chapter 6) of the maternal female. Eggs were kept in the bags and placed in a 40 L insulated container with fresh seawater and constant aeration for the 6 h journey to the aquarium facility. Using these methods 170 eggs were collected on 3 June, 50 eggs and 10 females on 17 June and 72 eggs and 11 females on 3 August.

The eggs were held in the transport container with constant aeration for less than 24 h before being transferred to the experimental set-up in the outdoor aquarium facility at SAASC (Chapter 3). Three temperature treatments were used, corresponding to the natural range of water temperatures at the aggregation area during the developmental period: (1) constant 16°C; (2) constant 20°C; and (3) variable ambient (Amb) water temperature. A fourth treatment of constant 12°C, equivalent to the coldest water temperatures recorded during winter, was trialed in a pilot study in 1999, but as egg development failed (contrary to Cronin 2000) the temperature was not used in 2000. The constant water temperature treatments of 16°C and 20°C were increased by 2°C on 5 October to 18°C and 22°C respectively, to simulate the rapid rise in ambient water temperature at that time (Fig. 7.2).

The eggs collected on 3 June formed the basis of the main experiment (Fig. 7.3a). Eggs were weighed (Egg Wt) and divided into two groups (large and small) based on maternal size or age class (i.e. type 2 or type 3 bones, respectively) for those where the female was also collected. Eggs from each maternal group were randomly allocated to the three temperature treatments resulting in 42 eggs from each per

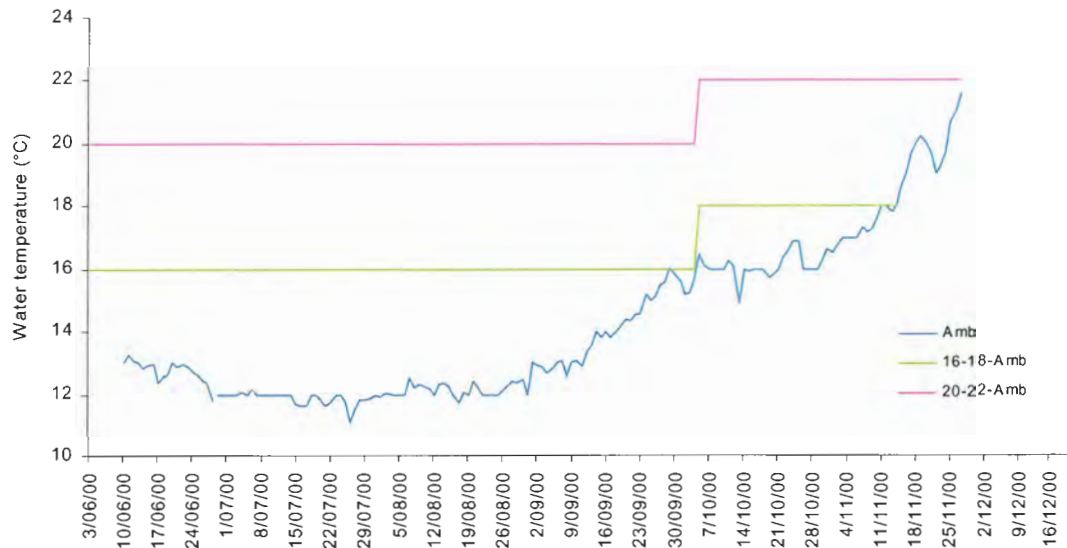


Figure 7.2 Tank water temperatures during egg development and juvenile rearing experiments. Constant water temperature treatments were increased by 2°C on 5 October to keep in line with the rapid rise in ambient water temperature.

treatment. To mimic the orientation of eggs in the wild, eggs were suspended from a small polystyrene float via nylon yarn looped around the stalk and each egg was individually labelled. Four replicate eggs were randomly assigned to each development “tub”, with seven tubs per temperature treatment. The tubs consisted of a 2 L plastic container fitted with a lid and a fine mesh bottom to allow for water circulation, floated in the larger tanks. Ambient temperature was 14.5°C at the time the experiment was set up, so eggs were gradually acclimated to the 16-18°C and 20-22°C treatments.

A further 48 eggs were collected on 17 June, to ensure adequate juvenile numbers for subsequent growth experiments in the event of either poor hatching success or hatchling survival. These eggs were processed according to the same experimental design as the first batch; however, there were only 2 development tubs in each temperature and maternal group combination, with four randomly allocated eggs in each (Fig. 7.3b). About 1 month into the experiment the water temperature in the 20-22°C treatment rose to over 25°C for over 24 h because of equipment failure, which resulted in the death of all eggs and complete loss of the treatment. Hence, a third batch of eggs was collected on 3 August to ensure some 20-22°C hatchlings would be available for juvenile experiments. Unfortunately, at that late stage in the spawning season very few large females with Type 3 bones were present (Chapter 4), so only eggs from small Type 2 females were available. These were assigned to the three temperature treatments, with 24 eggs per treatment (Fig 7.3c).

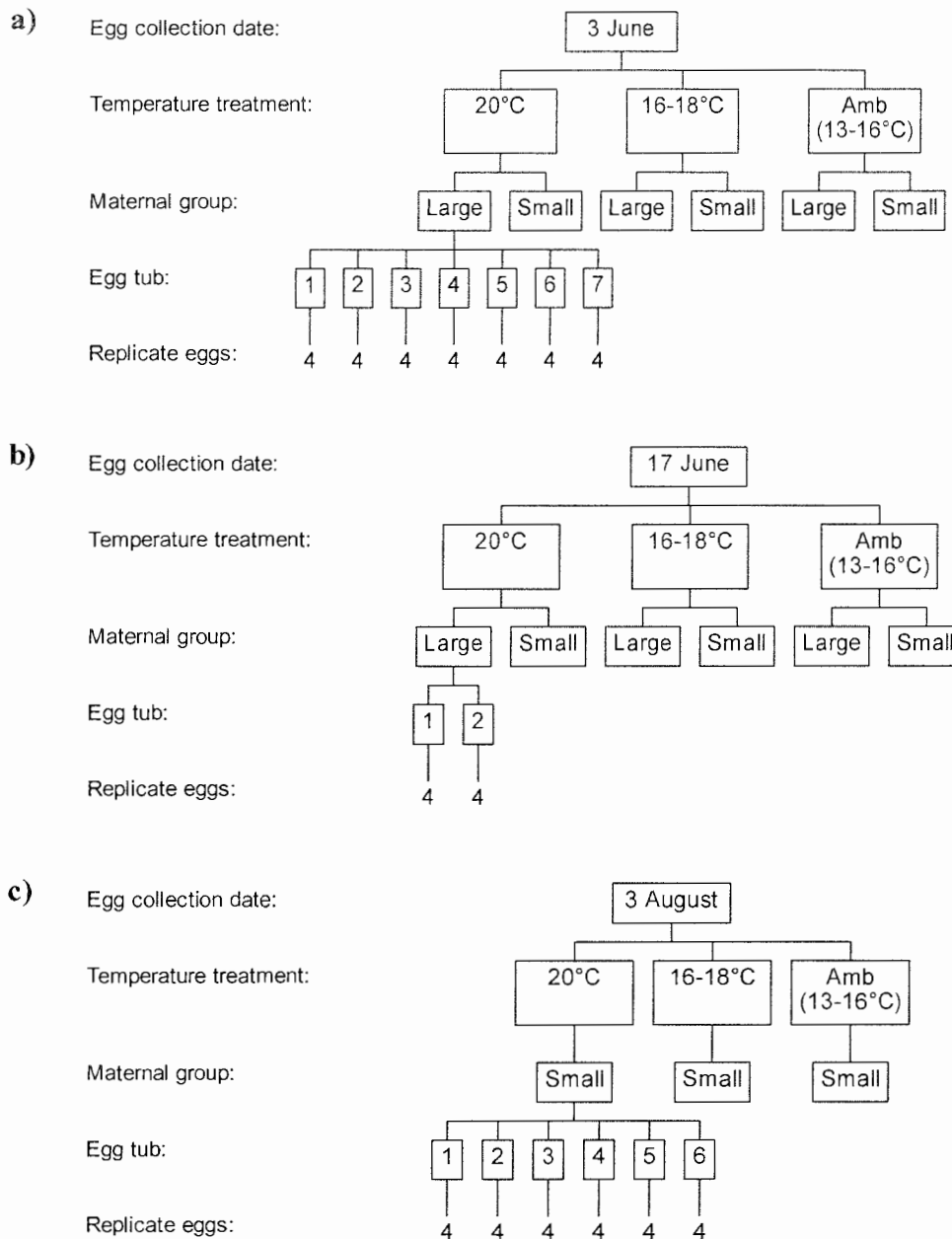


Figure 7.3 Schematic diagrams of the experimental designs used for egg development experiments: for *S. apama* eggs collected on 3 June (a); 17 June (b); and 3 August (c). NB: No large eggs were collected on 3 August.

Tubs were checked daily for hatchlings. Each new hatchling was removed with a small dip net and weighed alive (Hatch TWt) to the nearest 0.0001 g in a dish containing seawater from the treatment tank tared on an electronic Mettler balance. Since hatchlings were kept alive and used for juvenile growth experiments no other measurements were collected at this stage so as to minimize stress due to

handling. Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) methods were used to test for significant differences in development time and Hatch TWt respectively, between the different temperature treatments, egg collection dates, maternal groups and nested replicate tubs. The eggs collected on 3 August were analysed separately due to the absence of the large maternal group. Egg Wt was used as a covariate with Hatch TWt due to the significant correlation between the two variables. The ANOVA assumptions of normality of data and homogeneity of variance were verified by visual assessment of normal probability plots and residual plots.

7.3.3 Juvenile growth experiment

Juveniles from the egg development experiment were used in growth experiments. Once hatching was completed, a feeding experiment was set up in each temperature treatment (Fig. 7.4). In the 16-18°C and Amb treatments, juveniles were derived from eggs collected on two dates in June. Due to the small number of juveniles surviving from the eggs from the 17 June, those collected on the two dates were combined for the feeding experiments. Therefore, juveniles from each original maternal group were randomly allocated to the two different feeding regimes – “full” and “half” (Fig. 7.4a).

Those assigned to the “full” feeding regime were fed once every day, whereas, the “half” feeding regime were fed once every second day. A single feeding event consisted of two live rock pool shrimp, *Leander screnus*, of equivalent size to the mantle length of the hatchlings (Fig 7.5b). This ration level was considered adequate, as three shrimp per day resulted in the shrimp heads being left uneaten. As the juveniles grew the size of the shrimp were matched to the size of the juveniles. A representative sample of shrimp were individually weighed and measured to determine their length-weight relationship, to relate to the length-weight relationship of the juvenile cuttlefish to estimate the % body weight fed to juveniles of different sizes. Juveniles were maintained in individual plastic containers of the same construction as the egg development tubs (Fig. 7.5a), to prevent competition for food and to monitor individual food usage. Results from experiments in 1999, in which juveniles were initially held in pairs showed an uneven consumption of food such that one increased in size while the other eventually perished.

In the 20-22°C treatment, juveniles were only available from eggs collected in August because of the loss of this treatment for the June samples. These juveniles were also randomly assigned to the two feeding regimes (Fig. 7.4c). All hatchlings from the August eggs in 16-18°C and Amb treatments were sacrificed on the day of hatching due to the limited space available for juvenile rearing.

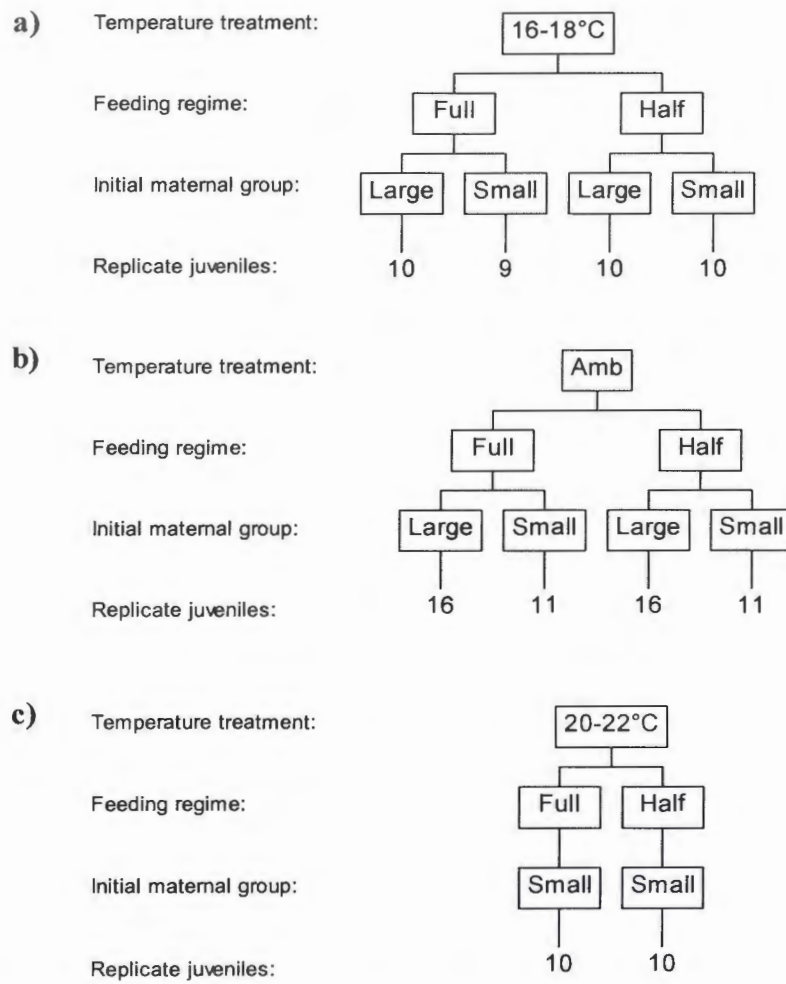


Figure 7.4 Schematic diagrams of the experimental design used for juvenile feeding experiments in each temperature treatment: (a) constant 16-18°C; (b) ambient water temperature; and (c) constant 20-22°C.

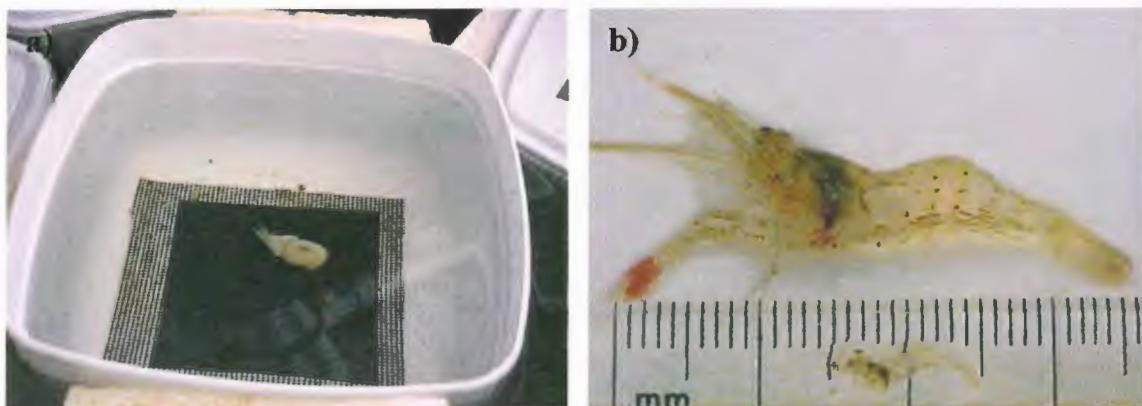


Figure 7.5 (a) Juvenile *S. apama* in an individual experimental rearing container, with mesh bottom to allow for water circulation. (b) Rock pool shrimp, *Leander screnus*, indicating the size range used for live juvenile cuttlefish food.

Due to the variation in egg development time and hatch dates between different temperature treatments, juveniles in different treatments were of different ages and some had undergone significant growth before the start of the juvenile feeding experiments. Thus, all juveniles were weighed (Pre-expt TWt) and their mantle length (Pre-expt ML) measured at the start of the experiment. Furthermore, they were chemically marked with calcein (Chapter 6) at the start of the experiment to distinguish between pre-experiment and experimental growth of the bone.

In a trial in 1999, 1-2 month old juvenile *S. apama* were immersed in two concentrations of calcein (250 mg.L^{-1} and 100 mg.L^{-1}) for immersion periods of 3 h and 6 h. These concentrations were chosen on the basis of ones considered to be non-lethal from previous studies with juvenile fish (Bumgardner and King 1996). Since 74% mortality occurred during these trials a lower concentration of 50 mg.L^{-1} was used in 2000 with an immersion time of 3 h, to minimize mortality and deleterious effects.

As calcein is difficult to dissolve in salt water a concentrated stock solution of 500 mg calcein in 50 mL distilled water was prepared using sodium bicarbonate to buffer the solution to a pH of 7 to increase the solubility of calcein (Wilson et al. 1987). This stock solution was diluted with filtered seawater to produce an immersion bath with a final concentration of 50 mg L^{-1} . Small containers of 10 cm diameter with a mesh bottom were immersed in the bath to keep juveniles separate and individually identifiable.

The growth experiment was started on the 31 October and continued for 49 days. The tubs were checked at each feeding time for dead individuals and cleaned of remaining food and waste. Dead individuals were removed and dissected. At the end of the experiment all remaining individuals were killed and processed fresh. The total wet weight (End TWt) and dorsal mantle length (End ML) were measured. The cuttlebones were removed, measured (End BL), air dried at room temperature, and stored for later analysis. The sex of individuals could not be determined.

The growth increments were visible through the dorsal shield with transmitted light under a dissecting microscope at low magnification. No calcein bands were evident in any of the juvenile bones. Therefore, it was not possible to separate pre-experimental growth of the cuttlebone from experimental growth and so all growth subsequent to the hatch mark was used for experimental comparisons.

Three variables were used to compare the growth of juveniles reared under different experimental treatments: (1) the number of increments following the hatching mark (Expt IncNo); (2) the average width of increments following the hatching mark (Expt AvIncWi), measured from digital images of the bones with Sigma scan image analysis software; and (3) the instantaneous relative growth rate for the

experimental period (Expt G; percent increase in total wet weight per day; % day⁻¹), calculated according to the equation:

$$ExptG = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \cdot 100$$

where, W_2 was the final weight at the end of the experiment, W_1 the initial weight at the start of the experiment and $t_2 - t_1$ the duration of the experiment in days (after Forsythe and Van Heukelem 1987).

ANOVA and ANCOVA statistical methods were used to test for significant differences in the three dependent variables between temperature treatments, feeding regimes and original maternal groups. Pre-expt Age or Pre-expt TWt was used as a covariate for tests when a significant correlation between the dependent variable and pre-experiment growth was detected. The ANOVA assumptions of homogeneity of variances and normality of data were confirmed via normal probability plots, residual plots and Levene tests ($p = 0.233-0.868$). Juveniles arising from June eggs were analysed separately for all sources of variation and then the data from the small maternal group of the June eggs were combined with the August eggs reared in the 20-22°C treatment to compare the variation of variables across all three temperature treatments and respective feeding regime combinations. However, it should be noted that any significant effects of temperature detected in this latter analysis may relate to the different egg collection date or temperature of the 20-22°C juveniles.

7.3.4 Pre-hatch bone analysis

The pre-hatch portion of the cuttlebones of juveniles reared in the different temperature treatments was analysed to determine if any characteristics related to the rearing conditions. Bones collected from field hatchlings and those killed on the day of hatching (August eggs reared in the 16-18°C and Amb treatments) were used to establish the criteria for distinguishing the pre-hatch portion of the bones from subsequent growth. The width of increments in the hatchling bones were consistently greater than 0.5 mm with the exception of the last increment which varied in size depending on the stage of formation at the time of hatching. Therefore, the pre-hatch portion of the bone was considered to consist of all increments greater than 0.5 mm in width prior to the very narrow increments associated with the change from endogenous to exogenous feeding just following hatching.

Three variables were used to describe the pre-hatch portion of the bones: (1) the length of the portion (Pre-hatch BL); (2) the number of increments in the portion (Pre-hatch IncNo); and (3) the average width of increments in the portion (Pre-hatch AvIncWi). ANOVA and ANCOVA methods were used

to analyse the data for these three variables between the different temperature treatments, collection dates and original maternal groups. Egg Wt was used as a covariate for tests where a significant correlation between the dependent variable and original egg size was detected. Data were tested for homogeneity of variances and normality and were found to conform to these statistical assumptions.

The pre-hatch portion of the cuttlebones from adults from the aggregation area in May 1998, 1999 and 2000 were also analysed and the variables compared between Type 2 and Type 3 bones (Chapter 6).

7.4 Results

7.4.1 Egg development in situ

Deposition of eggs

Eggs were found in cryptic locations, attached to the underside of flat rock slabs or within tight crevices within the broken reef bedrock, between 3 and 5 m depth. Very few were attached to exposed rock surfaces. There were occasional loose eggs, which were likely to have been dislodged following deposition.

Eggs were first observed in small numbers on marked egg rocks in late May (Fig. 7.6), after which numbers increased rapidly, such that by mid-June approximately half of the rocks supported over 100 eggs each. Further deposition occurred during July on most rocks and to a less extent in August. Some egg batches were very dense (up to 50 eggs.100 cm⁻²) and confined to only part of the rock under-surface. In very dense clumps the basal rings of the late eggs were often attached to those of eggs deposited earlier.

The total number of eggs deposited on an individual egg rock varied greatly from 0 to 453. Two rocks in each plot remained with no eggs throughout the entire spawning season. The number of eggs deposited per rock was not related to the area of the rock under-surface (correlation coefficient = -0.055; $p = 0.829$; $n = 18$).

Rocks in Plot 2 had lower numbers of eggs than those in Plots 1 and 3 (Fig. 7.6), although this difference was not statistically significant due to the high within-plot variation (ANOVA; $F = 16.48$; $p = 0.368$; $df = 2,15$).

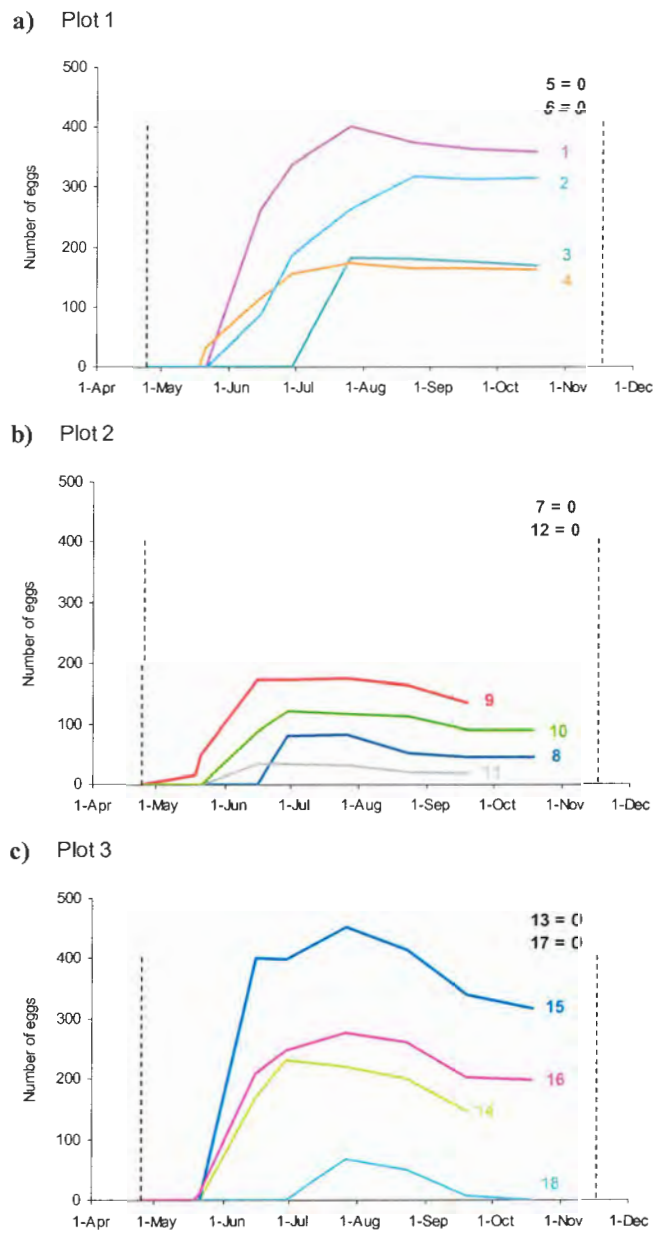


Figure 7.6 Number of *S. apama* eggs per rock at the three plots monitored at the Black Point site during the 2000 spawning season.

Survival of eggs

Females embedded each ovum in a thick capsule of gelatinous material prior to deposition, which presumably protected the ovum from potential predators by concealing the yolk-source within. No direct predation on the eggs of *S. apama* by fish was observed. However, sea urchins, *Heliocidaris erythrogramma*, were often present on the underside of rock slabs with the remains of capsule bases in their near vicinity (Fig. 7.7a). Some urchins were also found with dislodged eggs entangled within their spines. Examination of the contents of the mouthparts of urchins found on the underside of egg rocks revealed the presence of gelatinous material similar to the egg capsules, suggesting the urchins were consuming the eggs rather than inadvertently dislodging them.

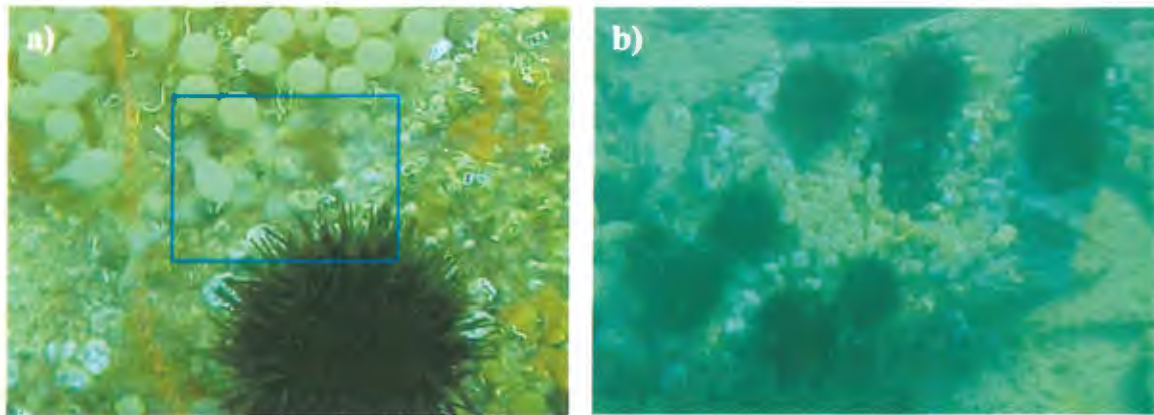


Figure 7.7 (a) Remains of egg bases and damaged eggs in the area (enclosed by the square) originally covered by the sea urchin. (b) High density of urchins present on the underside of an egg rock.

There was a slight decrease in the number of eggs per rock toward the end of the spawning season (Fig. 7.6). This was usually correlated with the presence of urchins, as indicated by the remains of egg bases nearby. In particular, for two rocks, i.e. Rock 8 and Rock 18, most eggs deposited were lost. Under most rocks, only one or two urchins were present (overall mean = 0.9 ± 0.3 SE urchin per rock ($n = 12$); however, high densities were found in some instances (Fig. 7.7b). The average number of urchins per rock varied between the three plots (significant difference; ANOVA; $F = 8.13$; $p = 0.010$; $df = 2,9$). There were consistently higher numbers of urchins found under rocks in Plot 2, which also had the lowest numbers of eggs deposited (Fig. 7.6b). Conversely, Plot 3 had the highest loss rate of eggs toward the end of the spawning season (Fig. 7.6c), but did not have more urchins per rock than Site 1 (Tukey HSD test; $p = 0.783$).

Development of eggs

Six macroscopic stages of egg development were distinguished for use in tracking the development of eggs in situ (Table 7.1; Fig. 7.8*b-d*). Just after deposition, the capsule was soft and transparent for approximately 1 h and the bright-yellow, yolk-filled ovum was clearly visible (Fig. 7.8*b*). Upon exposure to seawater the capsule contracted and became more solid and opaque. "Young" eggs were identified by their bright white capsule and relatively small compact size (Fig. 7.8*b*). As development progressed, the capsule expanded and the wall became thinner, more translucent and often discoloured (Fig. 7.8*c*). The developing embryo gradually became visible through the capsule. Just prior to hatching, the fully developed embryo was clearly visible and the external yolk sac was either greatly reduced, relative to the embryo size, or completely consumed (Fig. 7.8*d*).

Under each rock there were usually multiple batches of eggs of different developmental stages and sizes (Fig. 7.8*a*; Fig. 7.9). From the development of individual batches of eggs, it was clear that eggs deposited early in the season in May or June, were the first to hatch in September, and those laid later in July or August hatched from October. All viable eggs had hatched by early November.

Hatch success and survival of hatchlings

It was not possible to estimate the hatch success of eggs in situ, due to the degeneration or consumption of egg capsules after hatching. However, eggs that were not developing properly became obvious by September and their numbers were used to indicate the potential percentage of non-viable eggs per rock. The mean percentage was only 2.4% (SE = $\pm 0.5\%$; range = 0.9 - 5.9%; n = 12). This suggests that a high percentage of eggs developed through to hatching, except for losses due to predation. Hatching in response to disturbance caused by the over-turning of egg-rocks to do counts was observed, and the disturbance may have resulted in some premature hatching. However, a substantial proportion of hatching had already occurred when this was first noticed and in most cases no residual external yolk sac remained, indicating eggs were ready to hatch naturally. New hatchlings immediately swam toward the substrate and hid under overhangs or within crevices. Predation by magpie perch, *Cheilodactylus nigripes*, on new hatchlings was observed; however, the hatchlings were from rocks that had been turned over with the eggs exposed. Normally they would have more protection upon hatching due to the cryptic and confined location of the eggs.

Table 7.1 Macroscopic stages of egg development used to stage *S. apama* eggs in situ at the aggregation area and from images taken underwater.

| Stage | | Description |
|-------|----------------|---|
| I | Fresh | Soft transparent capsule; yellow ovum visible within; relatively large size |
| II | Young | Rigid opaque capsule; bright white; whole egg solid with no internal features discernible |
| III | Early-term | Slightly enlarged opaque capsule; discoloured to cream or yellow; no internal features clear |
| IV | Mid-term | Capsule enlarged and semi-transparent; embryo visible; external yolk sac larger or same size as embryo |
| V | Late-term | Capsule enlarged and semi-transparent; embryo clearly visible; external yolk sac smaller than embryo |
| VI | Ready to hatch | Capsule very expanded and transparent with very thin walls; embryo clearly visible; external yolk sac minimal in size, or completely absent |

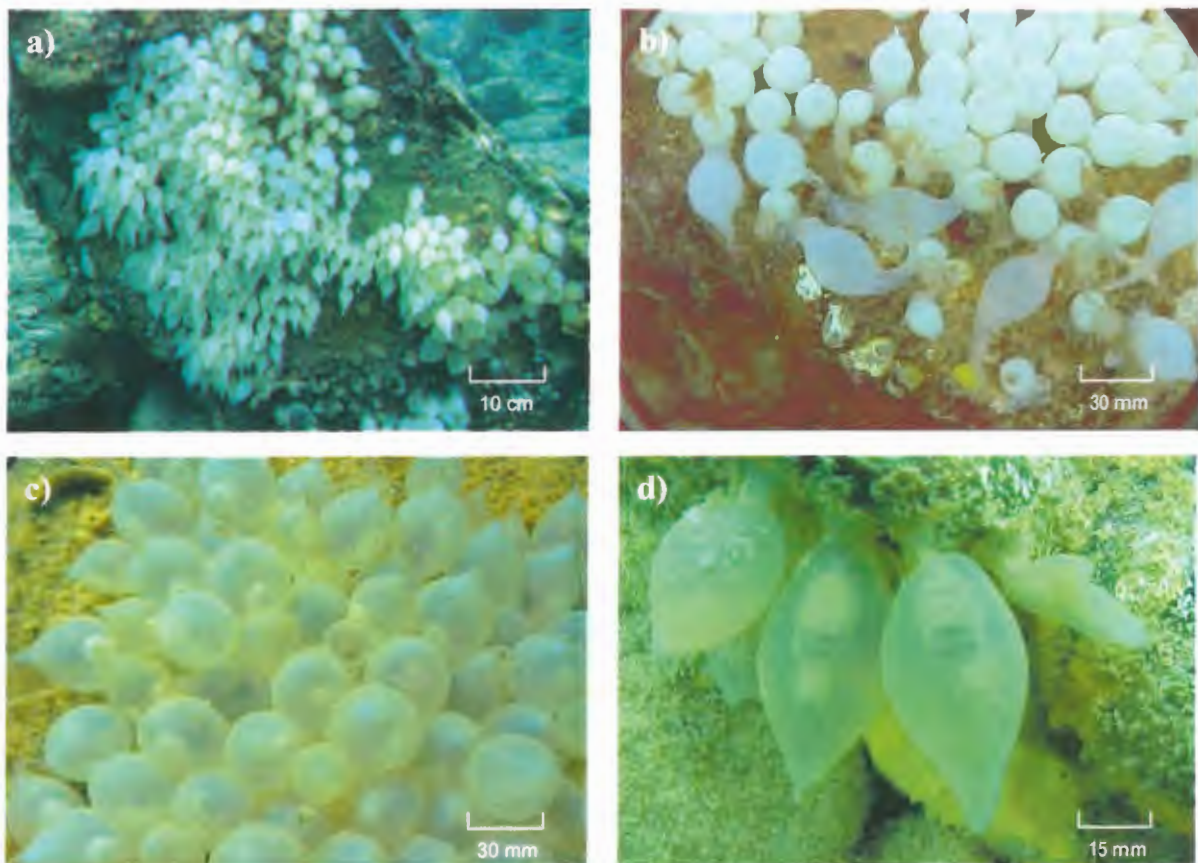


Figure 7.8 (a) Multiple batches of *S. apama* eggs of different stages and parentage on the underside of a single rock. Examples of different egg development stages: (b) transparent fresh eggs and “young” opaque bright white eggs; (c) late-term discoloured eggs with thin transparent capsule wall and embryo visible within; and (d) ready to hatch eggs with fully developed embryo clearly visible within and no external yolk sac remaining.

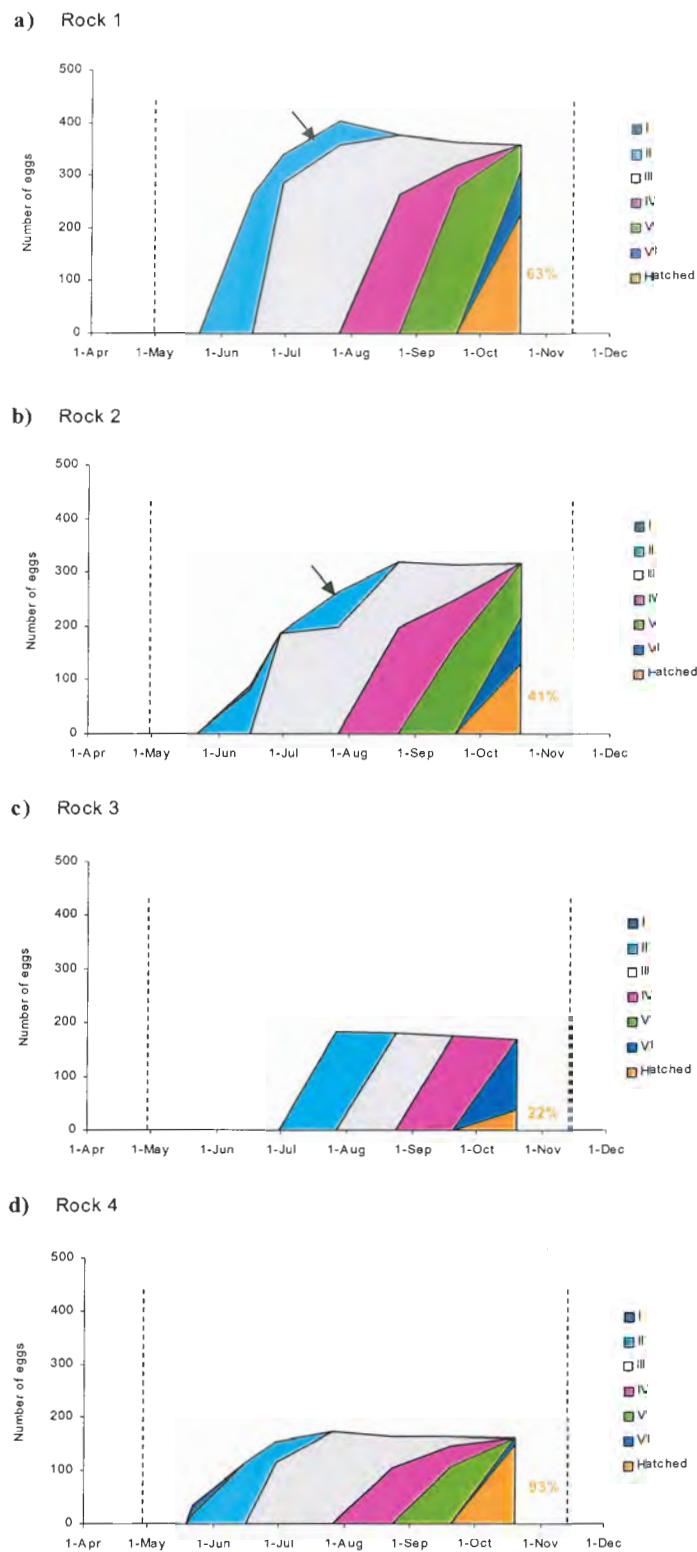


Figure 7.9 Number of *S. apama* eggs of each developmental stage on each egg rock (a-d) in Plot 1 of the Black Point site throughout the 2000 spawning season. Arrows indicate subsequent eggs deposited after initial batches. Percentage hatched on 20 Oct also indicated.

Water temperature

Water temperature at Black Point ranged from 25.7°C in late February to 12.4°C in July (Fig. 7.10). From May to June, when cuttlefish moved into the aggregation area and the peak period of egg-laying occurred, the temperature decreased from around 17°C to 14°C. Then during the winter months of July and August the temperature remained relatively constant between 12°C and 14°C, before increasing from below 14°C to above 20°C between September and November. Therefore, the eggs laid early in the spawning season would initially have experienced slightly warmer temperatures followed by the coldest temperatures of winter. Whereas, those laid later in the season would initially have experienced the coldest temperatures followed by rising temperatures through spring. The hatching season covered the period of September to November when water temperature increased substantially. Therefore, successive batches of hatchlings would have experienced vastly different temperature regimes.

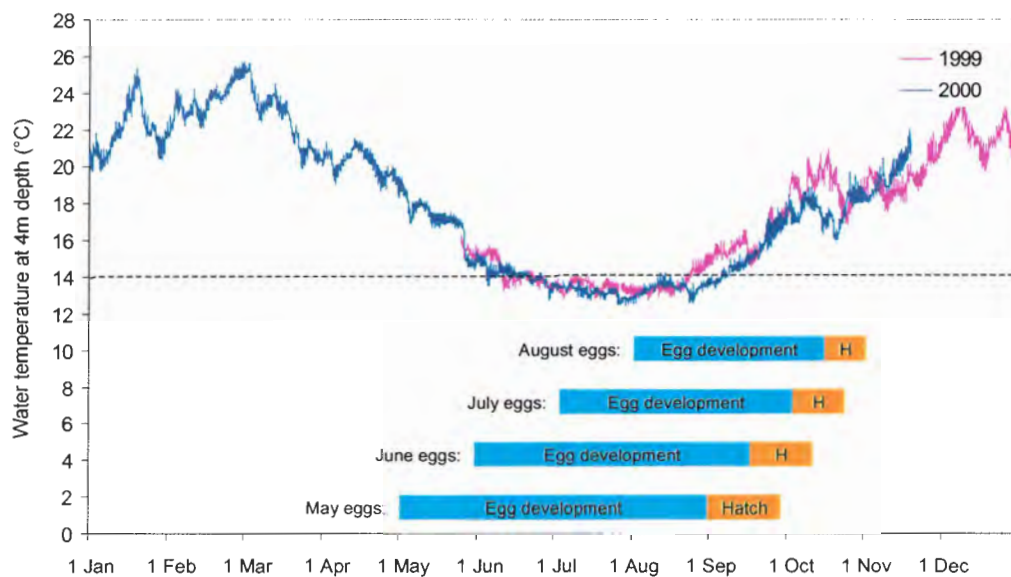


Figure 7.10 In situ water temperature profile at Black Point from 15 May 1999 to 6 November 2000. The timing of different egg development and hatching periods is indicated by the bars at the base.

7.4.2 Egg development and hatching experiment

Maternal group and egg size

For eggs collected in June there was a linear relationship between the Egg Wt and both maternal ML and maternal TWt ($r^2 = 0.7788$ and $r^2 = 0.8857$, respectively; Fig. 7.11). Although there was minimal

variation in the size of eggs laid by each female, evident as narrow error bars around each point in Fig. 7.11, there was substantial variation between similarly sized females. For example, consider the two points to the far right in Fig. 7.11.

The linear relationship between Egg Wt and maternal size was less significant for eggs collected in August ($r^2 = 0.2559$ and $r^2 = 0.3276$; Fig. 7.11). Only small Type 2 females were available at this time and eggs laid were small, irrespective of female size. Although the ML range of the females varied by over 50 mm (Fig. 7.11a), the corresponding range in TWT was relatively smaller (Fig. 7.11b), suggesting that egg size was more influenced by TWT than ML.

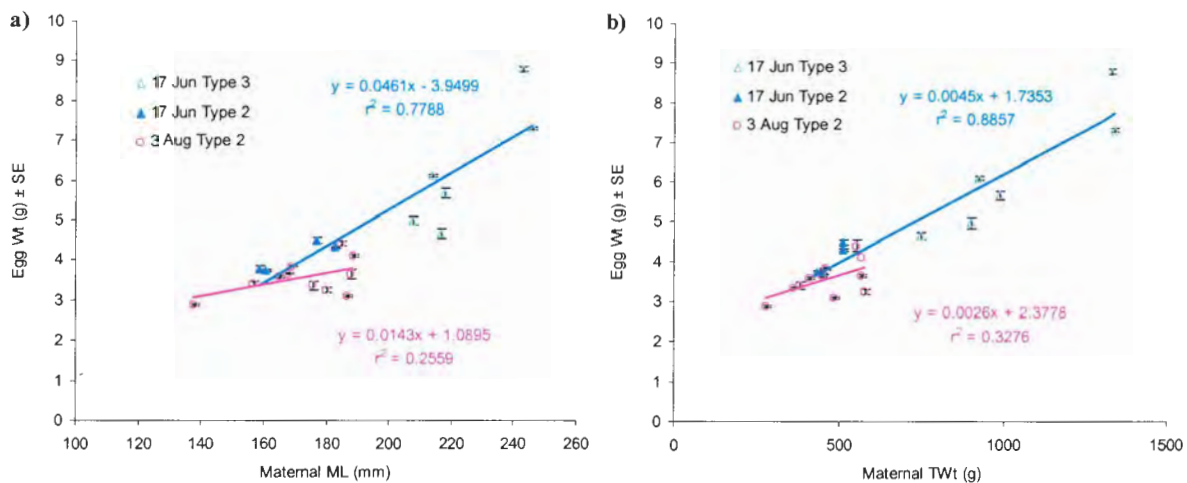


Figure 7.11 Variation in Egg Wt with respect to maternal ML (a) and maternal TWT (b) for eggs and females collected on 17 June and 8 August 2000. Each point represents a single female (Type 2 and Type 3 indicated in different colors) with error bars indicating SE of individual Egg Wt measurements. Linear regressions for the different collection dates are also shown, including the equation and r^2 value for each relationship.

Although initial Egg Wt represented a continuous variable in the egg development experiment, eggs were divided into two “maternal groups” (large and small) based on the size and age of the egg-laying female. This was to determine if the life cycle of the mother influenced egg development or juvenile growth. Egg weight was significantly different between the two maternal groups with the older and larger (Type 3) females depositing larger eggs (Fig. 7.12; Table 7.2). Since Type 3 females were present at the aggregation area in greater numbers at the start of the spawning season (Chapter 5), it follows that a greater number of large eggs were laid earlier in the season than later.

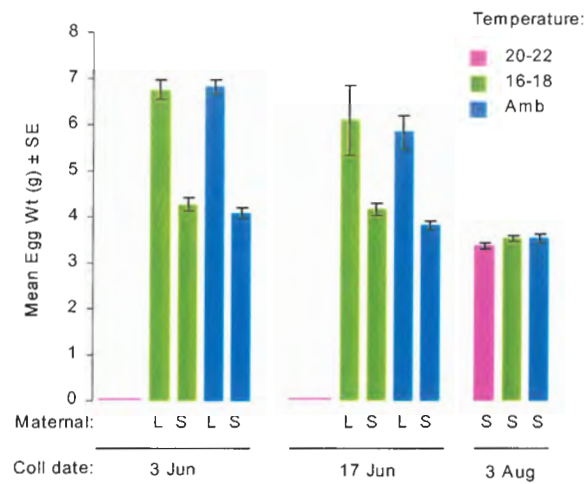


Figure 7.12 Mean size of eggs at the start of the experiment (Egg Wt) collected on different dates from large and small females allocated to the different temperature treatment regimes.

Table 7.2 Results of ANOVA tests for variation in the size of eggs at the start of the experiment (Egg Wt) with respect to temperature treatment (Temp), collection date (Coll Date), maternal group (Maternal) and tub number (Tub).

| Experiment | Source of variance | df | F Value | Prob < F |
|------------------|--------------------------|------|---------|----------|
| June eggs | Temp | 1 | 0.72 | 0.397 |
| | Coll Date | 1 | 10.72 | 0.002* |
| | Maternal | 1 | 195.72 | 0.000* |
| | Temp*Coll Date | 1 | 0.25 | 0.622 |
| | Temp*Maternal | 1 | 0.35 | 0.557 |
| | Coll Date* Maternal | 1 | 5.28 | 0.024* |
| | Temp*Coll Date* Maternal | 1 | 0.00 | 0.962 |
| | Tub (nested) | 28 | 2.07 | 0.005* |
| | Residual | 91 | | |
| | Extra August eggs | Temp | 2 | 1.34 |
| Tub (nested) | | 15 | 1.00 | 0.472 |
| Residual | | 44 | | |

* Significant at the $\alpha = 0.05$ significance level.

NB: The experimental design for June eggs was unevenly replicated with respect to the fixed factor Coll Date.

Egg Wt also differed significantly between the two collection dates in June, even though they were only two weeks apart (Table 7.2). Eggs collected on 17 June were smaller than those from the earlier collection date, although the significant interaction term indicates a more complicated relationship between collection date and maternal group (Fig. 7.12). Although eggs from the two maternal groups were randomly assigned to the development tubs there was also significant variation between individual tubs of the one treatment combination, although it only accounted for a relatively small proportion of the overall variation in egg size. This variation related to the large size range of eggs and the chance distribution of the small number of replicate eggs (4) between tubs.

Development time

Extreme care was taken to minimise disturbance of eggs to prevent premature hatching. Hatching occurred over a period of 31 days for each temperature and collection date combination (e.g. Fig. 7.13). Such variation is surprising since all eggs were laid on the same day. Both large and small eggs from both maternal groups showed a similar frequency distribution of hatch dates, with a distinct peak period of 2 days in the middle of the distribution (Fig. 7.13), that may have coincided with some unknown environmental cue.

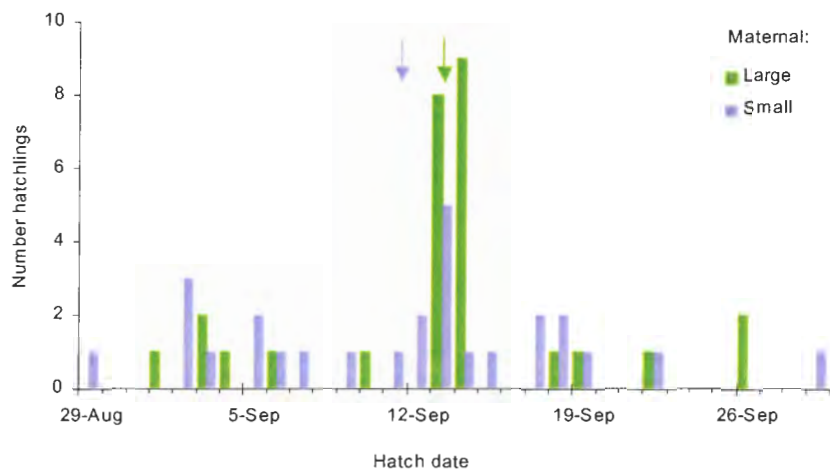


Figure 7.13 Frequency distribution of hatch dates of eggs collected from Black Point on 17 June 2000 and subsequently reared in aquaria at 16-18°C. Arrows indicate the mean hatch dates for eggs from each maternal group.

Water temperature during development had the greatest effect on egg development time, with a shorter mean development time at higher water temperatures (Fig. 7.14; Table 7.3). This relationship held irrespective of the collection date or maternal group. There was no apparent relationship between development time and original Egg Wt (Fig. 7.14b). The mean development time for each temperature treatment also varied between collection dates, with progressively shorter development times for eggs collected later in the season. This was probably related to the gradual increase in ambient water temperature during the experimental period, and the increase in the constant temperature treatments on 5 October. In particular, these factors would have had a large influence on the development times for the August batch of eggs, which had a much faster development time of eggs in the Ambient temperature treatment relative to the eggs collected in June.

A significant difference between the development times of eggs reared in different tubs within each treatment combination was also detected. However, this accounted for only a relatively small proportion of the total variance ($F = 1.99$; Table 7.3).

Hatching success

A high percentage of eggs in all temperature treatments successfully hatched (range = 75 to 100%). Hatching success of eggs collected on 17 June was marginally lower than of those collected on other dates. However, this is unlikely to represent a true difference in the viability of eggs laid at that time, but rather an artefact of the handling or treatment of those eggs during collection and transport.

Hatchling size

Hatch TWt was positively correlated with original Egg Wt (Fig. 7.15b), but also varied with temperature and collection date when the effect of egg size was removed (i.e. using Egg Wt as a covariate). Generally, eggs that developed at higher temperatures, hatched at larger sizes. The August eggs from the Amb treatment were the only ones that did not conform to this pattern. Some premature hatching in response to disturbance occurred in the 16-18°C treatment for August eggs causing mean Hatch TWt for that treatment to be well below that of the Amb treatment. When the significant effect of egg size was removed by using Egg Wt as a covariate in the ANCOVA, no significant difference in Hatch TWt was detected between maternal groups (Table 7.4). Repetition of the analysis using a simple ANOVA without Egg Wt as a covariate resulted in a significant difference between maternal groups ($F = 26.22$; $df = 1,91$; $p = 0.000$), which related to the original difference in egg size between the two groups.

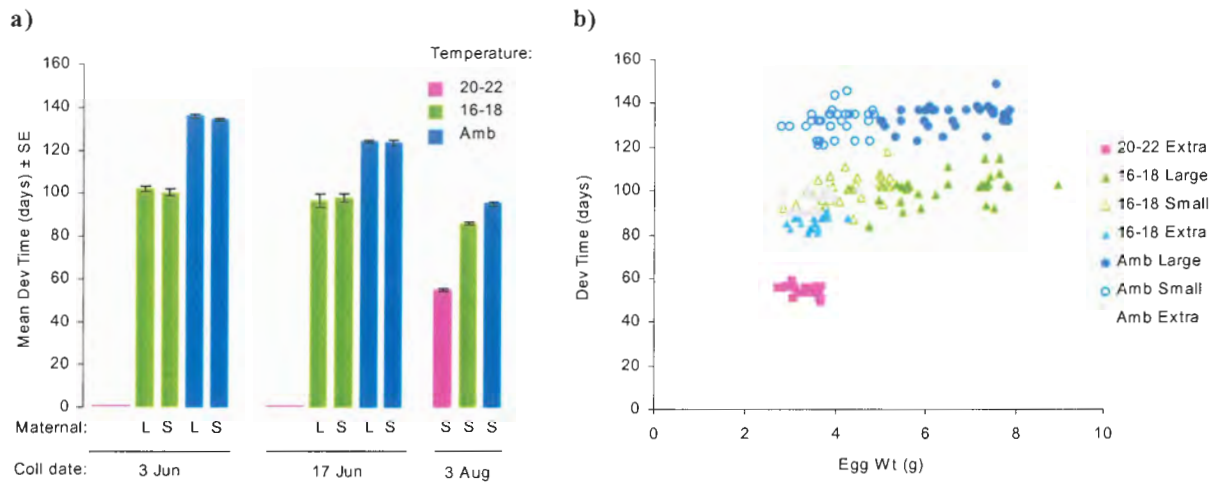


Figure 7.14 (a) Mean development time of eggs collected on different dates laid by small and large females, reared under different temperature regimes. (b) Bivariate scatterplot of egg development time with respect to original egg size (Egg Wt) with points grouped according to different temperature treatment and maternal group combinations.

Table 7.3 Results of ANOVA tests for variation in the development time of eggs with respect to temperature treatment (Temp), collection date (Coll Date), maternal group (Maternal) and tub number (Tub).

| Experiment | Source of variance | df | F Value | Prob < F |
|-------------------|--------------------------|----|---------|----------|
| June eggs | Temp | 1 | 782.95 | 0.000* |
| | Coll Date | 1 | 44.64 | 0.000* |
| | Maternal | 1 | 0.19 | 0.662 |
| | Temp*Coll Date | 1 | 8.38 | 0.005* |
| | Temp*Maternal | 1 | 0.20 | 0.658 |
| | Coll Date* Maternal | 1 | 1.28 | 0.261 |
| | Temp*Coll Date* Maternal | 1 | 0.10 | 0.753 |
| | Tub (nested) | 28 | 1.99 | 0.008* |
| | Residual | 91 | | |
| Extra August eggs | Temp | 2 | 853.37 | 0.000* |
| | Tub (nested) | 15 | 1.26 | 0.267 |
| | Residual | 44 | | |

* Significant at the $\alpha = 0.05$ significance level.

NB: The experimental design for June eggs was unevenly replicated with respect to the fixed factor Coll Date.

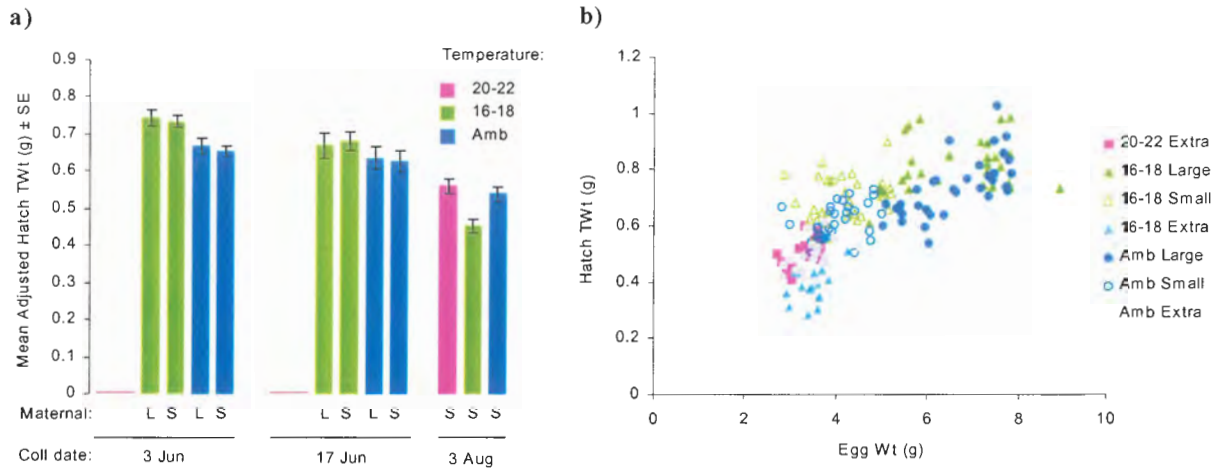


Figure 7.15 (a) Mean adjusted hatchling size (Hatch TWt) of eggs collected on different dates laid by small and large females, reared under different temperature regimes (values adjusted to an overall mean Egg Wt of 4.76g). (b) Bivariate scatterplot of Hatch TWt with respect to original egg size (Egg Wt) with points grouped according to different temperature treatment and maternal group combinations.

Table 7.4 Results of ANCOVA tests for variation in the size of hatchlings (Hatch TWt) with respect to temperature treatment (Temp), collection date (Coll Date), maternal group (Maternal) and tub number (Tub) with Egg Wt used as a covariate.

| Experiment | Source of variance | df | F Value | Prob < F |
|--------------------------|--------------------------|----|---------|----------|
| June eggs | Temp | 1 | 12.57 | 0.001* |
| | Coll Date | 1 | 4.36 | 0.040* |
| | Maternal | 1 | 0.02 | 0.901 |
| | Temp*Coll Date | 1 | 0.85 | 0.358 |
| | Temp*Maternal | 1 | 0.29 | 0.591 |
| | Coll Date* Maternal | 1 | 0.15 | 0.700 |
| | Temp*Coll Date* Maternal | 1 | 0.05 | 0.827 |
| | Tub (nested) | 28 | 1.33 | 0.156 |
| | Egg Wt (covariate) | 1 | 15.26 | 0.000* |
| | Residual | 90 | | |
| Extra August eggs | Temp | 2 | 29.09 | 0.000* |
| | Tub (nested) | 15 | 1.93 | 0.047* |
| | Egg Wt (covariate) | 1 | 7.76 | 0.008* |
| | Residual | 43 | | |

* Significant at the $\alpha = 0.05$ significance level.

NB: The experimental design for June eggs was unevenly replicated with respect to the fixed factor Coll Date.

Survival of hatchlings

Considerable mortality occurred across all treatments in the first week following hatching during the transition from endogenous feeding on residual internal yolk reserves to exogenous feeding. Exogenous feeding did not commence until 3 to 7 days following hatching and if appropriate food was not supplied during that period death ultimately resulted.

Initially high mortality resulted from the trial of different organisms as potential food sources, such as brine shrimp, amphipods, isopods and larval fish. Mysids were found to be an appropriate food source but were difficult to obtain in sufficient numbers. Eventually a reliable source of tiny rock pool shrimp of approximately the same length as the hatchlings was located, and these were readily consumed as a first food source. The hatchlings most affected by the delay in obtaining appropriate hatchling food were those from eggs collected in June and reared in the 16 to 18°C treatment, as they began hatching first. This resulted in lower survival in that temperature treatment (Table 7.5). All subsequent hatchlings were supplied with rock pool shrimp the day after hatching, resulting in a much higher rate of survival (Table 7.5).

Table 7.5 Survival of hatchlings in each temperature treatment prior to commencement of the juvenile growth experiment.

| Collection date | Temp | Original no. of eggs | No. hatchlings | No. alive at start of experiment | % Survival | Mean age (days) ± SE |
|-----------------|---------|----------------------|----------------|----------------------------------|------------|----------------------|
| June eggs | 16-18°C | 72 | 68 | 39 | 57% | 45.4 ± 1.2 |
| | Amb | 72 | 65 | 54 | 83% | 13.9 ± 0.7 |
| August eggs | 20-22°C | 24 | 23 | 20 | 87% | 34.9 ± 0.7 |

7.4.3 Juvenile growth experiment

At the start of the experiment the juveniles in the ambient temperature treatment were much younger than from the other two treatments (Fig. 7.16a); however, their TWt was only marginally smaller than those of the 20-22°C treatment (Fig. 7.16b). The only juveniles that had undergone appreciable growth were those from the 16-18°C treatment. Pre-expt TWt or Pre-expt Age were used as covariates for the analysis of all variables from the growth experiments to account for the different sizes and ages of juveniles used in each treatment.

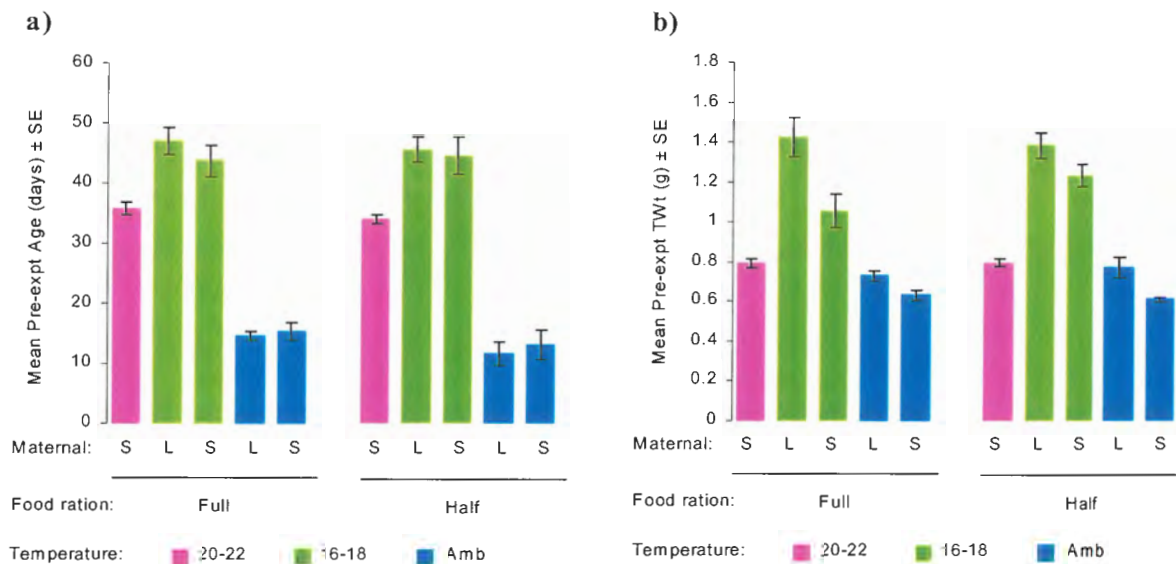


Figure 7.16 Mean age and TWt of juveniles randomly allocated to each experimental treatment.

Overall, juveniles maintained at the higher food level had higher survival rates (Table 7.6; Wald $\chi^2 = 5.02$; $df = 1$; $p = 0.0251$). The lowest survival occurred in the Amb treatment fed the half ration. This may have related to these juveniles being younger, rendering them more susceptible to nutritional stress and death at low food levels.

Table 7.6 Survival of juveniles in each temperature treatment and food level combination during the growth experiment.

| Food level | Temp | No. juveniles start of experiment | Mean age (days) ± SE | No. juveniles end of experiment | Mean age (days) ± SE | % Survival |
|------------|---------|-----------------------------------|----------------------|---------------------------------|----------------------|------------|
| Full | 20-22°C | 10 | 35.7 ± 1.1 | 7 | 82.7 ± 1.0 | 70% |
| | 16-18°C | 19 | 45.6 ± 1.7 | 18 | 94.9 ± 1.8 | 95% |
| | Amb | 27 | 14.8 ± 0.7 | 22 | 62.8 ± 0.7 | 81% |
| Half | 20-22°C | 10 | 34.0 ± 0.8 | 6 | 81.0 ± 0.8 | 60% |
| | 16-18°C | 20 | 45.1 ± 1.8 | 16 | 93.5 ± 1.9 | 80% |
| | Amb | 27 | 12.1 ± 1.5 | 12 | 60.1 ± 1.5 | 44% |

From the length-weight relationship of the rock pool shrimp (Fig. 7.17a) and that of the juvenile cuttlefish (Fig. 7.17b), it was possible to estimate the approximate daily ration in terms of % TWt for the two feeding levels used in the experiment (Fig. 7.17c). The results indicated the daily feeding ration would have increased as the body weight of the juveniles increased, such that by the time a TWt of 10g was reached the ration in terms of % TWt would have doubled from the initial feeding levels at hatching (Fig. 7.17c). Therefore, over the lifetime of a 10g individual, the mean daily feeding level would have been 10.7% TWt for the "full" ration and 5.3% TWt for the "half" ration. No partial food remains were found in any tub at either ration level.

The mean growth rate of juveniles (Expt G) fed the full ration was approximately double that of those reared on the half ration (Fig. 7.18; Table 7.7). Temperature also had a significant effect on growth rate although the results were not as clear cut. Juveniles derived from June eggs had higher growth rates in the 16-18°C treatment compared to the Amb treatment after correction for the difference in Pre-expt TWt of the treatments (Table 7.7). However, juveniles in the 20-22°C treatment had a lower mean growth rate than those in the 16-18°C treatment (Fig. 7.18a). This may have related to the complication of using eggs from two different collection dates for the comparison. The magnitude of difference between the means of the 16-18°C and 20-22°C treatments were much lower in all 3 variables investigated than between the 16-18°C and Amb treatments, which compared eggs collected on the same day, despite the fact the temperature difference between the latter two treatments was smaller. Alternatively, the use of equal rations across all temperature treatments may have influenced the results. Maintenance metabolic rates were likely to have been higher in the 20-22°C treatment but no extra food was given to compensate for this. The significant interaction terms between temperature and food relate to the relatively larger influence of temperature on growth rate at the higher food level. Growth rate also varied significantly with Pre-expt TWt (Fig. 7.18b), which was used as a covariate in the ANCOVA.

The difference in ration during the experimental period was also sufficient to produce a significant difference in both the average width and number of increments deposited in the cuttlebones between the two food levels, even with cuttlebone growth prior to the feeding experiment still included in both variables (Fig. 7.19a and Table 7.8; Fig. 7.20a and Table 7.9; respectively). Juveniles maintained on the full ration had wider and more numerous increments in the cuttlebone. Temperature also had a significant effect on both cuttlebone growth variables but accounted for a much smaller proportion of the overall variance (Table 7.8 and Table 7.9). The significant interaction term between temperature and maternal group related to the greater effect of temperature on the number of increments in cuttlebones of juveniles derived from large eggs compared to small ones.

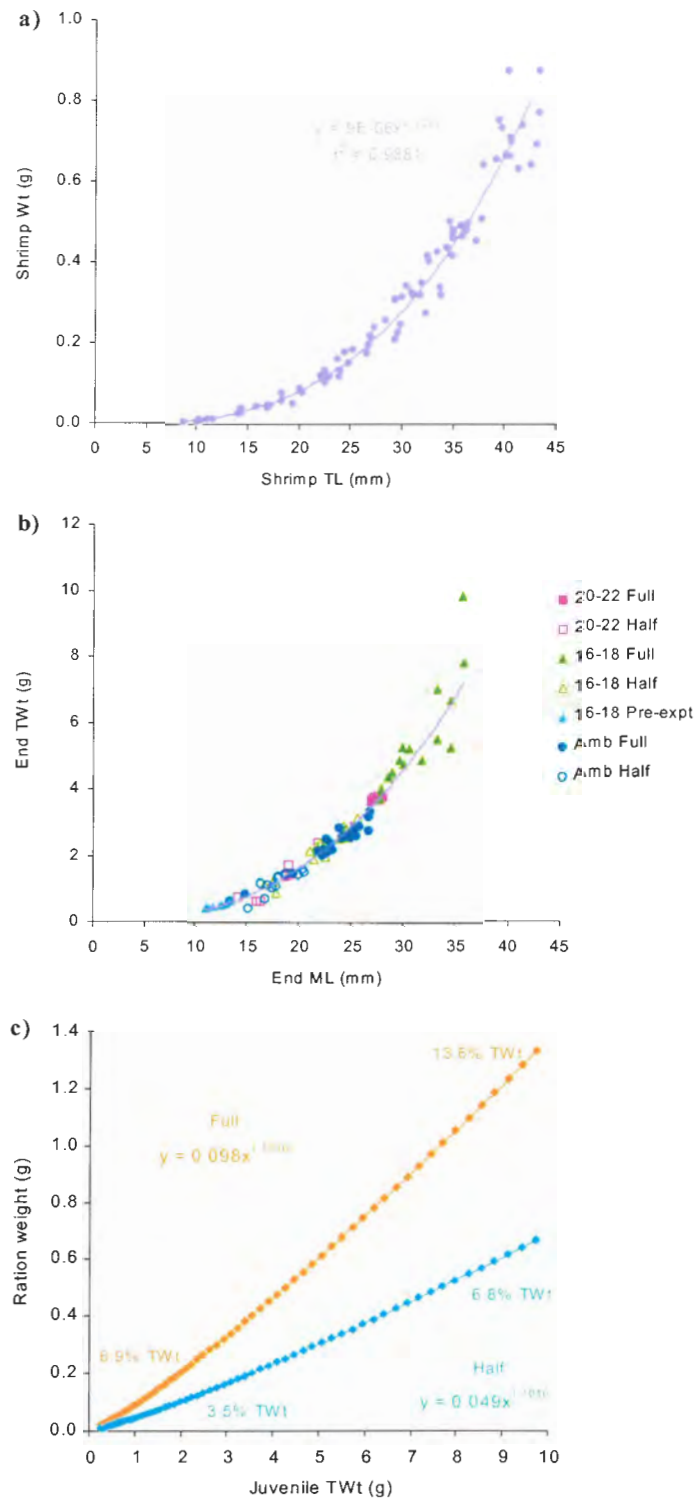


Figure 7.17 Length-weight relationship of rock pool shrimp, *Leander screnus* (a) and *S. apama* juveniles (b) over the size range used in the juvenile growth experiments. (c) Calculated ration levels, based on the “Full” ration of two shrimp of similar total length to juvenile mantle length fed once per day.

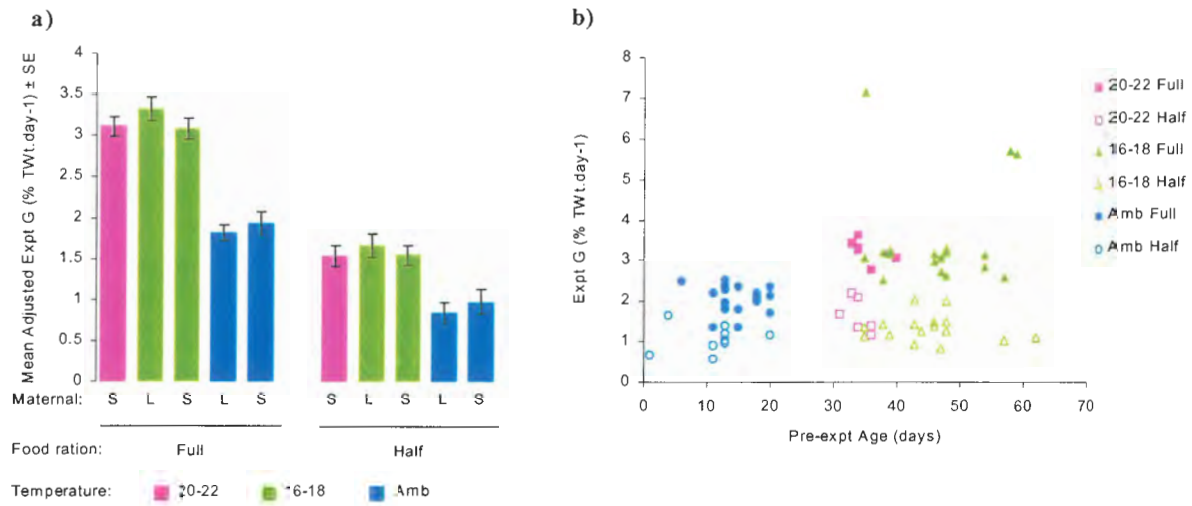


Figure 7.18 (a) Mean adjusted growth rate during the experimental period (Expt G) of juveniles reared from the eggs of small and large females under different temperature and feeding regimes (values adjusted to an overall mean Pre-expt TWt of 0.96g); (b) Bivariate scatterplot of Expt G with respect to Pre-expt TWt with points grouped according to different feeding regimes, temperature treatments and maternal group combinations.

Table 7.7 Results of ANCOVA tests for variation in the Expt G of juveniles with respect to temperature treatment (Temp), food regime (Food) and original maternal group (Maternal) with Pre-expt TWt used as a covariate.

| Experiment | Source of variance | df | F Value | Prob < F |
|-----------------------------|--------------------------|----|---------|----------|
| June eggs | Temp | 1 | 42.55 | 0.000* |
| | Food | 1 | 272.55 | 0.000* |
| | Maternal | 1 | 0.10 | 0.755 |
| | Temp*Food | 1 | 16.55 | 0.000* |
| | Temp*Maternal | 1 | 3.74 | 0.058 |
| | Food* Maternal | 1 | 0.23 | 0.637 |
| | Temp*Food* Maternal | 1 | 0.07 | 0.790 |
| | Pre-expt TWt (covariate) | 1 | 10.77 | 0.002* |
| | Residual | | 53 | |
| Small Maternal Group | Temp | 2 | 20.51 | 0.000* |
| | Food | 1 | 181.21 | 0.000* |
| | Temp*Food | 2 | 3.55 | 0.041* |
| | Pre-expt TWt (covariate) | 1 | 5.28 | 0.029* |
| | Residual | | 31 | |

* Significant at the $\alpha = 0.05$ significance level.

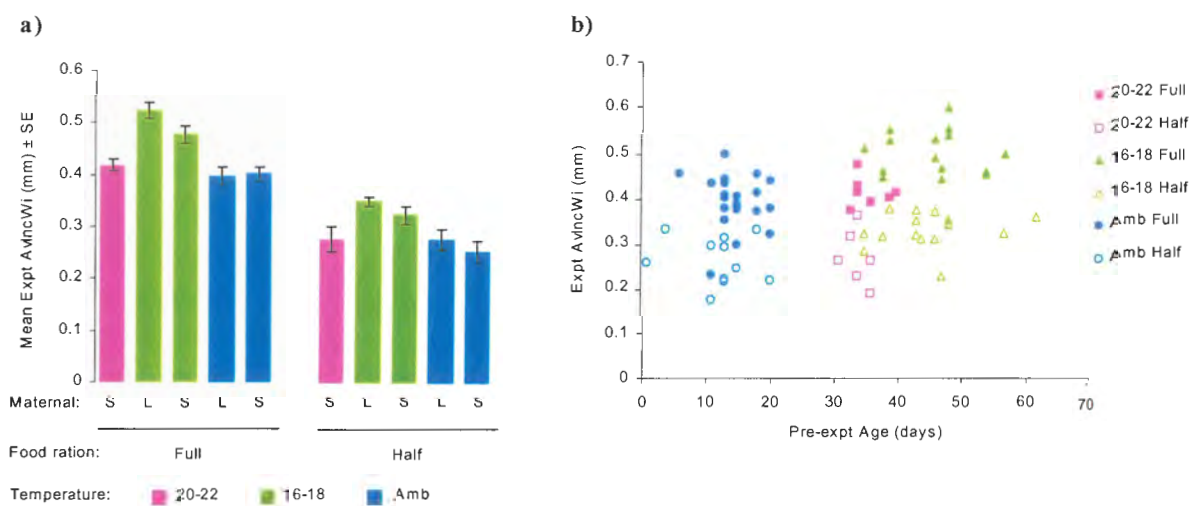


Figure 7.19 (a) Average width of increments deposited during the experimental period (Expt AvIncWi) in the cuttlebone of juveniles reared from the eggs of small and large females under different temperature and feeding regimes. (b) Bivariate scatterplot of Expt AvIncWi with respect to Pre-expt Age with points grouped according to different feeding regimes, temperature treatments and maternal group combinations.

Table 7.8 Results of ANCOVA tests for variation in the Expt AvIncWi of juveniles with respect to temperature treatment (Temp), food regime (Food) and original maternal group (Maternal) with Pre-expt Age used as a covariate.

| Experiment | Source of variance | df | F Value | Prob < F |
|----------------------|--------------------------|----|---------|----------|
| June eggs | Temp | 1 | 7.30 | 0.009* |
| | Food | 1 | 133.96 | 0.000* |
| | Maternal | 1 | 3.43 | 0.069 |
| | Temp*Food | 1 | 1.22 | 0.275 |
| | Temp*Maternal | 1 | 1.07 | 0.305 |
| | Food* Maternal | 1 | 0.00 | 0.997 |
| | Temp*Food* Maternal | 1 | 0.76 | 0.388 |
| | Pre-expt Age (covariate) | 1 | 0.23 | 0.633 |
| | Residual | 56 | | |
| Small Maternal Group | Temp | 2 | 5.39 | 0.009 |
| | Food | 1 | 110.19 | 0.000* |
| | Temp*Food | 2 | 0.03 | 0.927 |
| | Pre-expt Age (covariate) | 1 | 1.39 | 0.247 |
| | Residual | 33 | | |

* Significant at the $\alpha = 0.05$ significance level.

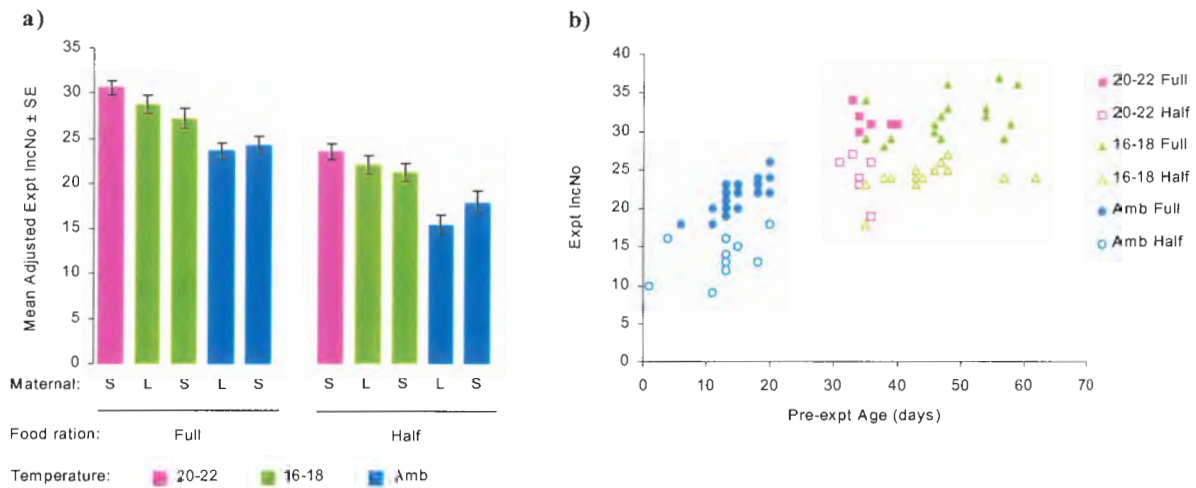


Figure 7.20 (a) Mean adjusted increment number deposited during the experimental period (Expt IncNo) in the cuttlebones of juveniles reared from the eggs of small and large females under different temperature and feeding regimes (values adjusted to a overall mean Pre-expt Age of 29.9 days). (b) Bivariate scatterplot of Expt IncNo with respect to Pre-expt Age with points grouped according to different feeding regimes, temperature treatments and maternal group combinations.

Table 7.9 Results of ANCOVA tests for variation in the Expt IncNo of juveniles with respect to temperature treatment (Temp), food regime (Food) and original maternal group (Maternal) with Pre-expt Age used as a covariate.

| Experiment | Source of variance | df | F Value | Prob < F |
|-----------------------------|--------------------------|----|---------|----------|
| June eggs | Temp | 1 | 6.58 | 0.013* |
| | Food | 1 | 176.87 | 0.000* |
| | Maternal | 1 | 0.15 | 0.698 |
| | Temp*Food | 1 | 0.70 | 0.405 |
| | Temp*Maternal | 1 | 6.71 | 0.012* |
| | Food* Maternal | 1 | 1.43 | 0.237 |
| | Temp*Food* Maternal | 1 | 0.32 | 0.575 |
| | Pre-expt Age (covariate) | 1 | 16.84 | 0.000* |
| | Residual | | 56 | |
| Small Maternal Group | Temp | 2 | 15.15 | 0.000* |
| | Food | 1 | 89.72 | 0.000* |
| | Temp*Food | 2 | 0.21 | 0.810 |
| | Pre-expt Age (covariate) | 1 | 7.45 | 0.010* |
| | Residual | | 33 | |

* Significant at the $\alpha = 0.05$ significance level.

Mean Expt AvIncWi showed a similar pattern across treatment combinations as mean growth rate (Fig. 7.19a and Fig. 7.18a, respectively) and there was also a strong correlation between the two variables (Pearson correlation coefficient = 0.831; $p = 0.0000$; $n = 75$). Therefore, the width of increments deposited in the cuttlebones varied according to growth rate. Mean Expt IncNo was also correlated with growth rate (Pearson correlation coefficient = 0.765; $p = 0.0000$; $n = 75$), but showed a different pattern across treatment combinations (Fig. 7.20a). Cuttlebones from juveniles reared in the 20-22°C treatment had the highest mean Expt IncNo, despite having a lower mean growth rate and Expt AvIncWi than juveniles in the 16-18°C. This suggests that the two cuttlebone variables respond differently under different conditions, with the rate of increment deposition increasing at higher temperatures independent of growth rate.

Total increment number in the cuttlebones of juveniles was linearly related to post-hatch age (Fig. 7.21a), with the slope of the regression line varying according to the experimental conditions of temperature treatment and food regime (Fig. 7.21b). All regression lines differed significantly from the $y = x$ relationship which would indicate formation of one increment per day (Fig. 7.21b). Therefore, the number of increments in the cuttlebone does not equate to age in days, but rather is a function of both age and growth rate.

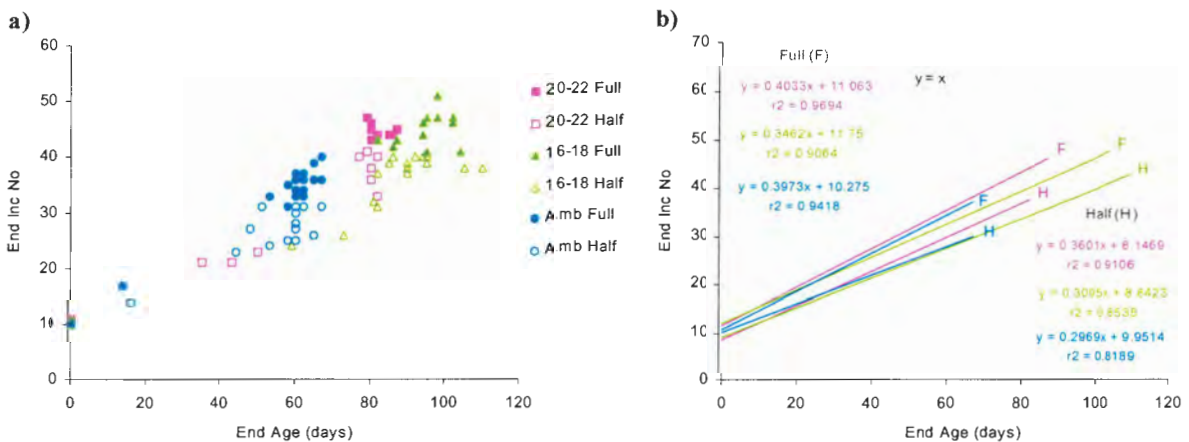


Figure 7.21 Relationship between increment numbers within juvenile cuttlebones with age. Regression relationships for each of the temperature and feeding regime combinations.

7.4.4 Pre-hatch bone analysis

No variable measured on the pre-hatch portion of the juvenile bones showed a clear relationship with temperature, collection date or maternal group. However, the Pre-hatch BL, and to a less extent Pre-

hatch IncNo, showed a significant positive correlation with Egg Wt (Fig. 7.22b and Table 7.10; Fig. 7.23b and Table 7.11; respectively), suggesting that the pre-hatch portion may indicate whether an adult cuttlefish was derived from the egg of a small or large female. However, the accuracy with which these parameters could be measured on the pre-hatch portion of the adult cuttlebones was questionable due to the high degree of calcification in the forked region. So although the technique was attempted on Type 2 and Type 3 bones collected from the aggregation area in May 1998, 1999 and 2000 the results were considered too dubious to warrant further attention.

7.5 Discussion

This may be the first documented study of the development of cephalopod eggs in situ in their natural environment. Furthermore, the concurrent monitoring of water temperature enabled the development and hatching times of eggs to be related to the prevailing environmental conditions. The results verified that for *S. apama*, egg development and hatching occurred over a range of temperatures despite the distinct annual spawning season and relatively narrow hatching period. Field and aquarium data clearly demonstrated a decrease in egg development time with increasing temperature. Eggs laid early in the spawning season had a long development time, due to the cold winter temperatures, and hatched at the start of spring when temperatures were still low. On the other hand, eggs laid later in the season had a shorter development time due to the rising water temperatures, and hatched later in spring when temperatures were much warmer. Similar trends have been described for many other cephalopod species based on laboratory studies (Boletzky 1989). Nevertheless, these results are the first to demonstrate the effect in the wild and provide strong support for the highly cited conceptual model developed by Forsythe (1993), which relates the different temperature conditions experienced by successive hatchlings to subsequent juvenile growth.

The only experimental manipulation of the eggs in the field was the lifting and lowering of rocks for egg-counting and the slight elevation of one side of each rock to prevent damaging the eggs upon replacement. However, even these minor variations from the natural situation have potential consequences. Firstly, some premature hatching may have resulted from the disturbance of eggs during rock movement. However, the resulting variation in hatch date would have been minor relative to the frequency of sampling, as premature hatching only occurs in eggs relatively close to hatching (Boletzky and Hanlon 1993). Secondly, the slight elevation of rocks may have influenced the numbers of eggs deposited in two opposing ways. Fewer eggs may have been deposited if tight dark spaces were preferred for egg-laying or alternatively, more eggs if the elevation increased the accessibility of the

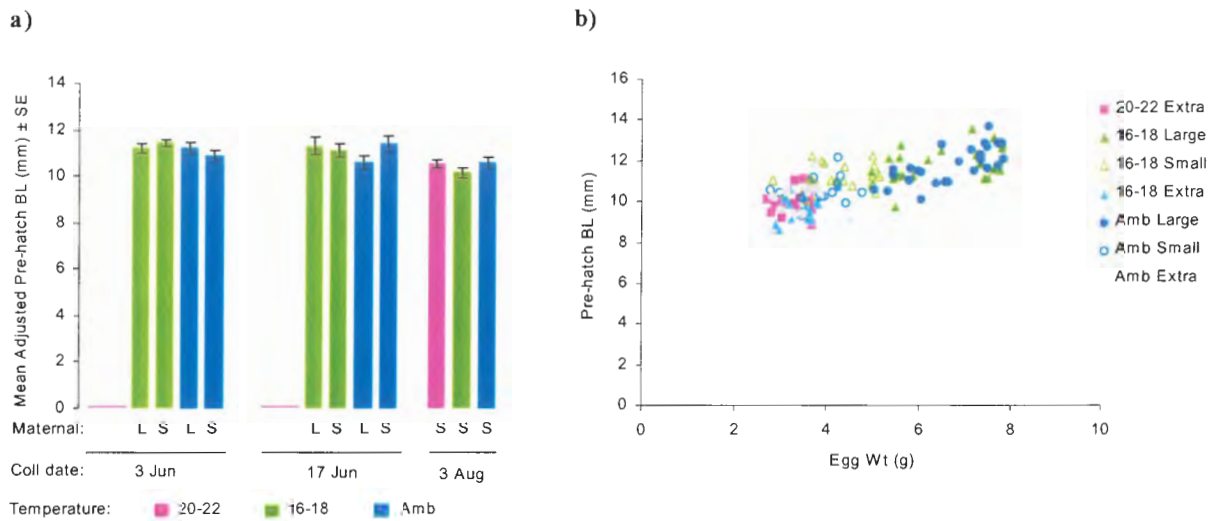


Figure 7.22 (a) Mean adjusted length of the pre-hatch bone (Pre-hatch BL) of juveniles reared from the eggs of small and large females under different temperature and feeding regimes (values adjusted to an overall mean Egg Wt of 0.96g). (b) Bivariate scatterplot of Pre-hatch BL with respect to Egg Wt with points grouped according to different feeding regimes, temperature treatments and maternal group combinations.

Table 7.10 Results of ANCOVA tests for variation in the Pre-hatch BL of juveniles with respect to temperature treatment (Temp), food regime (Food), original maternal group (Maternal) and tub number (Tub) with Egg Wt used as a covariate.

| Experiment | Source of variance | df | F Value | Prob < F |
|--------------------------|--------------------------|----|---------|----------|
| June eggs | Temp | 1 | 1.03 | 0.314 |
| | Coll Date | 1 | 0.03 | 0.864 |
| | Maternal | 1 | 0.69 | 0.409 |
| | Temp*Coll Date | 1 | 0.48 | 0.491 |
| | Temp*Maternal | 1 | 0.36 | 0.549 |
| | Coll Date* Maternal | 1 | 2.94 | 0.092 |
| | Temp*Coll Date* Maternal | 1 | 7.48 | 0.008* |
| | Tub (nested) | 27 | 1.54 | 0.088 |
| | Egg Wt (covariate) | 1 | 13.75 | 0.000* |
| | Residual | 55 | | |
| Extra August eggs | Temp | 2 | 1.67 | 0.207 |
| | Tub (nested) | 15 | 1.68 | 0.112 |
| | Egg Wt (covariate) | 1 | 0.02 | 0.895 |
| | Residual | 29 | | |

* Significant at the $\alpha = 0.05$ significance level.

NB: The experimental design for June eggs was unevenly replicated with respect to the fixed factor Coll Date.

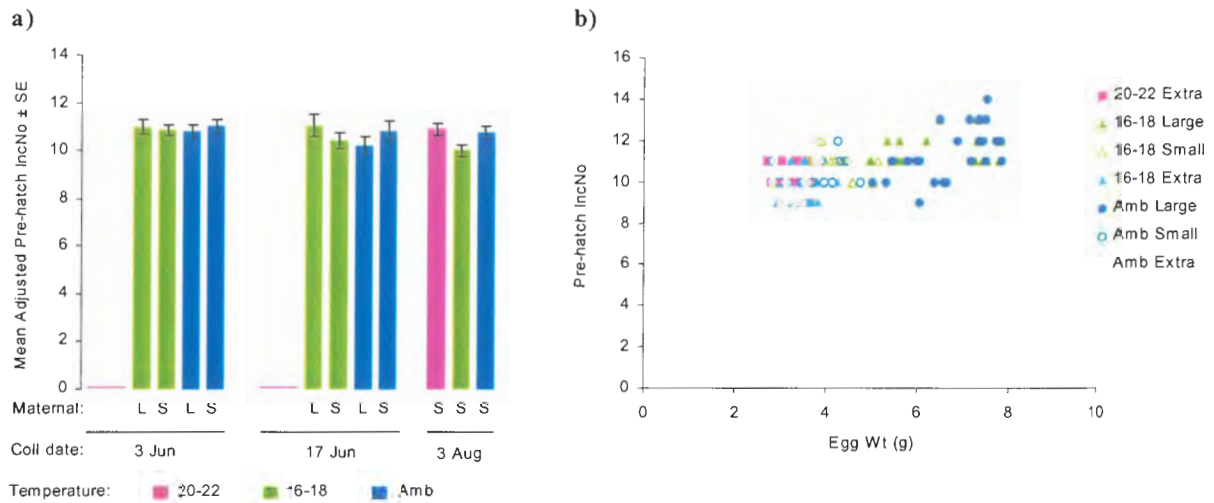


Figure 7.23 (a) Mean adjusted increment number of the pre-hatch bone (Pre-hatch IncNo) of juveniles reared from the eggs of small and large females under different temperature and feeding regimes (values adjusted to an overall mean Egg Wt of 4.76g). (b) Bivariate scatterplot of Pre-hatch IncNo with respect to Egg Wt with points grouped according to different feeding regimes, temperature treatments and maternal group combinations.

Table 7.11 Results of ANCOVA tests for variation in the Pre-hatch IncNo of juveniles with respect to temperature treatment (Temp), food regime (Food), original maternal group (Maternal) and tub number (Tub) with Egg Wt used as a covariate.

| Experiment | Source of variance | df | F Value | Prob < F |
|-------------------|--------------------------|----|---------|----------|
| June eggs | Temp | 1 | 0.02 | 0.880 |
| | Coll Date | 1 | 1.16 | 0.288 |
| | Maternal | 1 | 0.28 | 0.601 |
| | Temp*Coll Date | 1 | 0.11 | 0.738 |
| | Temp*Maternal | 1 | 5.78 | 0.021* |
| | Coll Date* Maternal | 1 | 1.12 | 0.296 |
| | Temp*Coll Date* Maternal | 1 | 3.25 | 0.079 |
| | Tub (nested) | 26 | 2.03 | 0.020* |
| | Egg Wt (covariate) | 1 | 6.90 | 0.012* |
| | Residual | 43 | | |
| Extra August eggs | Temp | 2 | 5.22 | 0.012* |
| | Tub (nested) | 15 | 0.91 | 0.564 |
| | Egg Wt (covariate) | 1 | 0.14 | 0.720 |
| | Residual | 29 | | |

* Significant at the $\alpha = 0.05$ significance level.

NB: The experimental design for June eggs was unevenly replicated with respect to the fixed factor Coll Date.

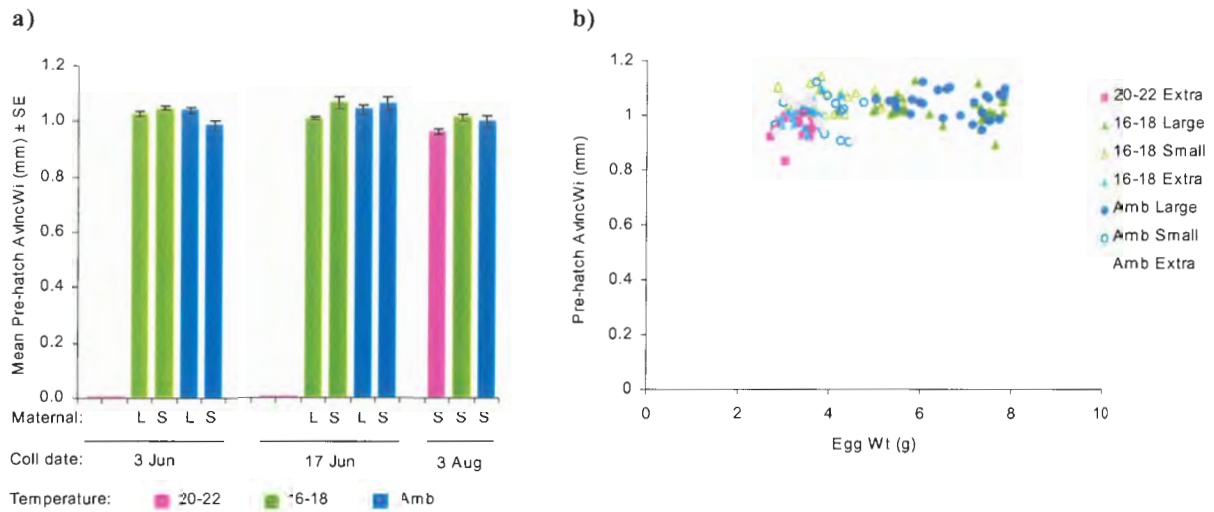


Figure 7.24 (a) Average increment width of pre-hatch bone (Pre-hatch AvIncWi) of juveniles reared from the eggs of small and large females under different temperature and feeding regimes. (b) Bivariate scatterplot of Pre-hatch AvIncWi with respect to Egg Wt with points grouped according to different feeding regimes, temperature treatments and maternal group combinations.

Table 7.12 Results of ANOVA tests for variation in the Pre-hatch AvIncWi of juveniles with respect to temperature treatment (Temp), food regime (Food), original maternal group (Maternal) and tub number (Tub).

| Experiment | Source of variance | df | F Value | Prob < F |
|-------------------|--------------------------|----|---------|----------|
| June eggs | Temp | 1 | 0.75 | 0.397 |
| | Coll Date | 1 | 3.77 | 0.057 |
| | Maternal | 1 | 0.06 | 0.813 |
| | Temp*Coll Date | 1 | 3.54 | 0.065 |
| | Temp*Maternal | 1 | 10.31 | 0.002* |
| | Coll Date* Maternal | 1 | 1.97 | 0.166 |
| | Temp*Coll Date* Maternal | 1 | 0.02 | 0.901 |
| | Tub (nested) | 27 | 2.72 | 0.001* |
| | Residual | 56 | | |
| Extra August eggs | Temp | 2 | 3.71 | 0.036* |
| | Tub (nested) | 15 | 0.62 | 0.839 |
| | Residual | 30 | | |

* Significant at the $\alpha = 0.05$ significance level.

NB: The experimental design for June eggs was unevenly replicated with respect to the fixed factor Coll Date.

underside of the rocks to females for egg-laying. These points would be of concern if the absolute quantification of eggs was the aim rather than relative comparisons. Similarly, the increased elevation of rocks may have resulted in an increased rate of predation on eggs.

Numerous observations suggested that sea urchins, *Heliocidaris erythrogramma*, fed on the eggs of *S. apama*. Although urchins tend to be mostly herbivorous, some can be omnivorous or entirely carnivorous depending on the primary food source available (Lawrence and Sammaros 1982). For example, eggs of the gastropod, *Anachis floridana*, were one of the preferred prey species of the omnivorous sea urchin, *Arbacia punctulata*, in experimental trials (Wahl and Hay 1995). *H. erythrogramma* are present at the aggregation area in very large numbers (4-11 urchins.m⁻²; SANTOS Ltd. 1985) in the same depth zone (3-5m) and habitat as the cuttlefish eggs. Therefore, predation by urchins may have a significant effect on the survival of *S. apama* eggs at the aggregation site, which ultimately affects recruitment to the population.

Suitable habitat for egg-laying did not appear to be limited as some rocks were not used throughout the entire spawning season. However, some unknown factor may have rendered those particular rocks unsuitable for egg deposition. The criteria used by females for choice of egg deposition sites were not investigated in this study. Perhaps the presence of high numbers of urchins on a rock deterred egg-laying as the plot that consistently recorded the highest number of urchins also recorded the lowest density of eggs. The hatching success of eggs in aquaria was relatively high (75-100%) and the low numbers of deformed eggs in the field indicated that most eggs developed successfully even when laid in very dense clusters, excluding losses due to predation.

Larger and older females deposited larger eggs, which produced larger hatchlings. A difference in hatchling size was also found between different rearing temperatures, with larger hatchlings resulting from development at higher temperatures. This result was opposite to that previously recorded in the literature. Bouchaud and Daguzan (1989) found hatchlings from eggs developed at higher temperatures were smaller and less resistant to food shortages following hatching. They attributed this to a decrease in the efficiency of yolk conversion at higher temperatures such that hatchlings had smaller internal yolk sacs and relatively large external yolk sacs at the time of hatching (Bouchaud 1991). No leftover external yolk sacs were noticed in any treatments in the current experiments except for some of the August eggs in the 16-18°C treatment, which prematurely hatched due to a disturbance event and consequently had a significantly lower mean hatch weight. Cronin and Seymour (2000) proposed that hatching for *S. apama* possibly occurred in response to low previtellic oxygen, which is a condition more likely to occur with higher water temperatures. Hence, the smaller hatchlings at higher

temperatures observed by Bouchaud (1991), may have resulted from premature hatching in response to oxygen limitation.

The results of the juvenile growth experiment suggested that growth rate varied according to the pre-experimental size of the individual, but which accounted for less variation than the two main treatment factors of food and temperature. Nevertheless, it does suggest that in similar environmental conditions, larger hatchlings will achieve higher growth rates than smaller hatchlings. Thus, original maternal size and age class would have an effect on subsequent juvenile growth rates.

The ration levels used for the juvenile feeding experiment were probably below optimal feeding rations in both food level treatments. The “full” and “half” levels administered equated to a mean daily feeding ration of 10.7% TWt.day⁻¹ and 5.3% TWt.day⁻¹, respectively. All food offered at these levels was consumed with no wastage. The maintenance ration for juvenile *S. officinalis* reared at temperatures of around 20°C varied between 2 to 3.5 % TWt.day⁻¹, below which animals lost weight and ultimately died within 40 days (Koueta and Boucaud-Camou 2001). Hence, the “half” ration levels used here were only just above these maintenance levels at the start of the experiment, particularly for the younger juveniles in the Amb treatment. This may have contributed to the lower survival rates recorded for that treatment compared to the others. However, it should be noted that the *S. officinalis* hatchlings used to establish the maintenance levels were much smaller than *S. apama* hatchlings used here (0.05-0.18g compared to 0.4-0.85g; Koueta and Boucaud-Camou 2001; the present study).

Juvenile *S. officinalis* fed unlimited food in captivity consumed ration levels of 10 to 46% TWt.day⁻¹ depending on the density of food offered and age (Koueta and Boucaud-Camou 2001). The optimal level of consumption decreased with age from 16.2% at 10 days of age to 10% at 40 days. Based on the methods used in this study, the juveniles had a gradual increase in ration level with age imposed on them instead of a natural decrease. As the juveniles that were used in the different temperature treatments were of different ages, those that were older probably received a ration closer to their optimum than younger ones. This fact may have further confounded the comparisons between different temperature treatments. Ideally ad libitum feeding would have been used for the “full” ration and the “half” ration calculated based on the daily consumption rate of the “full” treatment. However, the supply of live food was limited in this study and thus ad libitum feeding was not possible.

Nevertheless, some important conclusions can be drawn from the juvenile growth experiments despite the loss of one temperature treatment in the main experiment, the use of different aged juveniles in each temperature treatment and the limitations with respect to ration levels between treatments. Overall, variation in ration level produced a significant effect on the somatic growth rate of juveniles

and on cuttlebone growth in all temperatures tested. Juveniles maintained on higher food levels showed higher growth rates and had wider and more numerous growth increments in their cuttlebones. These results were consistent with the current interpretation of the increment width patterns in cuttlebones of the wild-caught adults, as presented in Chapter 6.

The effect of temperature on cuttlebone growth was not as conclusive. Further studies are necessary to verify that increased growth rates and hence cuttlebone growth actually occur at higher temperatures (i.e. during summer) as assumed in the age estimation interpretations. Without this verification it is difficult to relate the variation in increment width in the cuttlebones of adult *S. apama* collected from the wild with time of year. Other factors may influence growth rate and cuttlebone growth that do not relate to seasonal variation in temperature, including migration and variation in prey abundance (discussed in Chapter 6). Nevertheless, the results from this study do not contradict and in most cases support the current interpretation of aging results.

The results were also in general concordance with those from previous studies on juvenile growth rates of other cephalopods in captivity. Most studies have investigated the influence of only one variable, particularly temperature with unlimited food supply (e.g. Richard 1969; Forsythe and Hanlon 1988; Forsythe et al. 1994; Re and Narcisco 1994; Durholtz and Lipinski 2000; Villanueva 2000; Bettencourt and Guerra 2001; Forsythe et al. 2001; Hatfield et al. 2001) or the effects of different food rations at a single temperature level (e.g. Boletzky 1974a; Koueta and Boucaud-Camou 1999; Moltzschaniwskyj and Jackson 2000; Domingues et al. 2001; Koueta and Boucaud-Camou 2001). Few studies have investigated the interactive or simultaneous effects of food and temperature on juvenile growth rates (e.g. Richard 1967; Moltzschaniwskyj and Martínez 1998; Martínez et al. 2000). The latter studies indicate that the interactive effects of both variables are quite complex and affect different parts of the body in different ways (Martínez et al. 2000). Variation in food level usually accounts for more variation in growth rate than does temperature, as the increase in growth rate at higher temperatures is usually caused by a concurrent increase in ingestion rate. Under conditions of food limitation the effects of temperature become less apparent (Escribano et al. 1997; Moltzschaniwskyj and Martínez 1998; Brockington and Clarke 2001).

Relating laboratory results from feeding experiments back to the wild is always problematical due to the limited knowledge of food availability in the wild. Hence it is difficult to evaluate whether the food levels used in experiments were realistic (Escribano et al. 1997; Moltzschaniwskyj and Martínez 1998). In the case of juvenile *S. apama* this is particularly true as the diet of wild individuals is not known. Most juvenile *Sepia* feed on mysids or other small crustaceans (Boletzky and Hanlon 1983; Hanlon and

Messenger 1988; Nixon and Mangold 1998). Large swarms of mysids were noticed at the aggregation area during the hatching period. A study of three species of temperate mysids in similar habitat in Tasmania found that the abundances of the different species fluctuated throughout the year, with distinct peaks at different times for each species (Fenton 1992). Each species was low in abundance during August, September and October due to the death of adults at the end of the peak summer breeding period, with no substantial increase in abundance until their offspring matured in November and December. It is not known if similar species or patterns in abundance of mysids are found in the northern Spencer Gulf, but the winter depression of breeding is a common feature of most species in moderately cold temperate environments (Fenton 1992). This suggests that juvenile *S. apama* that hatch later may experience higher prey abundance due to the warmer spring temperatures.

In conclusion, the results of this Chapter showed that: (1) eggs were deposited in the aggregation area from May to August, with peak accumulation rates occurring in May and June; (2) the timing of egg development and hatching in relation to prevailing water temperatures means successive hatchlings experience different environmental conditions after hatching; (2) that large variation in the growth rates of juvenile *S. apama* can occur in response to variable environmental conditions such as temperature and food availability; (3) that variation in growth rate may influence the internal pattern of growth increments in the cuttlebone; and (4) that the patterns of variation in measured variables determined from the current experiments supported the interpretation of cuttlebone microstructure patterns as presented in Chapter 6, i.e. that two alternative life cycles for *S. apama* exist in the northern Spencer Gulf with life spans of one or two years.

8 Reproductive biology

K.C. Hall

8.1 Introduction

The reproductive output of a population is related to the reproductive strategy of the species and individual variation in the level of energy invested in gamete production (fecundity). Many life history characteristics together comprise the reproductive strategy of a species, including the age or size at first maturity, the frequency and duration of reproductive events and life span. Reproductive strategies have been traditionally classified according to two main types: (1) semelparous, where lifetime gamete production is confined to a single spawning event at the end of a brief life cycle; and (2) iteroparous, where lifetime gamete production is distributed over multiple spawning events over a large proportion of the life cycle, with feeding, somatic growth and regeneration of gonads between each event (Kirkendall and Stenseth 1985). However, these are now considered to represent the two extreme ends of a wide spectrum with many possible variations in between (Kirkendall and Stenseth 1985; Rocha et al. 2001).

Until recently most cephalopods were considered semelparous (Arnold and Williams-Arnold 1977; Mangold et al. 1993). However, the gradual discovery that many species do not conform to the traditional definition of semelparity has led to the realisation of a much broader range of reproductive strategies within this group (Mangold et al. 1993; Rocha et al. 2001). Furthermore, plasticity of life history characteristics amongst individuals of a single species in response to variation in environmental conditions (Chapter 7) has led to the recognition of multiple reproductive strategies for some species (e.g. Boyle et al. 1995; Collins et al. 1995b; Pecl 2001). Semelparity has generally been assumed for *S. apama* (Lu 1988b) but the reproductive biology has never been studied. Large numbers of cuttlebones wash up on some beaches of southern Australia in spring, which may coincide with mass mortality at the end of the spawning season (Lu 1988b). Obviously, understanding the reproductive strategy of the species would assist in formulating the appropriate management plan for this natural resource.

Semelparous species tend to allocate a high proportion of their body weight to reproductive tissue, often at the expense of somatic growth, which may decline or cease while energy resources are redirected to reproductive growth (Guerra and Castro 1994). Furthermore, food ingestion may decrease during or immediately following maturation or spawning and energy requirements during this final

period of the life cycle are met by endogenous sources, such as from somatic tissues (Castro et al. 1992). Thus, a decline in somatic condition often accompanies maturation or spawning in semelparous species (Cortez et al. 1995).

For cephalopods, the two organs most commonly assessed for declines in condition are the mantle and digestive gland (O'Dor and Wells 1987). The mantle of cephalopods consists primarily of muscular tissue, with proteins making up the principal component. Therefore, under conditions such as starvation or reproductive growth, the mantle proteins are likely to be utilised as an energy source (O'Dor and Webber 1986). The digestive gland of cephalopods is generally considered a “storage organ” for significant amounts of lipid (Blanchier and Boucaud-Camou 1986). Starvation resulted in a rapid decrease in the size of the digestive gland with respect to body size of *S. officinalis* (Castro et al. 1992). In this study, the mantle weight and thickness, and digestive gland weight were examined for cuttlefish collected from the aggregation area, to determine if a decline in condition was evident during the spawning season, which might indicate exhaustive spawning and a semelparous reproductive strategy.

The other important factor involved in the reproductive output of a population is the variation in individual fecundity. This was traditionally estimated by counting mature or maturing gametes within the reproductive organs of mature specimens just prior to the spawning season (Boletzky 1987). However, this technique can be unreliable when there is progressive production of gametes over a prolonged spawning season. A simple count of the number of mature ova in the ovary and/or oviduct grossly underestimated the total fecundity of an individual for any cephalopod species also spawned in captivity (e.g. Boletzky 1987; Lewis and Choat 1993; Maxwell and Hanlon 2000). Hence, in this study, spawning experiments using mature *S. apama* held in captivity were attempted to estimate the individual fecundity of female *S. apama* over the 3 month spawning season.

8.2 Aims

The analysis of the growth patterns found in the internal microstructure of the cuttlebone of wild caught *S. apama* adults suggested two alternative life cycles, neither of which involved spawning in more than one season (Chapter 6). This suggests that all individuals are semelparous even though some have an annual life cycle and others have a biennial life cycle. This chapter is concerned with verifying the reproductive strategy of *S. apama* and providing estimates of fecundity of individuals of the two different life cycles and relating this to the potential reproductive output of each and the spawning population at the aggregation area. The specific aims were:

- (1) to investigate the maturity status of different sized and aged individuals from the aggregation area to verify whether individuals from both year classes are mature at the same time and for the whole spawning season;
- (2) to determine if mature individuals are present at other locations in northern Spencer Gulf at other times of the year, which would indicate a more prolonged or year-round spawning season;
- (3) to determine the proportion of body weight devoted to reproductive tissue in comparison with other cephalopod species in relation to their reproductive strategy;
- (4) to determine if a decline in gonad tissue occurs during the spawning season, a possible indication of the same individuals being present for the entire spawning season;
- (5) to determine if a decline in condition of common storage tissues such as mantle muscle and digestive gland occurs during the spawning season, which might indicate senescence and semelparity;
- (6) to estimate the fecundity of different sized or aged females and to relate the findings to the size and age structure of the aggregation population;
- (7) similarly, to determine if different sized or aged males have different levels of investment in reproductive tissue which might indicate different "male quality".

8.3 Methods

8.3.1 Samples

The locations, dates and methods used to collect samples considered in the reproductive analysis were detailed in Chapter 3. General mensurative methods used for laboratory processing of samples were also provided.

8.3.2 Reproductive assessment

Terminology of reproductive organs follows that of Mangold (1987). *For males*, the testis weight (TestisWt) and spermatophoric complex weight (SCompWt; comprising the sperm duct, spermatophoric organ, spermatophoric duct, spermatophoric sac and penis) were recorded. For each male collected at the start of the spawning season in May 2000, a random sub-sample of 10

spermatophores were measured and their average length calculated ($AvSpermL$) to assess gamete size relations. *For females*, the ovary ($OvaryWt$) and proximal oviduct ($OductWt$) were carefully separated and weighed. The number and weight of mature yolk-filled eggs in the oviduct were recorded ($EggNo$ and $EggWt$, respectively). For each female collected in May 1999, the largest diameter of a sub-sample of 10 eggs was also measured and the average was calculated ($AvEggD$) to assess gamete size. The nidamental gland complex ($NidWt$; comprised of the paired nidamental glands and accessory nidamental glands) was also weighed.

8.3.3 Condition assessment

The intact head (including buccal mass and arms) was weighed before the hard structures were removed for ageing analysis (Chapter 6). The digestive gland was carefully removed and weighed ($DGWetWt$), before being stored in a petri dish and frozen. These were later thawed and dried in an oven at 60°C for up to a week to evaporate all moisture, and then reweighed for dry weight ($DGDryWt$). The stomach was graded visually for degree of fullness, where 1 was empty and 5 fully distended.

The remaining internal organs were removed and the total wet weight of the mantle including the funnel apparatus (MWt) was recorded. An incision was made along the midline of the anterior mantle and the mantle thickness ($Mthick$) was measured approximately one third of the way along the incision from the anterior end.

8.3.4 Data analysis

The relationship between each variable and body size, either TWt or ML , was assessed by plotting bivariate scatterplots. Most had a linear relationship with size or conformed to the simple allometric equation:

$$y = bx^{\alpha}$$

where, x represents body size, y the dependent variable and α the allometric coefficient. In the latter instance, log-transformation of variables x and y , resulted in a linear-regression relationship of the general form:

$$\log y = \alpha \log x + \log b.$$

Isometry, the special case where $\alpha = 1$ indicating y is directly proportional to x , was rarely observed and in most cases immature and mature individuals showed significantly different linear-regression relationships for the same variable. Therefore, the calculation of gonad indices using ratios to correct for body size was inappropriate in all cases (Klingenberg 1996) and the appropriate linear regression relationship was used to adjust each individual value to the overall mean size of the group to correct for the effect of size (after Packard and Boardman 1987). Means of adjusted values were used for graphical comparisons and analysis of covariance (ANCOVA) tests, using the original values (or log-transformed values) with the appropriate size variable (or log-transformed variable) as a covariate, were used for statistical tests between different samples.

8.3.5 Spawning experiments

The methods used for the collection and maintenance of adult cuttlefish in aquaria in 1998 and 1999 were provided in Chapter 6. The alterations to the experimental design in 1999, were mainly in response to the lack of egg-laying observed in the 1998 experiment. These were as follows: (1) in 1998 adults were collected from Myponga Reef, in Gulf St Vincent due to its proximity to the aquarium facility. Variation in the timing of spawning between the two Gulfs may have contributed to the lack of spawning in experiments in 1998. Therefore, in 1999, adults were collected from the spawning aggregation area at the start of the spawning season in May to ensure all were sexually mature and ready to spawn; (2) in 1998 the cuttlefish were maintained in the outdoor set-up with ambient water temperatures, lest a change in water temperature or light regime interfered with egg-laying, whereas in 1999 cuttlefish were maintained in the indoor set-up under four different temperature regimes as required by the age validation experiment (Chapter 6); and (3) in 1998, there was one male-female pair per tank, whereas in 1999 there was one female in every tank, resulting in 3 per temperature treatment, but only one male was rotated between the three females of each treatment. This provided females with a chance to lay eggs in the absence of a male, in case the continual presence of a male prevented the females from spawning.

In both years, two types of substrate were provided for egg deposition: (1) flat rock slabs collected from the aggregation area with the natural suite of algae and benthic organisms in tact; and (2) a cement brick of 40x15x15 cm in size with two large oval cavities. In the three ambient tanks in 1999, rock slabs from the aggregation area with *S. apama* eggs already attached were also added to the tanks in case the presence of previously laid eggs encouraged egg deposition.

Unfortunately, neither experiment resulted in egg deposition in captivity. Some essential cue or substrate feature required for spawning may have been absent in the captive situation; therefore, experiments were attempted in the field in May 2000 at the start of the spawning season. Four polyethylene cages with a mesh size of 3 mm and dimensions of 2x2x2 m were deployed in the 4 m depth zone at the Black Point site. Large rock slabs similar to those used in the egg monitoring experiment were placed within each cage to provide habitat for egg-deposition. When females were first noticed in the aggregation area one female and male pair were placed in each cage and the rocks monitored daily for egg deposition.

8.4 Results

8.4.1 Sexual dimorphism

A distinct sexual dimorphism was evident between male and female *S. apama*. The largest males were much larger than the largest females (Fig. 8.1b). This discrepancy in size was not as obvious for the smaller Type 2 males and females (Fig. 8.1a). Males of both sizes had longer arms than the females and a distinctly larger fourth arm, which was used in reproductive behaviours that were unique to males (Chapter 9). This was evident in the greater percentage of body weight accounted for by the head, arms and buccal mass of males compared to females of a similar size (Fig. 8.1a-b).

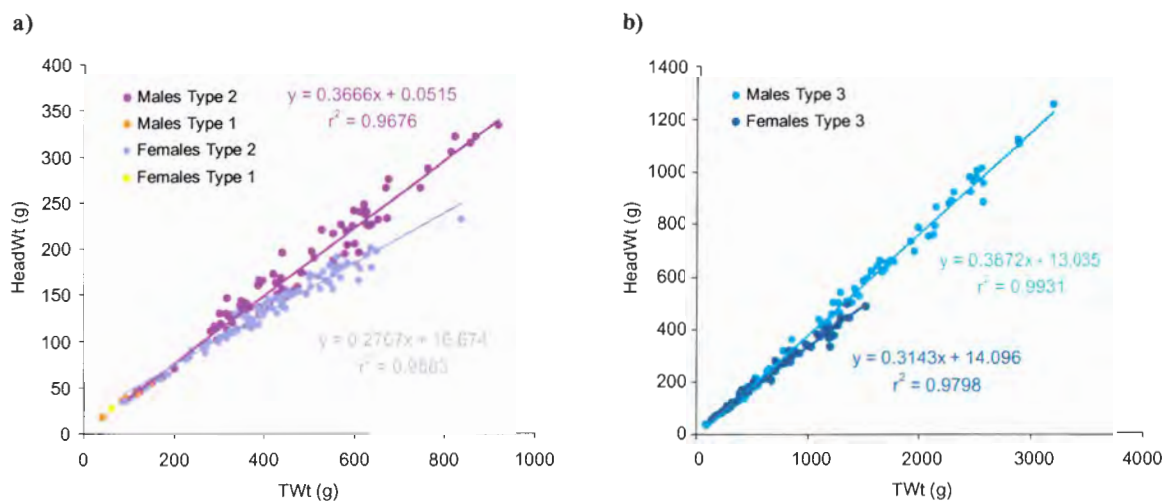


Figure 8.1 The relationship between HeadWt and TWt for male and female *S. apama*, grouped according to bone type and pooled across all samples.

The most reliable external feature used to distinguish female *S. apama* was the presence of a seminal receptacle in the buccal membrane below the beak musculature, which often had spermatangia (ejected spermatophore packets) attached to the surface. Spermatangia were also often found attached to the buccal membrane adjacent to the sperm receptacle in freshly mated females. The only external feature that conclusively identified males was the presence of a hectocotylus on the fourth arm, which consisted of a modification of the sucker rows near the base of the arm. This modified region was used to transfer spermatophores to the buccal region of the female during mating (described in Chapter 9).

8.4.2 General morphology of reproductive systems

The reproductive system of female *S. apama* consisted of a single posterior ovary, comprised of eggs at various stages of development and sizes held together by membranous connective tissue. This was connected to a single membranous oviduct, situated dorsal to the ovary. In mature individuals large yellow yolk-filled ova were stored within the oviduct. The oviduct opened into the anterior mantle cavity through the oviducal gland, which is apparently responsible for encapsulating each ovum in an individual protective capsule. The oviducal gland was connected to two large white nidamental glands, which contained thick white gelatinous material used to embed each ovum. Adjacent to these were two accessory nidamental glands, the function of which is unknown.

The reproductive system of male *S. apama*, consisted of a single posterior testis located within the forked region of the cuttlebone. The testis was connected by a duct to the spermatophoric complex, which consisted of the spermatophoric duct and organ, spermatophoric sac and penis. The sperm was packaged into spermatophores, consisting of long thin membranous packets accompanied by an ejaculatory apparatus. Spermatophores were stored within both the spermatophoric sac and penis of mature individuals.

8.4.3 Reproductive assessment

Maturity

The level of maturity of individuals was not staged according to a maturity scale based on macroscopic or histological characteristics, as is common practice for cephalopod studies (Lipinski and Underhill 1995). Only a distinction between mature and immature individuals was made based on the presence or absence of mature gametes in the storage compartments of the reproductive tracts. Stored mature gametes were considered a positive indication that the individuals were ready to spawn (as per

Mangold 1987). Thus, males were considered mature if spermatophores were present in the penis section of the spermatophoric complex (all but the smallest males had spermatophores in the spermatophoric sac), and females were considered mature if yolk-filled ova were present in the proximal oviduct.

All cuttlefish collected from the aggregation area during the spawning season were sexually mature (Fig. 8.2), with the exception of one female collected in June 1998 that had no eggs in her oviduct. She was not considered in further analyses. Several females at the end of each spawning season also had no ova in their oviducts but these were assumed to be spent, and thus were included as mature females in the analyses. In contrast, only a small percentage of females in NSG samples were mature, even those collected in late April, not long before the start of each spawning season (Fig. 8.2). Females appear to mature rapidly in late April-May, which would equate to an age at maturity of 6-8 months for Type 2 females and 18-20 months for Type 3 females. The size at maturity similarly varied according to life cycle type. All females collected from the aggregation area had fresh spermatangia attached to the surface of their sperm receptacles, indicating recent mating. In contrast, spermatangia were not detected on the receptacles of any females from the NSG samples.

Most males were mature (84.6%; n = 512). They appear to be more precocious than females, with many mature ones found in NSG as early as February, well before the start of the spawning season (Fig. 8.2). This suggests males mature at a younger age than females, possibly as early as 4 to 6 months old for Type 2 individuals and 16 to 18 months old for Type 3 individuals. No Type 1 individuals of either sex were mature in any sample. This is consistent with the hypothesis that Type 1 individuals do not mature and spawn in the first season following hatching.

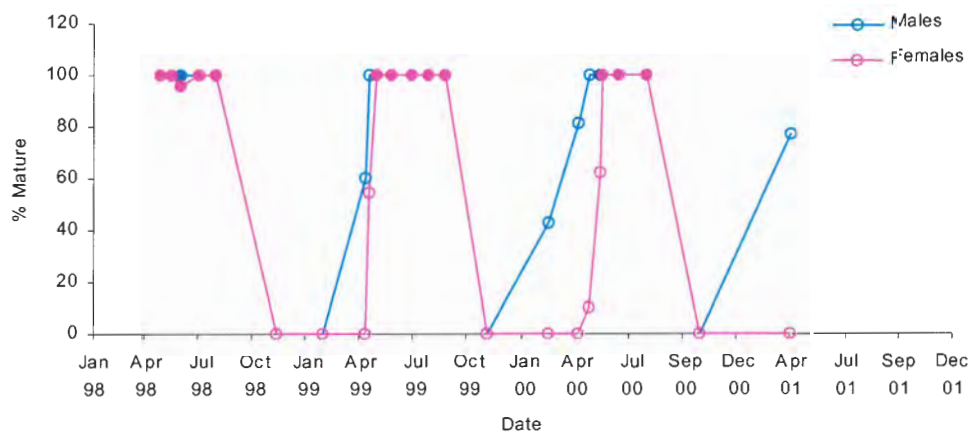


Figure 8.2 Variation in the percentage of mature males and females recorded in NSG (open circles) and aggregation (solid circles) samples collected from May 1998 to April 2001.

Gonad-body weight relationships

All but one of the measured components of the reproductive systems showed a positive log-linear relationship with TWt, when all samples were pooled (Fig. 8.3 to Fig. 8.5). The exact relationship, however, varied considerably between mature and immature individuals and between individuals of different year classes (bone types). OductWt was the one variable that did not show a consistent relationship with body size (log TWt) (Fig. 8.5); therefore, no adjustment for size was made and original values were used in all subsequent analyses. For all other variables, the individual values were scaled with respect to TWt according to the corresponding linear regression relationship between the log variable and log TWt, and adjusted to an overall mean size of 494.2 g (SE = \pm 16.0 g; n = 396; range = 16 – 1623 g; log mean = 2.694) for females and 709.8 g (SE = \pm 17.8 g; n = 512; range = 25 – 3208 g; log mean = 2.851) for males. Due to the large differences between the relationships of mature and immature individuals with respect to all variables, the two groups were treated separately in all subsequent analyses. Not all samples were analysed for age estimation, so it was not possible to separate all samples according to bone type.

For all reproductive variables examined, the linear relationship was positive, such that larger individuals had more gonad tissue. Consequently, Type 3 individuals tended to have larger gonads than Type 2 individuals due to their larger body size. However, for any given size, Type 2 individuals actually had larger gonads (Fig. 8.3 to Fig. 8.5). Indeed the grouping of data according to bone type helped explain more variation around the general relationship in all cases. NidWt showed the least variation around the log-linear regression relationships with TWt (Fig. 8.4*d-f*), which suggests that the size of the nidamental glands is depends more on body size than stage of maturity or spawning condition.

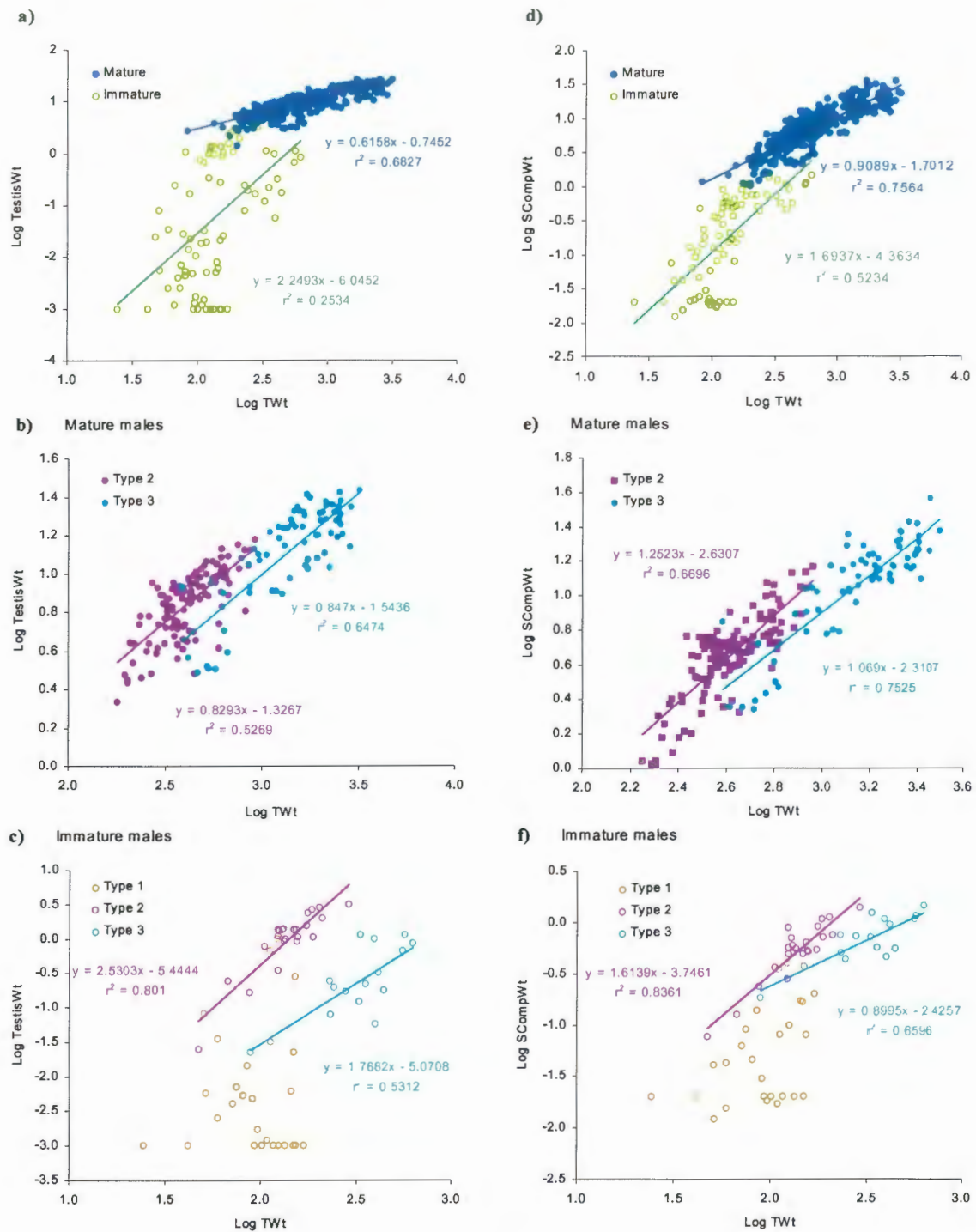


Figure 8.3 The relationship between log TestisWt and log TWt (a-c) and log SCompWt and log TWt (d-f) for male *S. apama* with respect to maturity (a,d) and bone type (b-c,e-f) and pooled across all samples.

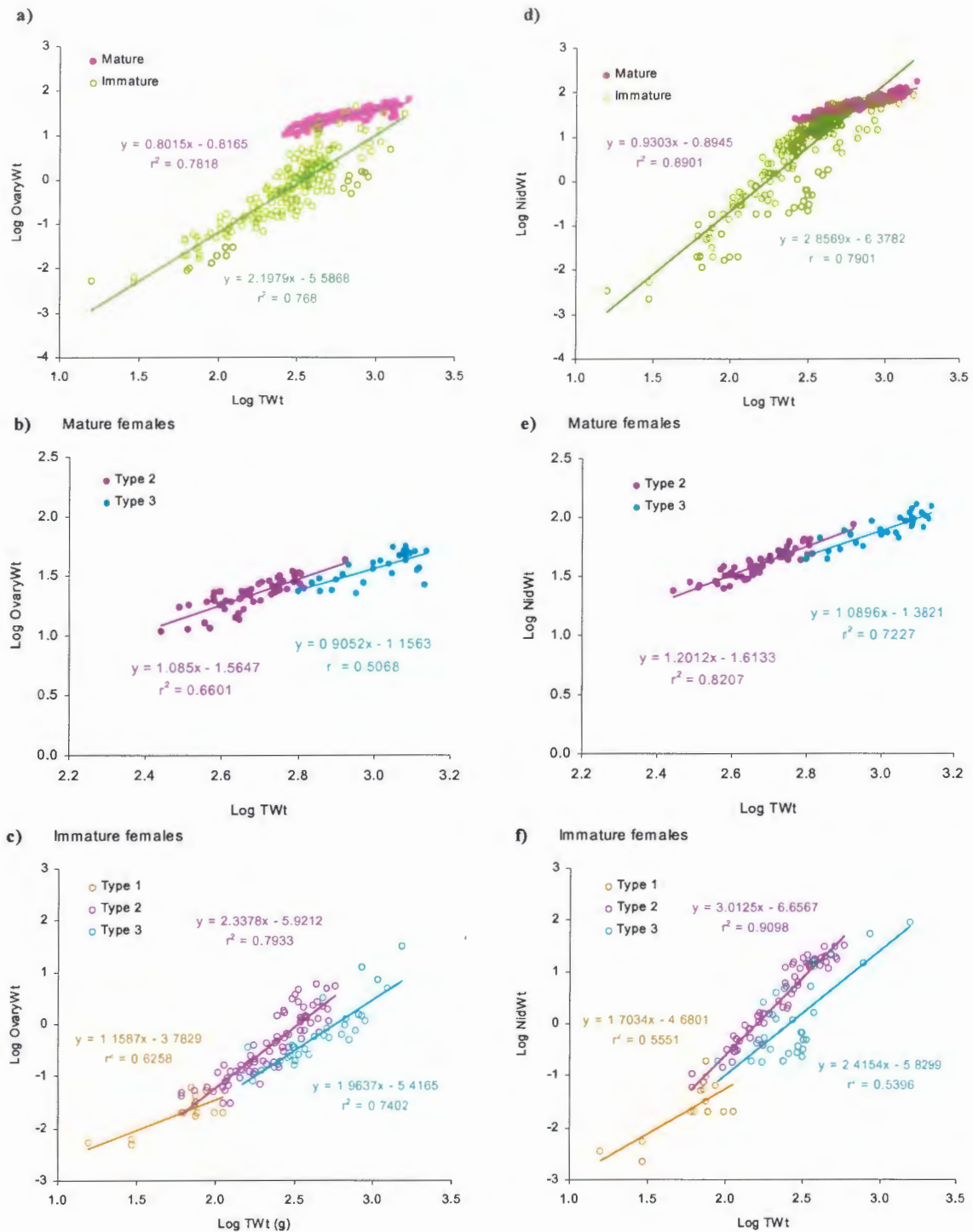


Figure 8.4 The relationship between log OvaryWt and log TWt (a-c) and log NidWt and log TWt (d-f) for female *S. apama* with respect to maturity (a,d) and bone type (b-c,e-f) and pooled across all samples.

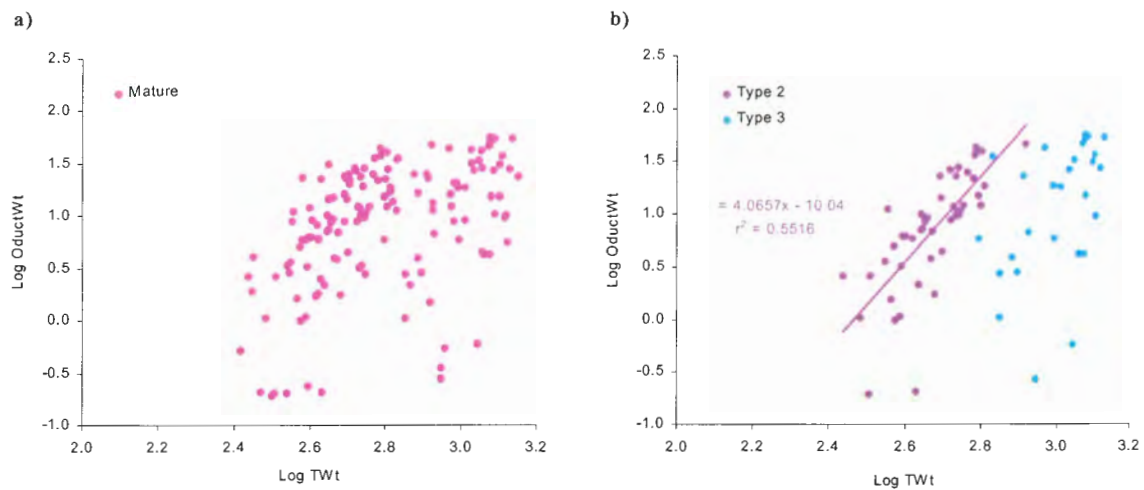


Figure 8.5 The relationship between log OductWt and log TWt for female *S. apama* with respect to maturity (a) and bone type (b) and pooled across all samples.

Females had a much larger TGonadWt for a given size than males (Fig. 8.6). Mean TGonadWt in females varied greatly with maturity status (Fig. 8.6d). Mature individuals had between 35.8 g and 485.3 g (mean 110.4 ± 5.5 g SE; $n = 170$) of body weight invested in reproductive tissue, which accounted for between 8.8% and 39.6% (mean 15.6 ± 00.4 % SE; $n = 170$) of TWt. A much smaller percentage of TWt was devoted to reproductive tissue in immature females (mean 3.5 ± 0.2 % SE; $n = 226$; range 0.02 – 15%). The mean total weight of reproductive tissue in males was much less, irrespective of maturity status. Mature males had between 3.3 to 61.7 g (mean 19.7 ± 11.4 g SE; $n = 433$) of body weight invested in reproductive tissue, which accounted for only 1.0% to 5.4% (mean 2.8 ± 0.8 % SE; $n = 433$) of TWt. This figure was smaller again for immature males, with only between 0.01% and 2% (mean 0.5 ± 0.1 % SE; $n = 79$) of TWt comprised of reproductive tissue.

TGonadWt varied according to TWt with a log-linear relationship (Fig. 8.6) for both sexes, similar to the relationships for the individual components of the reproductive systems. The exact relationships between the log-transformed variables also varied according to maturity status and bone type, so individual values were adjusted according to size using the corresponding log-linear relationship as described for the individual components.

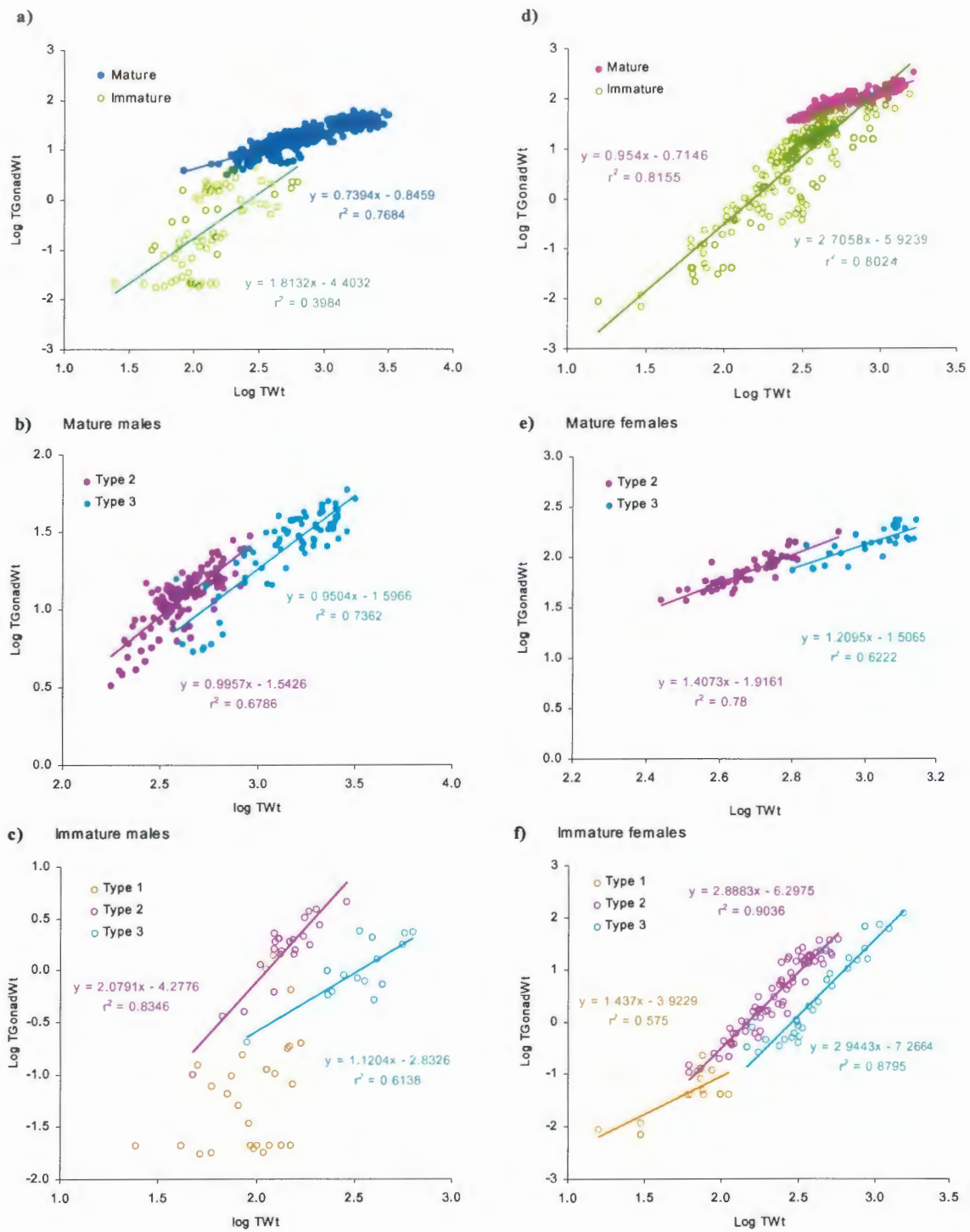


Figure 8.6 The relationship between log TGonadWt and log TWt for male (a-c) and female (d-f) *S. apama*, with respect to maturity (a) and bone type (b-c) and pooled across all samples.

Temporal variation in reproductive variables

The measured variables of the reproductive system were averaged according to maturity status for analysis of temporal trends across all samples (Fig. 8.7 to Fig. 8.9). The mean values for individuals held in captivity for the duration of the spawning seasons in 1998 and 1999 were also included, as the conditions experienced by these individuals were known and might serve as a basis for comparison with those collected from the wild.

The mean TWt varied considerably across different samples for both sexes (Fig. 8.7). Immature individuals were considerably smaller than mature individuals collected from either the aggregation area or NSG population. Mature females in NSG samples were of a similar size to those collected from the aggregation area (Fig. 8.7*b*), whereas this was not so for males (Fig. 8.7*a*). The very large Type 3 males found at the aggregation area were relatively rare in NSG samples, even in April just before the start of the spawning season. There was a general decline in mean TWt of mature individuals collected from the aggregation area as the season progressed from May to August, which was particularly pronounced for females (Fig. 8.7*b*).

There was a distinct decline in mean adjusted TGonadWt during the spawning season for males collected from the aggregation area (Fig. 8.8*a*). This trend was also reflected in the mean adjusted TestisWt and to a less extent, the mean adjusted SCompWt (Fig. 8.8*b* and Fig. 8.8*c*, respectively). These findings were consistent with a decline in reproductive condition that might be expected if the same individuals were present for the duration of the spawning season. Immature males had a surprisingly high mean adjusted Testis Wt relative to TWt in February samples in 1999 and 2000 (Fig. 8.8*b*), which suggested testis maturation occurred around that time and little of the weight was converted to SCompWt. The subsequent lower values for all mature individuals in April suggested that TestisWt declined relative to TWt and SCompWt once maturity was reached later in the season.

Mean adjusted TGonadWt of mature females also varied in samples from the aggregation area over the spawning season but the trend was less pronounced than for males and showed greater variability between years. However, this may be due to the overriding influence of NidWt on the estimates (accounts for the largest proportion of TGonadWt), as mean adjusted Ovary Wt and Oduct Wt both declined during the spawning seasons (Fig. 8.9*b* and Fig. 8.9*c*). Immature females had much smaller ovaries than the mature ones, which did not show significant increase until April (Fig. 8.9*b*). This was consistent with the trends in percent maturity, which indicated that females underwent rapid maturation later than males, in April or May as compared to February. After being held in captivity for the

duration of the spawning season, the mean adjusted OvaryWt of females was very low, with a corresponding vast increase in the mean OductWt (Fig. 8.9). Although females did not lay eggs in captivity they apparently continued to produce eggs and store them in the oviduct.

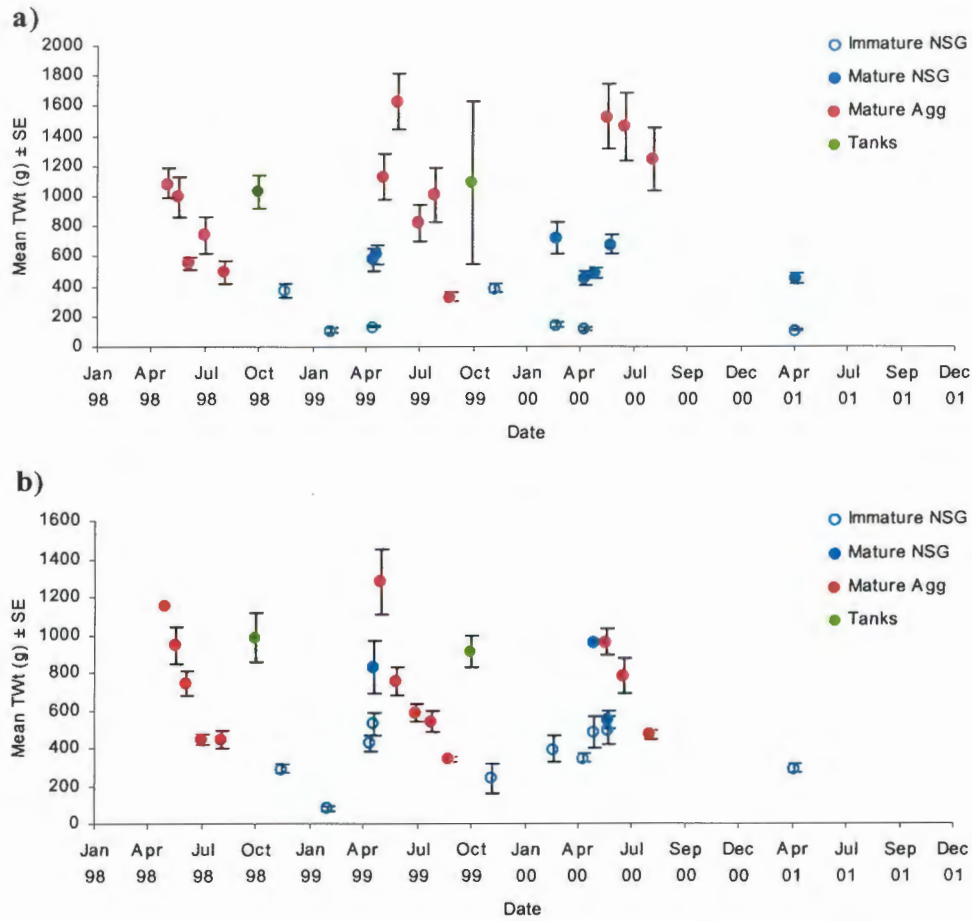


Figure 8.7 Mean TWt for male (a) and female (b) *S. apama* for each sampling date from May 1998 to April 2001.

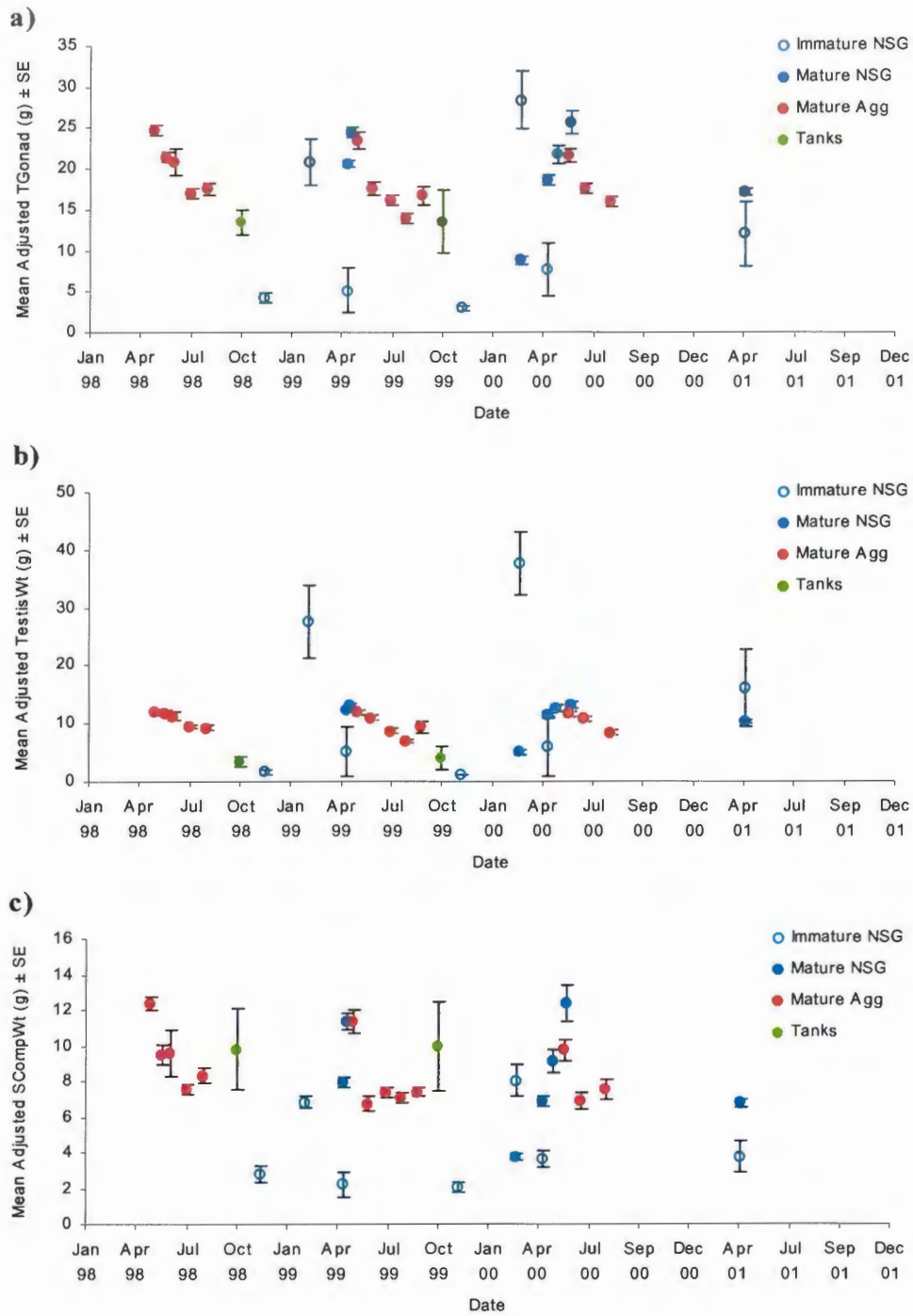


Figure 8.8 Mean adjusted TGonadWt (a) TestisWt (b) and SCompWt (c) of male *S. apama* for each sampling date from May 1998 to April 2001.

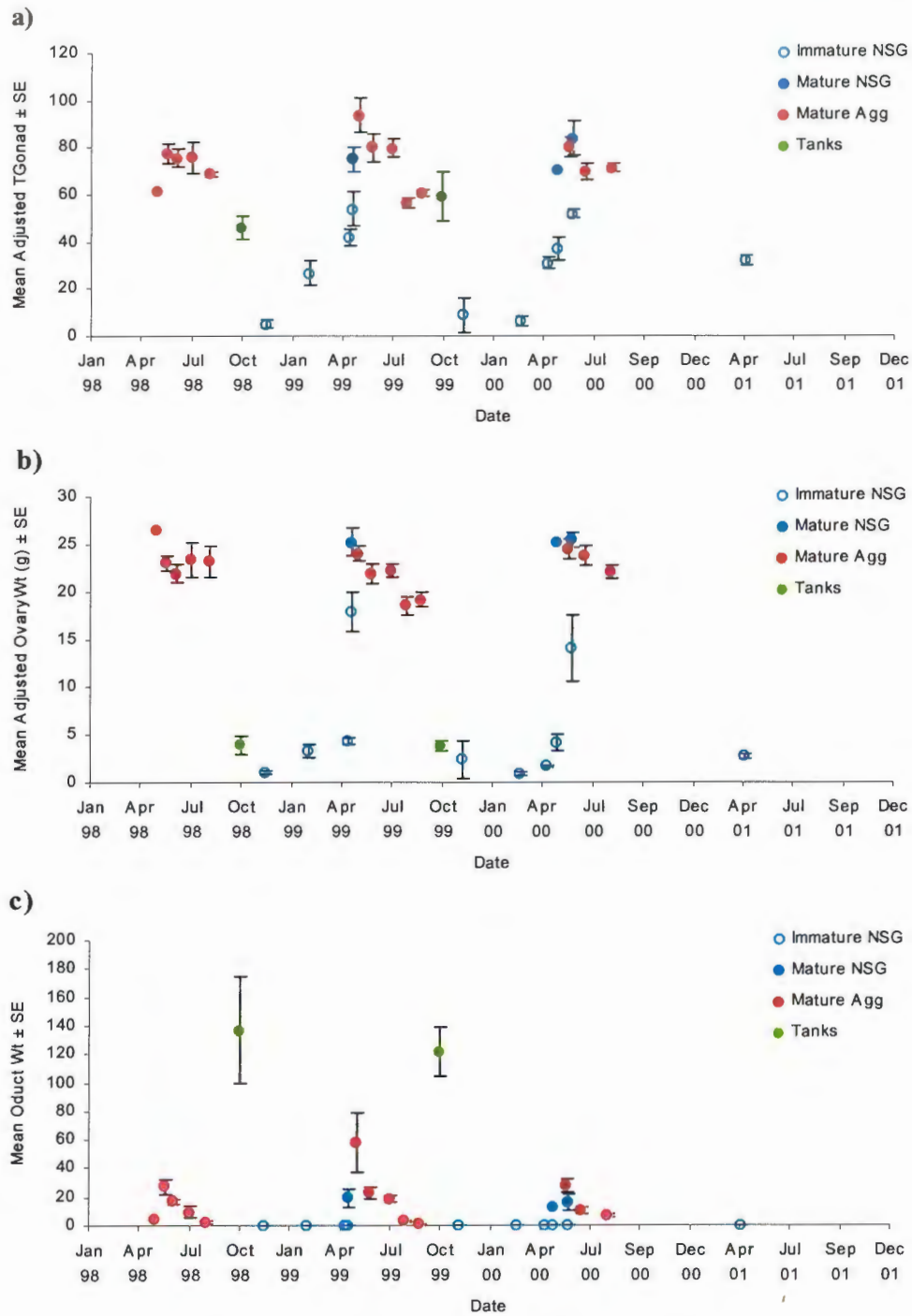


Figure 8.9 Mean adjusted TGonadWt (a), adjusted OvaryWt (b) and OductWt (c) of female *S. apama* for each sampling date from May 1998 to April 2001.

Comparison between bone types

The mean TWt's of individuals with Type 3 bones were significantly larger than those with Type 2 bones for both sexes and in all samples (Fig. 8.10a and Fig. 8.11a; Table 8.1 and Table 8.2). The mean TWt of each Type significantly decreased from the start to the end of the spawning season for both sexes. These differences were still significant when the log-transformed variable was tested via ANCOVA with log ML as the covariate. This suggests that the change in TWt was not only related to a change in overall size (ML), but also in the TWt of similarly-sized individuals, which may have related to either a decline in reproductive or somatic tissue mass.

Type 2 males had a higher mean adjusted TGonadWt relative to Type 3 males once the effect of the size difference between the two groups was removed (Fig. 8.10b). Mean TestisWt was significantly greater in Type 3 males than Type 2 males (Fig. 8.10c; Table 8.1), but after correction for the effect of size, Type 2 males had significantly greater mean adjusted TestisWt relative to body weight (Fig. 8.10d; Table 8.1). A similar trend for mean SCompWt and mean adjusted SCompWt (Fig. 8.10e and Fig. 8.10f, respectively) was also evident. Therefore, the smaller Type 2 males invested a larger proportion of body weight into reproductive tissue than did Type 3 males, but due to their smaller size the TGonadWt was smaller.

Due to the small numbers of Type 3 females in all August samples, statistical comparisons were only made between the two bone types for the May samples, and monthly comparisons were restricted to the data for Type 2 bones. No significant difference was detected between the mean adjusted TGonadWt of females of the two different bone types. And although OvaryWt was significantly larger in Type 3 females compared to Type 2 females (Fig. 8.11c; Table 8.2), the difference was not significant once the effect of relative body size was accounted for (Fig. 8.11d; Table 8.2). Therefore, Type 3 females did not invest a greater proportion of body weight into reproductive tissue but did have greater overall potential reproductive output due to their larger size. Mean adjusted OvaryWt did not significantly decline from May to August but TGonadWt and OductWt did (Table 8.2). Hence, females did not show as consistent a decline in reproductive tissue as did males toward the end of the spawning season.

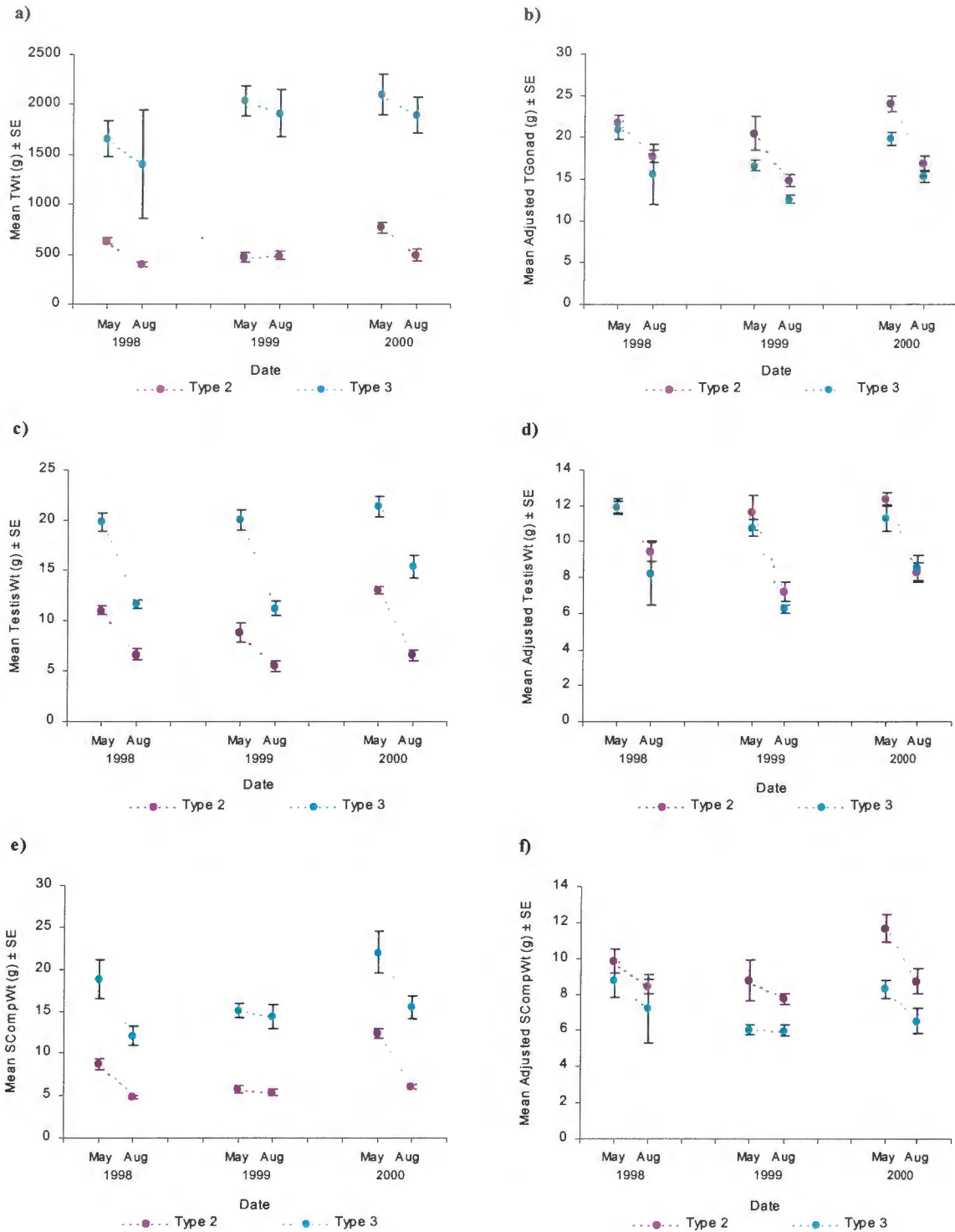


Figure 8.10 Mean weights and adjusted weights of the reproductive tissues of male *S. apama* with Type 2 and Type 3 bones, collected from the aggregation area at the start (May samples) and toward the end (August samples) of each spawning season.

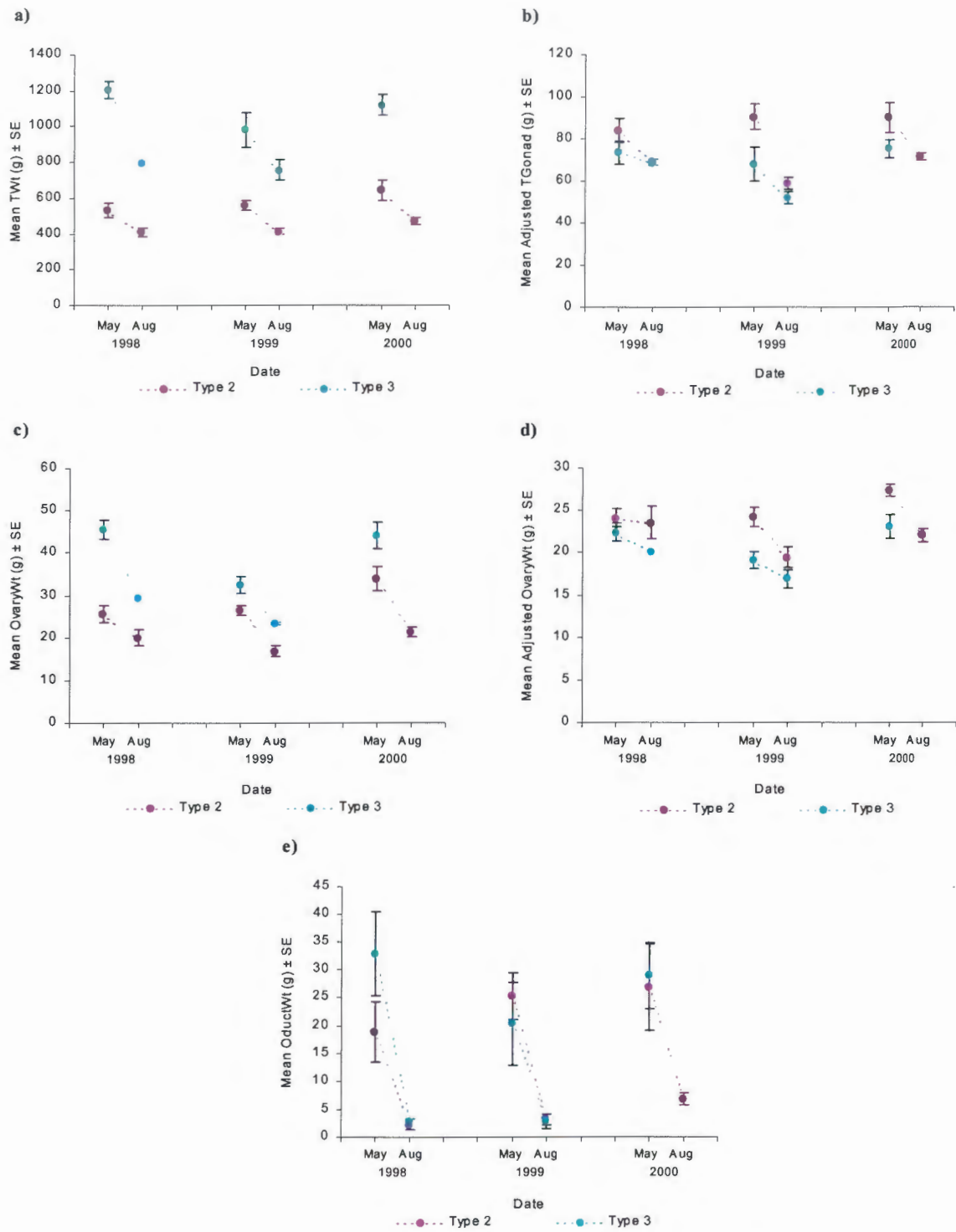


Figure 8.11 Mean weights and adjusted weights of the reproductive tissues of female *S. apama* with Type 2 and Type 3 bones, collected from the aggregation area at the start (May samples) and toward the end (August samples) of each spawning season.

Table 8.1 Results of analysis of variance (ANOVA) and covariance (ANCOVA) tests between the different bone types, collection months and years for male *S. apama* reproductive variables, using log TWt as a covariate for log-transformed variables.

| Sources of Variance | df | TWt | | log TGonadWt | | TestisWt | | log TestisWt | | SCompWt | | log SCompWt | |
|---------------------|---------|--------|--------|--------------|--------|----------|--------|--------------|--------|---------|--------|-------------|--------|
| | | F | P>F | F | P>F | F | P>F | F | P>F | F | P>F | F | P>F |
| BType | 1 | 207.02 | 0.000* | 10.75 | 0.001* | 200.07 | 0.000* | 4.69 | 0.033* | 155.77 | 0.000* | 8.13 | 0.005* |
| Month | 1 | 3.99 | 0.049* | 117.62 | 0.000* | 119.18 | 0.000* | 96.07 | 0.000* | 31.58 | 0.000* | 35.74 | 0.000* |
| Year | 2 | 3.19 | 0.045* | 20.12 | 0.000* | 9.15 | 0.000* | 7.69 | 0.001* | 11.28 | 0.000* | 16.31 | 0.000* |
| Btype*Month | 1 | 0.03 | 0.869 | 1.03 | 0.312 | 7.09 | 0.009* | 0.07 | 0.793 | 0.62 | 0.434 | 1.56 | 0.215 |
| Btype*Year | 2 | 2.35 | 0.100 | 0.18 | 0.837 | 0.84 | 0.435 | 0.09 | 0.917 | 0.12 | 0.890 | 1.19 | 0.308 |
| Month*Year | 2 | 0.54 | 0.587 | 0.96 | 0.385 | 0.02 | 0.983 | 0.84 | 0.434 | 7.28 | 0.001 | 7.35 | 0.001* |
| Btype*Month*Year | 2 | 0.14 | 0.874 | 1.34 | 0.267 | 2.86 | 0.062 | 1.11 | 0.334 | 0.29 | 0.752 | 0.34 | 0.717 |
| log TWt (covariate) | (1) | - | | 88.13 | 0.000* | - | | 35.02 | 0.000* | - | | 60.18 | 0.000* |
| Residual | 97 (96) | | | | | | | | | | | | |

* Significant at the $\alpha = 0.05$ significance level.

Table 8.2 Results of analysis of variance (ANOVA) and covariance (ANCOVA) tests[#] between the different bone types, collection months and years for female *S. apama* reproductive variables, using log TWt as a covariate for log-transformed variables.

| Sources of Variance | df | TWt | | log TGonadWt | | OvaryWt | | log OvaryWt | | OductWt | |
|---------------------|---------|--------|--------|--------------|--------|---------|--------|-------------|--------|---------|--------|
| | | F | P>F | F | P>F | F | P>F | F | P>F | F | P>F |
| BType | 1 | 111.89 | 0.000* | 3.99 | 0.054 | 29.77 | 0.000* | 0.01 | 0.944 | 0.44 | 0.512 |
| Year | 2 | 2.06 | 0.143 | 0.19 | 0.825 | 6.46 | 0.004* | 3.96 | 0.028* | 0.29 | 0.748 |
| BType*Year | 2 | 2.32 | 0.113 | 0.45 | 0.644 | 3.56 | 0.039* | 1.63 | 0.211 | 0.97 | 0.391 |
| log TWt (covariate) | (1) | - | | 30.67 | 0.000* | - | | 12.87 | 0.001* | - | |
| Residual | 35 (34) | | | | | | | | | | |
| Month | 1 | 30.84 | 0.000* | 15.78 | 0.000* | 37.83 | 0.000* | 4.03 | 0.051 | 60.68 | 0.000* |
| Year | 2 | 4.16 | 0.022* | 0.92 | 0.408 | 6.06 | 0.005* | 1.71 | 0.193 | 2.09 | 0.136 |
| Month*Year | 2 | 0.29 | 0.747 | 2.44 | 0.099 | 1.89 | 0.163 | 1.13 | 0.333 | 0.38 | 0.685 |
| log TWt (covariate) | (1) | - | | 90.41 | 0.000* | - | | 45.04 | 0.000* | - | |
| Residual | 46 (45) | | | | | | | | | | |

* Significant at the $\alpha = 0.05$ significance level.

[#] The analysis of female data was divided into two tests due to the small sample sizes of Type 3 bones in August samples. Hence, the effect of bone Type was tested using May samples only and the effect of sample date was tested using Type 2 bone data only.

Gamete size

Gamete size varied according to body size (ML), with larger Type 3 individuals producing larger ova or spermatophores than the smaller Type 2 individuals (Fig. 8.12). Furthermore, there was little variation in the size of ova or spermatophores of a single individual, but there was substantial variation between individuals of a similar size.

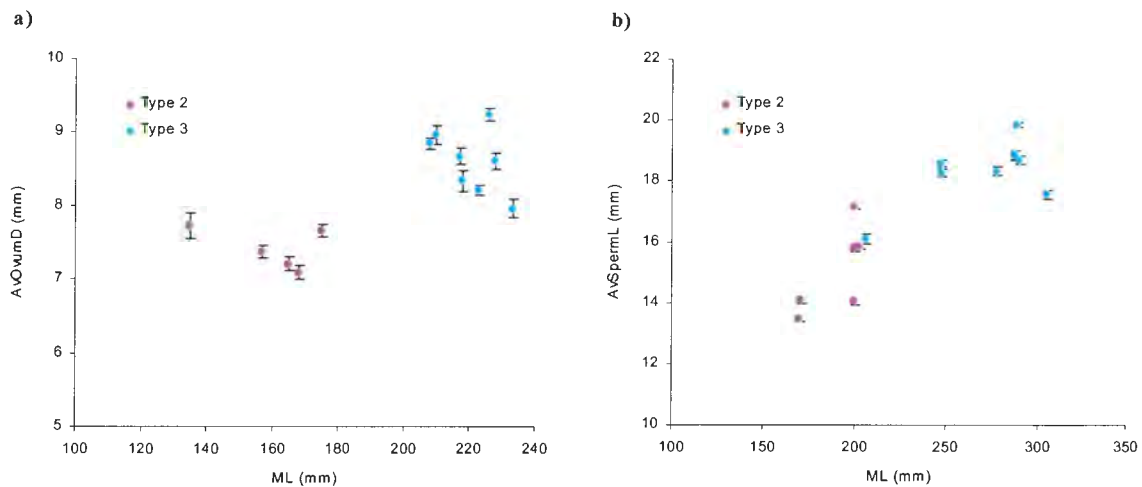


Figure 8.12 (a) The relationship between mean AvOvumD and ML for female *S. apama*. (b) The relationship between mean AvSpermL and ML for male *S. apama*.

8.4.4 Condition assessment

Condition variable-body weight relationships

All the condition variables measured showed a positive linear relationship with TWt or ML without the need for log-transformation, when data were pooled across all samples (Fig. 8.13). MWt showed a strong linear regression relationship to TWt with little variation (Fig. 8.13a-b), whereas Mthick and DGWetWt showed much greater variation around the linear relationships (Fig. 8.13c-d and Fig. 8.13e-f, respectively). These variables were analysed further for possible variation that related to maturation or spawning. It would have been preferable to use DGDryWt rather than DGWetWt, as the potential confounding effects of water replacement of consumed tissues is removed from the former by the drying process. However, the dry weights could not be used for the final analysis as a large number were lost due to oven malfunction during drying which resulted in some batches being destroyed by exposure to temperatures of over 160°C.

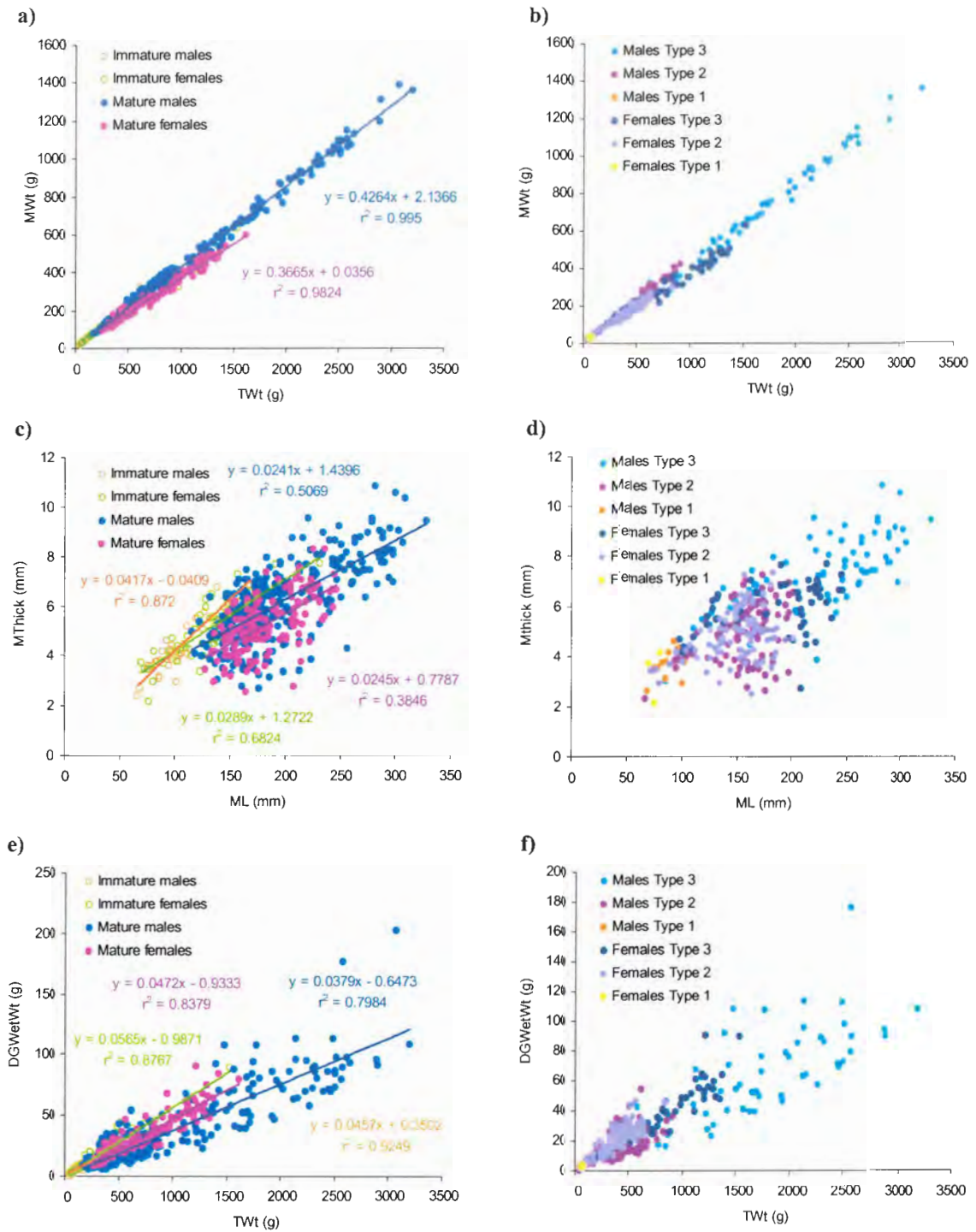


Figure 8.13 The relationship between MWT and TWt (a-b), Mthick and ML (c-d) and DGWetWt and TWt (e-f) for male and female *S. apama* with respect to maturity status and bone type and pooled across all samples.

The linear relationship of each variable to size varied considerably between mature and immature individuals and between individuals of different bone types (Fig. 8.13). Therefore, individual values were scaled with respect to body size according to the corresponding linear regression relationship, and adjusted to an overall mean size of 590.5 g (SE = \pm 62.1 g; n = 255; range = 62 – 1623 g) for females and 832.5 g (SE = \pm 34.7 g; n = 363; range = 48 – 3208 g) for males.

Temporal variation in condition variables

The highest mean adjusted Mthick was found in mature males in NSG samples and aggregation samples in April and May around the start of the spawning season (Fig. 8.14a). Immature males in NSG samples from Feb and April, comprised of Type 1 and Type 2 individuals had low mean values, whereas, those from Nov, comprised solely of Type 3 individuals, were marginally higher. The mean values for mature individuals in the aggregation samples declined throughout both the 1998 and 1999 spawning seasons but remained fairly constant in 2000. Individuals held in captivity did not feed well and grew little, such that most were thought to have died of starvation. The mean adjusted Mthick of these individuals was comparable to those of the aggregation samples toward the end of the spawning seasons.

The mean adjusted DGWetWt was highest in all NSG samples, both mature and immature, and aggregation samples from the start of the spawning season (Fig. 8.14b). The values declined throughout each spawning season. The mean values for the tank individuals were the lowest, even much lower than those for the aggregation samples at the end of the season.

The stomach fullness index (where 1 = empty and 5 = fully distended) was highest in the NSG samples, particularly for immature males; and very low, generally between 1 and 2, for all aggregation samples (Fig. 8.14c). This suggests that individuals in the wider NSG area actively feed during the summer months, whilst those at the aggregation area show little evidence of feeding.

In contrast to males, the highest mean adjusted Mthick for females was found in immature and mature females from NSG samples (Fig. 8.15a). Mature females in the aggregation samples had slightly lower mean values in May which subsequently declined throughout the spawning season. Females maintained in tanks had the lowest recorded mean Mthick, particularly those from the 1999 experiments. In comparison, mean adjusted DGWet Wt varied little across samples, with one mature individual in the NSG sample from April 2000 and the females maintained in captivity the only notable exceptions (Fig. 8.15b). For the stomach fullness index the immature females in NSG samples had the highest mean values, whilst very low values were attained for all aggregation samples (Fig. 8.15c).

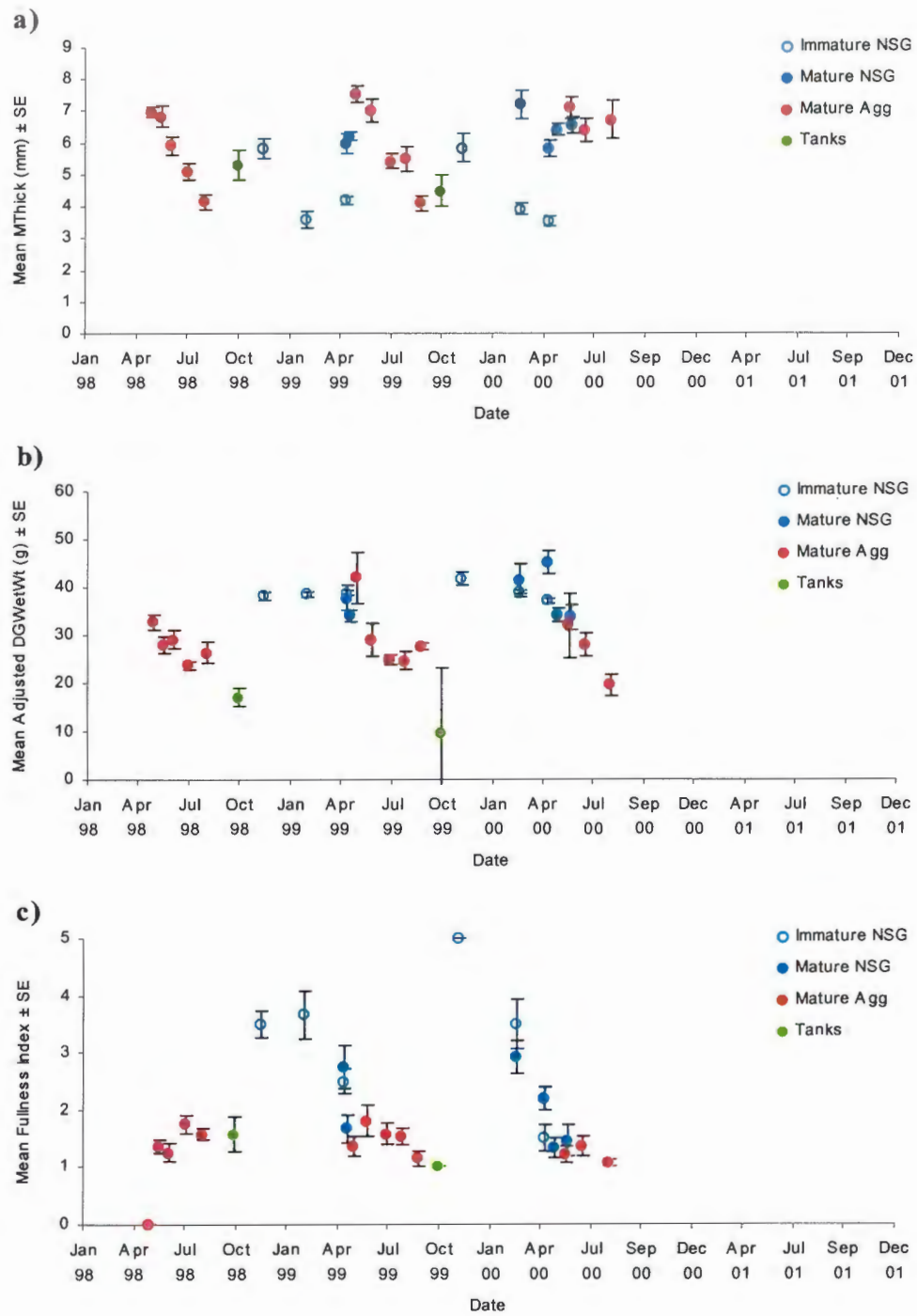


Figure 8.14 Mean adjusted Mthick (a), adjusted DGWetWt (b) and index of stomach fullness (c) of male *S. apama* for each sampling date from May 1998 to April 2001.

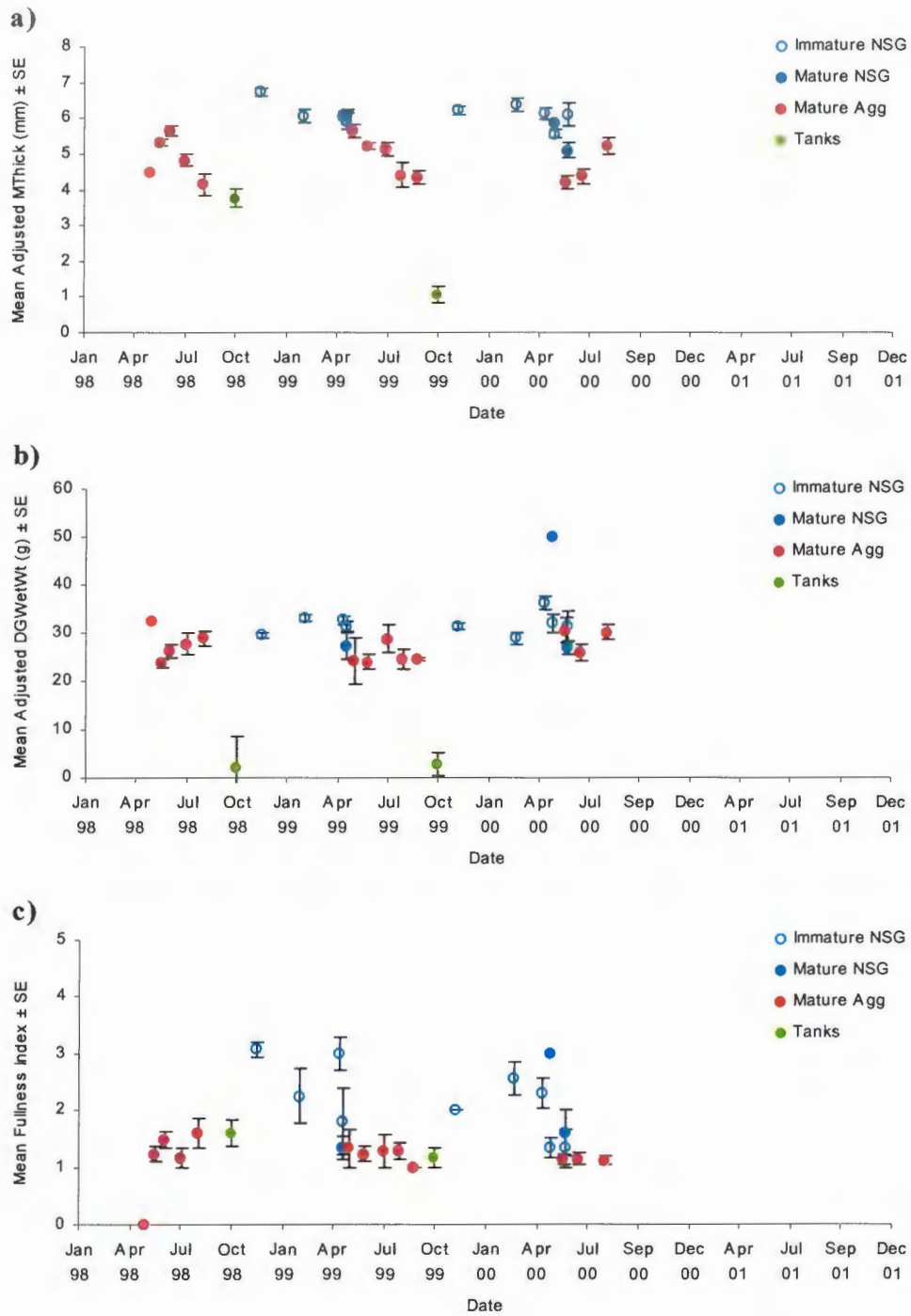


Figure 8.15 Mean adjusted Mthick (a), adjusted DGWetWt (b) and index of stomach fullness (c) of female *S. apama* for each sampling date from May 1998 to April 2001.

Comparison between different bone types

Neither condition variable differed significantly between Type 2 and Type 3 individuals of either sex, after adjustment for differences in size (Fig. 8.16; Table 8.3 and Table 8.4). However, both did differ significantly between the start and the end of the spawning season for males (Fig. 8.16a-b; Table 8.3). In particular, Type 3 males had a very pronounced decrease in mean adjusted DGWetWt toward the end of the spawning season relative to Type 2 males (Fig. 8.16b). There was no consistent trend for either condition variable with respect to collection month or bone type for females, but there was a significant difference between years (Fig. 8.16c-d; Table 8.4). The above results suggest that the same males, particularly Type 3 males, may have been present for the duration of the spawning season, based on the consistent decline in condition but that the situation was less clear for females.

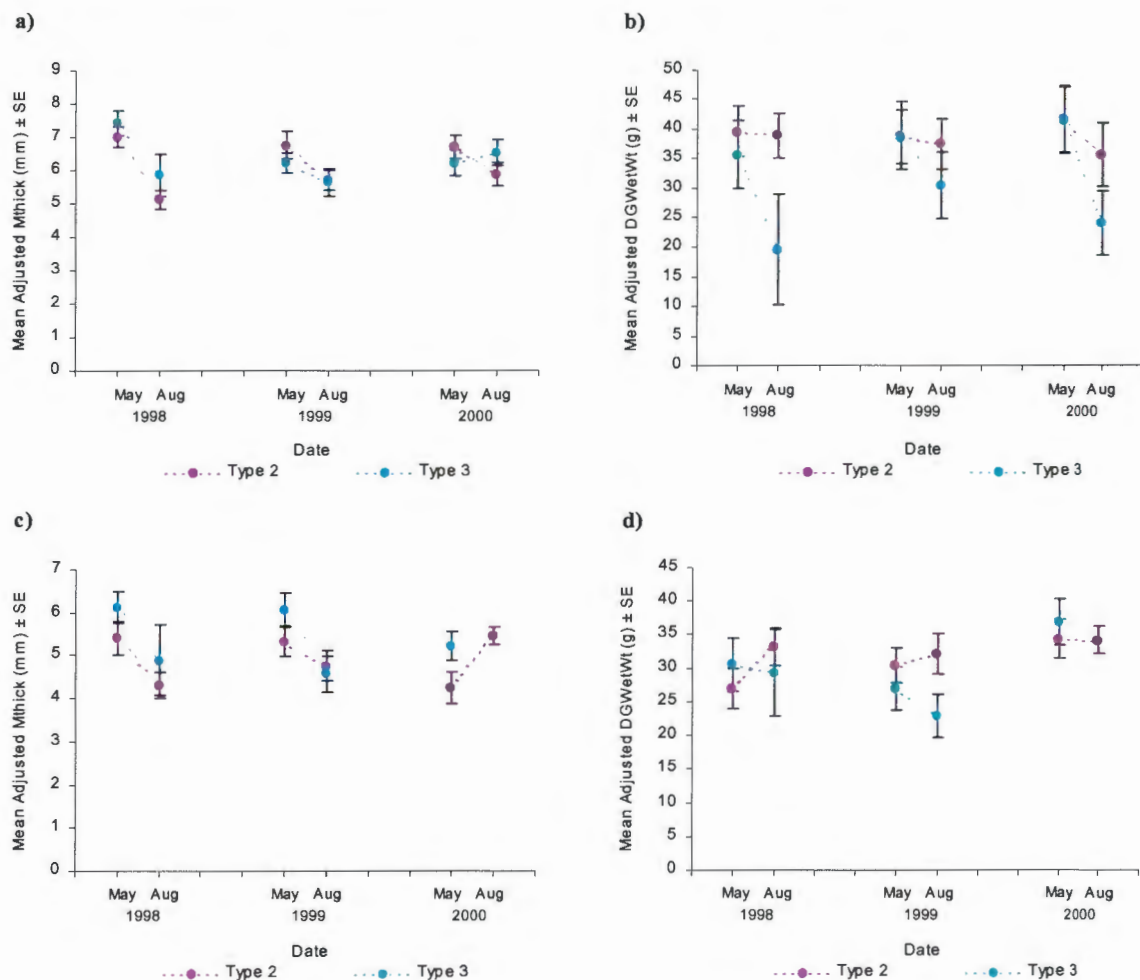


Figure 8.16 Mean adjusted Mthick and DGWetWt for male (a-b) and female (c-d) *S. apama* with Type 2 and Type 3 bones, collected from the aggregation area at the start (May samples) and toward the end (August samples) of each spawning season.

Table 8.1 Results of analysis of covariance (ANCOVA) tests between the different bone types, collection months and years for male *S. apama* condition variables Mthick and DGWetWt, using ML and TWt as a covariate respectively.

| Sources of Variance | df | Mthick | | DGWetWt | |
|---------------------|----|--------|--------|---------|--------|
| | | F | P>F | F | P>F |
| BType | 1 | 0.10 | 0.752 | 1.75 | 0.189 |
| Month | 1 | 22.18 | 0.000* | 7.50 | 0.007* |
| Year | 2 | 0.92 | 0.401 | 0.30 | 0.738 |
| Btype*Month | 1 | 2.61 | 0.109 | 3.55 | 0.063 |
| Btype*Year | 2 | 1.59 | 0.210 | 0.55 | 0.578 |
| Month*Year | 2 | 4.16 | 0.019* | 0.54 | 0.583 |
| Btype*Month*Year | 2 | 0.41 | 0.668 | 0.18 | 0.837 |
| log ML or logTWt | 1 | 47.75 | 0.000* | 127.14 | 0.000* |
| Residual | 93 | | | | |

* Significant at the $\alpha = 0.05$ significance level.

Table 8.2 Results of analysis of covariance (ANCOVA) tests[#] between the different bone types, collection months and years for female *S. apama* condition variables Mthick and DGWetWt, using ML and TWt as a covariate respectively.

| Sources of Variance | df | Mthick | | DGWetWt | |
|---------------------|----|--------|--------|---------|--------|
| | | F | P>F | F | P>F |
| BType | 1 | 1.99 | 0.168 | 0.27 | 0.607 |
| Year | 2 | 18.83 | 0.000* | 3.97 | 0.028* |
| BType*Year | 2 | 0.76 | 0.474 | 0.93 | 0.405 |
| log ML or logTWt | 1 | 16.92 | 0.000* | 25.75 | 0.000* |
| Residual | 34 | | | | |
| Month | 1 | 0.24 | 0.624 | 4.41 | 0.041* |
| Year | 2 | 0.31 | 0.738 | 1.37 | 0.265 |
| Month*Year | 2 | 8.23 | 0.001* | 1.24 | 0.300 |
| log ML or logTWt | 1 | 3.16 | 0.082 | 41.65 | 0.000* |
| Residual | 34 | | | | |

* Significant at the $\alpha = 0.05$ significance level.

[#] The analysis of female data was divided into two tests due to the small sample sizes of Type 3 bones in August samples. Hence, the effect of bone Type was tested for May samples only and the effect of sample month was tested for Type 2 bones only.

8.4.5 Spawning experiments

The attempts to cage females in the wild to monitor egg-laying through the 2000 spawning season were unsuccessful. Fine micro-algae was present as thick slime floating above the substrate throughout the aggregation area during the spawning season, which rapidly fouled the cages. Constant maintenance was required to provide adequate water flow through the cages. A week after the cages were deployed a severe storm dislodged and battered the cages resulting in the death of all experimental animals. The

experiment was not attempted again due to the logistic difficulties of running the experiment in such an exposed, shallow, subtidal habitat.

Females maintained in aquaria for the duration of the 1998 and 1999 spawning seasons, mated but did not deposit any eggs, even when presented with rocks from the aggregation area with conspecific eggs attached. The females died with up to 478 ova (mean 371 ± 101 SE in 1998; $n = 5$; mean 338 ± 40 SE in 1999; $n = 6$), in their oviducts compared to similar sized individuals from the aggregation area, which only had from 0 to 136 ova. This suggests that the females kept producing ova even in the absence of egg-deposition. Therefore, the best estimate of fecundity from this study is between 340 to 370 for an averaged sized female.

8.5 Discussion

Reproductive assessment

The results presented in this Chapter confirmed that all individuals at the aggregation area during the winter months were sexually mature, and that all females and many males in the wider northern Spencer Gulf during summer were immature and unlikely to be spawning. The higher stomach fullness indices of individuals for NSG samples suggest that they were actively feeding and that the area might represent the summer feeding grounds of the population. Obviously, the summer NSG samples came from the trawlable ground in the centre of the Gulf and if mature or spawning individuals were present at that time they would likely have been in the coastal areas, as they are during winter. The only coastal area monitored during the summer months was the aggregation area, and certainly no spawning cuttlefish were present there at that time. Overall, the evidence to date suggests that spawning does not occur at other times of the year and I conclude that *S. apama* has a restricted annual spawning season from May to August, with a peak in reproductive condition between May and June.

Males were more precocious than females, maturing at a younger age. Precocity of males has been reported for other *Sepia* species (e.g. Boletzky 1983; Guerra and Castro 1988; Gabr et al. 1998). Age and size at maturity of *S. apama* also varied according to life cycle type. Type 2 individuals of both sexes matured at a younger age and smaller size than Type 3 individuals based on the "two life cycle" model. No mature Type 1 individuals were recorded. This was consistent with the current hypothesis that Type 1 individuals do not spawn in the first year following hatching, but delay maturity for another 12 months to return as the larger Type 3 individuals in the following year (Chapters 6 and 7).

The potential reproductive output of Type 2 and Type 3 individuals differed due to the size differences between groups. Overall, the larger Type 3 individuals had larger gonads and produced larger gametes for both males and females. However, Type 2 individuals invested more in reproductive tissues relative to their body weight. These results suggest a difference between the two life cycle types in "reproductive quality", a difference which might offset the likely increased predation risk of surviving and growing for another year before returning to spawn at a larger size. This difference in potential reproductive output between the two groups also has implications for the overall reproductive output of the population. Type 3 individuals were present in greater numbers at the start of the spawning season than at the end (Chapter 5), but accounted for a smaller proportion of the population (Chapter 6). Therefore, intense fishing pressure early in the spawning season may disadvantage Type 3 individuals by providing them little opportunity to achieve much of their potential reproductive output before being captured.

The mean total weight of reproductive tissue in females was much higher than for males. This suggests that females invest a greater proportion of their energy budget into the production of reproductive tissue (Cortez et al. 1995), whereas males direct relatively more energy toward somatic growth. This might be expected if sexual selection for larger body size resulted due to a fitness advantage in large body size for competition between males for access to females. Loss in reproductive output due to a lower level of gamete production may be counteracted by increased fertilization success gained by increased access to females by larger males. Alternatively, smaller Type 2 males had significantly greater investment in reproductive tissue relative to body weight than larger Type 3 males. They may use an alternative reproductive tactic of transferring more sperm per mating to account for their lower competitive ability in dominating females relative to large males.

Eggs within the ovaries of *S. apama* covered a range of developmental stages and sizes, which suggests that females may spawn more than one batch of eggs in a season. There was poor correlation between oviduct weight and female size, suggesting that not all eggs mature simultaneously and laid in one batch (Harman et al. 1989). Furthermore, one tagged female was present for an extended period of time at the aggregation area (Chapter 4) and females during behavioural sampling were not always engaged in continuous oviposition but often moving about the aggregation area (Chapter 9). It is not yet known how many of the ovarian eggs present at the start of the season would become mature during the spawning season and over what time period an individual female may spawn. Eggs of a wide variety of sizes were also observed in the ovaries of other *Sepia* species including *S. officinalis*, *S. pharaonis* and *S. dollfusi* (Gabr et al. 1998), which was thought to indicate an intermittent or batch spawning strategy.

Fecundity

The fecundity of *Sepia* species can rarely be estimated by a direct count of mature ova in the gonads due to the prolonged nature of the spawning season and the potential for eggs to be sequentially produced over time. The numerous spawning experiments that attempted to answer this question resulted in no egg-deposition in captivity. In contrast, other *Sepia* species have readily deposited eggs in captivity on any available substrate. Boletzky (1987) observed a prolonged intermittent or batch spawning in a female *S. officinalis* held in aquaria. She laid considerably greater numbers of eggs than counts of mature ovarian eggs would have suggested. Spawning experiments with *Idiosepius pygmaeus* suggested that female fecundity was related to the duration of female survival rather than original ovarian egg numbers (Lewis and Choat 1993). In both cases the experimental females died with some immature ova still in their ovaries.

Although the experiments with *S. apama* failed to result in spawning, the captive females died with higher numbers of mature ova in their oviducts than any females collected from the field. The wide variation in the number of ova in the oviducts of mature females collected from the aggregation area probably reflected the different stages of egg laying that each female had reached when collected rather than overall fecundity. As eggs are laid, they are removed from the oviduct and are not immediately replaced. Hence, a female collected towards the end of laying a batch of eggs would have very few eggs remaining in her oviduct. Many of the females collected at the end of the spawning season in August were in such a condition. Thus, fecundity could not be reliably estimated from counts of mature ova in the oviducts of wild-caught individuals, as this would have vastly underestimated the number of mature eggs that a female might potentially lay throughout the spawning season.

Individual fecundity may even be in excess of the number of ova found in the captive females at the end of the spawning season (i.e. around 340 to 370). It is not known how many eggs the females may have further produced if egg-laying had occurred. The ovaries of individuals at the end of the captive period still contained eggs of various sizes and stages; however, they were very small in comparison with wild females of a similar size, suggesting that the captive females may have completed their spawning potential.

Condition indices

Male condition showed a more pronounced decline in both reproductive tissues and general condition during the spawning season than did females. There were few observations of feeding at the

aggregation area and all individuals collected had low stomach fullness indices. Cuttlefish in the area readily attacked squid jigs when presented, which suggests that the low feeding levels probably related to a lack of food rather than a lack of appetite. So, if the same individuals were present and spawning for the duration of the three month spawning season, a decline in their condition would be expected. Accordingly, the little decline in condition of the females was surprising. This might be interpreted in a number of different ways: (1) the same females were present all season but underwent no consistent loss in condition as a result of spawning; (2) new females arrived at the spawning aggregation area toward the end of the season replacing those that were in poorer condition that left; or (3) females were metabolically different to males, and the variables measured for condition were inappropriate for females. The fact that the variables detected a significant decline in the condition of captive females suggests that the latter hypothesis was unlikely. The highly male-biased sex ratios of the aggregation population suggest that there may be a higher turnover of females there relative to males, such that not all females are present at the one time. The results of the condition assessment support this hypothesis. The tagging study at the aggregation area showed that at least some females remained there for up to 6 weeks, accounting for a large proportion of the spawning season. Still, this does not preclude new females from arriving later in the season as others leave. A more detailed study, preferably using radio-telemetry tagging to determine precise female turn-over rates and residence times would aid in the interpretation of these results for assessment of condition.

Only a small number of moribund animals were ever observed during the visual surveys towards the end of the season. It is possible, however, that spent moribund animals migrated out of the area directly following spawning, and died elsewhere. This has been postulated for a number of other *Sepia* species (Gabr et al. 1998). Alternatively, dead cuttlefish may float to the surface and be consumed by predators such as sea birds or dolphins. Only in the case of *Loligo opalescens* have dead or dying individuals been found in large numbers at the spawning site (Fields 1965).

In conclusion, the results indicate *S. apama* of both sexes and life cycle types have semelparous reproductive strategies, but that spawning may occur over a substantial period at the end of the life cycle. Following the definitions outlined in Rocha et al. (2001), an intermittent terminal spawning mode is proposed for *S. apama*, similar to that described for *S. officinalis*; however, further research is required to ascertain better estimates of fecundity and verify the batch formation of eggs.

9 Mating system[‡]

K.C. Hall and R.T. Hanlon

9.1 Introduction

The mating system of a population refers to the behavioural strategies used by individuals to maximise their reproductive success (Davies 1991; Reynolds 1998). The evolution of mating systems is driven by mechanisms of sexual selection (reviewed by Alcock 1998). Sexual selection was described first by Darwin in 1871 as a complement to natural selection (Andersson and Iwasa 1996) and deals solely with selection forces associated with reproduction. Stated explicitly, it is the difference in individual reproductive success caused by heritable differences in traits or tactics that affect success in gaining access to mates and fertilisations (Andersson 1994).

The intensity and direction of sexual selection is influenced by the operational sex ratio (OSR) – the average ratio of sexually active males to females at any given time (Emlen and Oring 1977). The OSR can also be expressed as the average ratio of mature female to male gametes available for fertilisation at any given time (Kvarnemo and Ahnesjö 1996). So even if the sex ratio of mature adults is 1:1, if females produce relatively few costly eggs and males produce millions of low cost sperm (as is the case for many species), the OSR will still be biased towards males, due to the excess of sperm available to fertilise relatively few eggs (Kvarnemo and Ahnesjö 1996). This is the basis of sexual selection theory. If the OSR is biased towards one sex, intrasexual competition between members of that sex for access to mates (and their gametes) may be more intense (Emlen and Oring 1977; Andersson and Iwasa 1996), and traits or tactics that enhance success in competition will be favoured. Alternatively, sexual selection may be influenced by mate choice exercised by members of the limiting sex, because they can be selective due to an abundance of potential mates (Andersson and Iwasa 1996; Ryan 1997). Accordingly, traits or tactics that enhance “attractiveness” will be favoured. Furthermore,

[‡] The results presented in this chapter were derived from a collaboration with Dr Roger Hanlon of the Marine Biological Laboratory, Woods Hole, Massachusetts, U.S.A. and form the basis of the following publication: Hall K.C. and Hanlon R.T. (2002). Principal features of the mating system of a large spawning aggregation of the giant Australian cuttlefish *Sepia apama* (Mollusca: Cephalopoda). *Mar. Biol.* 140: 533-545.

multiple reproductive tactics may be used by either sex to solve the problems of mate acquisition and successful fertilisation (Hensen and Warner 1997; Gross 1998).

Sexual selection processes and mating systems in cephalopods are not well documented, especially in the field (cf., Moynihan and Rodaniche 1982; Hanlon and Messenger 1996). In particular, there have been few studies of cuttlefish reproductive behaviour, and only one species, i.e. *Sepia officinalis*, has been studied in detail in the laboratory (e.g., Bott 1938; Tinbergen 1939; Adamo and Hanlon 1996; Boal 1996; Boal 1997; Boal and Marsh 1998; Boal et al. 1999; Hanlon et al. 1999; Adamo et al. 2000). The mating systems described for *Loligo* squid species are complex with the different sized males using different tactics to obtain matings with females (Hanlon 1998). The reproductive success of the males was found to relate to behavioural tactics and was not proportional to the number of each size class present in the population (Buresch et al. 2001). Therefore, reproductive behaviour and the prevailing mating system may significantly influence the overall reproductive output of a population.

The possibility of there being two alternative life cycle types for *S. apama* (Chapter 6), each with different quality with respect to potential reproductive output and gamete size (Chapter 8), suggests that different reproductive strategies may exist. Furthermore, the highly male-biased sex ratio of the spawning population (Chapter 5) suggests there may be a high level of competition between males for access to females and a strong gradient for sexual selection. Therefore, a complex mating system with different reproductive tactics used by the different life cycle types might be expected for *S. apama* at the aggregation area. Since the fishery for this species specifically targets the spawning aggregation, an understanding of the mating system and the mechanisms of sexual selection of the aggregation population should facilitate appropriate management of the fishery.

9.2 Aims

The reproductive behaviour was sampled to describe: (1) the nature of resources defended or offered; (2) number of mates acquired by each sex; (3) nature and duration of pair formations; (4) forms of courtship; (5) methods of copulation and fertilisation; (6) frequency and mode of egg-deposition; (7) the form and duration of parental care; (8) behavioural tactics used for mate and fertilisation acquisition; and (9) the extent of mate choice.

9.3 Methods

The behavioural study was limited to Black Point at between 3-8 m depth. To determine the relative proportion of each component of the population present at the time of behavioural sampling, four replicate transects were completed on 15 May 1999, using the same methods described in Chapter 4. The paired status (lone or paired) of the animal was also noted.

9.3.1 Behavioural sampling

Behavioural sampling was completed during 6-14 May 1999. Digital video cameras (Sony VX-1000) in underwater housings were used to record behaviour during SCUBA dives. Two divers completed 34 dives (44 diver h) and produced 26 h of video footage, all done between daylight hours. The sampling protocol of Martin and Bateson (1986) was followed to assign behavioural sampling methods. This involved a small amount of ad libitum sampling initially to determine the main components of the mating system, followed by replicate focal animal sampling on each of the identified components. This entailed following a chosen individual for as long as possible without interruption (up to 1.5 h), and recording all behaviours and interactions. Individuals were chosen haphazardly at random locations within the site and were classified as either paired or lone, and by size. Females were considered to constitute a single size class (i.e., 150-250 mm ML), whereas males were classified as small if their estimated length was less than or equal to 250 mm ML (i.e. similar to female size) or large when greater than 250 mm ML. Only males that were confidently classified as either small or large were chosen for focal animal sampling.

Cuttlefish habituated quickly to the presence of divers. Generally the diver maintained a distance of 1-3 m from the subject, depending on visibility. The video wide-angle function allowed us to record all cuttlefish and habitat in the immediate vicinity of the focal individual to place their behaviour in context, and the zoom function was used to record details associated with a particular behaviour.

9.3.2 Analysis of video

Video was played back on a multi-motion digital VCR to quantify the duration and frequency of the main behaviours. An ethogram of 50 different behaviours was used to score the videotapes. Analysis of variance (ANOVA) was used to test for differences in duration of pair formation or mating with respect to male size. A Wilk-Shapiro/rankit plot and histogram plot of residuals were used to check the normality of data before statistical analysis. Residuals for pair duration showed a negative exponential

distribution and consequently were transformed to natural logarithms to normalise error values. Similarly, residuals for mating duration showed a lognormal distribution and were transformed to base-10 logarithms to normalise error values. Residuals were plotted against fitted values to check for homogeneity of variances. A Chi-squared goodness-of-fit test was used to test if observed frequencies of non-successful matings differed from expected frequencies with respect to male size.

9.4 Results

9.4.1 Main components of the population

Most females encountered on transects were paired ($n = 44$; 88%; Fig. 9.1a), and there were only six lone females (12%). In contrast, most males were lone ($n = 131$; 73%; Fig. 9.1b), and 89% ($n = 117$) of the lone males were small. Only 27% of males ($n = 49$) were paired, and of these 53% ($n = 26$) were large males, despite them being less numerous ($n = 40$; 22%) than small males ($n = 140$; 78%).

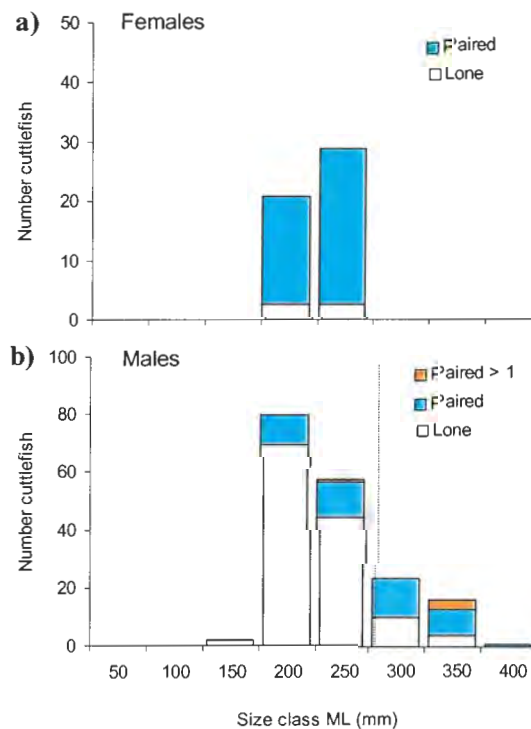


Figure 9.1 Length-frequency histograms of female (a) and male (b) *S. apama* at the Black Point site in May 1999 when the behavioural sampling was completed, indicating number of individuals in each size class that were lone, or paired with one or more mate; data pooled for four replicate transects.

Over 20 h of video footage were analysed. Table 9.1 indicates the breakdown of the footage according to focal individual categories and status (i.e. lone or paired). It was difficult to maintain focal sampling on lone females and large lone males because they remained lone for only short periods of time. Conversely, there were few small paired males. Hence, there was more total focal time directed toward large paired males, paired females and small lone males.

Table 9.1 Number of individuals and period of time sampled using underwater video for reproductive behaviour description.

| Focal category | n | Total focal time (h) | Time spent lone (h) | Time spent paired (h) | Mean time / individual (min \pm SE) |
|----------------|----|----------------------|---------------------|-----------------------|---------------------------------------|
| Small male | 14 | 4.6 | 3.0 | 1.6 | 19.5 \pm 4.1 |
| Large male | 28 | 8.7 | 2.6 | 6.1 | 18.6 \pm 3.3 |
| Female | 19 | 7.0 | 2.0 | 5.0 | 22.0 \pm 3.9 |
| Total | 61 | 20.3 | 7.6 | 12.7 | 19.1 \pm 2.2 |

9.4.2 Female defence vs. habitat defence

At first inspection, it seemed as though males might be defending egg-laying sites (i.e. large flat rocks under which females laid eggs). However, males paired with and guarded females rather than defend egg-laying sites, since when a female moved, the paired male moved with her, irrespective of habitat.

9.4.3 Number of mates and pair formation

Both sexes sought and accepted multiple mates sequentially; most pair formations were temporary. Only four large males (2% of all males) defended more than one female (up to three) simultaneously. Total number of sequential mates per individual cuttlefish could not be determined by the sampling method, which would require 24 h focal samples.

Pair formation encompassed the time from when a male started guarding a female until displaced by another male, or the female moved away and the male ceased to follow and guard her. Males often spent a long time guarding a female before gaining an opportunity to mate, and could be displaced without completing a successful copulation. Eighty-six pair formations were recorded during focal sampling and in 35 of these mating did not take place before the male was displaced or filming ceased.

Duration of pair formation varied greatly, from as little as 7 s to 90 min ($n = 86$). Most were short (5-10 min; Fig. 9.2a); however, the maximum duration of pair formation was limited by the length of focal sampling, which in turn was restricted by the length of a single dive (generally 90 min). Hence, estimates of average pair duration (Fig. 9.2b) may underestimate the true duration of pairings. The average pair duration was longer for large males (Fig. 9.2b), but the difference between the two size categories was not significant due to the large variation between individuals within the categories (ANOVA; $p = 0.1448$; $n = 86$). There was also no assortative pairing with respect to size of males and females (Fig. 9.2c), as any sized female paired with any sized male.

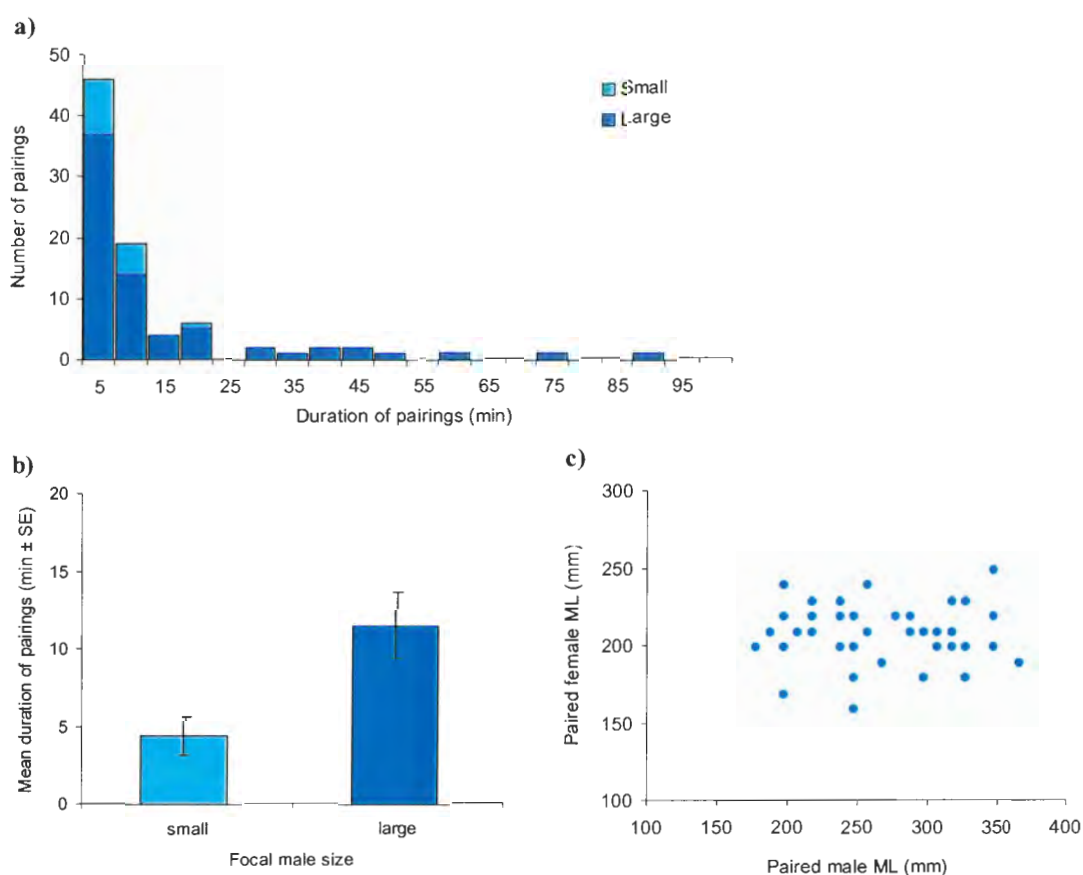


Figure 9.2 Details of pair formation: (a) frequency distribution of duration of pair formations filmed during behavioural sampling; (b) mean duration of pairings filmed with respect to size of focal male; and (c) size of paired females encountered on transects with respect to size of paired male.

9.4.4 Courtship

Courtship is defined as any behaviour exhibited by either sex that increases the receptivity of the partner to mating or fertilization. Little courtship was observed from either sex. On approach to a

female, males directed a low intensity unilateral passing cloud colour display, which entails moving waves of chromatophores, towards the female, followed soon after by a mating attempt. However, the time between a male encountering or acquiring a female and his attempt to mate with her varied enormously from 1 s to 10.2 min ($n = 65$), due to interference by other males. For uninterrupted encounters/takeovers, where the pair were subsequently left undisturbed, the mean time until an attempted mating was very short (mean = 19 s; SE = 4 s; $n = 35$). There was, thus, little time for courtship. Once paired and mated with a female, a male sometimes ($n = 19$) brushed his fourth arms over the female's dorsal mantle. However, this behaviour usually occurred post-copulation and may not be associated with courtship, but rather with mate guarding.

9.4.5 Mating

All matings ($n = 39$) occurred in the head-to-head position (Fig. 9.3a). Males generally initiated mating by spreading their arms and grasping the side of the female's head. The female then accepted the mating by opening her arms and overlapping them with the male's. Mating occurred in three stages: (1) the first 71% (SE = 1.9%; $n = 25$) of the mating duration involved the male jetting water from his funnel forward into the buccal area of the female, accompanied by movements of the male's second and third arms into this area; (2) followed by a rapid (ca 1 s) transfer of spermatophores with the hectocotylus on the left fourth arm to the sperm receptacle and buccal lining below the female's beak; and (3) concluding with a period of breaking open the spermatophores with quick forceful forward movements of the fourth arm in the buccal area of the female. The duration of each stage varied with respect to size of male (Fig. 9.3b). Large males spent more of the total mating time in the first stage before sperm transfer. However, the mean duration of mating varied little with respect to size of male (Table 9.2; ANOVA; not significant; $p = 0.0783$; $n = 25$; only successful matings with one transfer of sperm were used in the analysis) and was on average 2.4 min in duration (SE = 0.1 min; $n = 25$).

A mating was considered successful if a transfer of spermatophores occurred. Both large and small males achieved successful matings (Table 9.2). However, some matings were interrupted by a challenging male before spermatophores could be transferred (31% of small male matings and 13% of large male matings; no significant difference; $\chi^2 = 1.92$; $p = 0.1660$). There was usually only one transfer of spermatophores; however, on six occasions (19% of successful matings) there were two transfers. Females were not observed to remove spermatophores following mating; however a period of jetting water forward through the arms and buccal area while flaring the arms followed mating in both sexes.

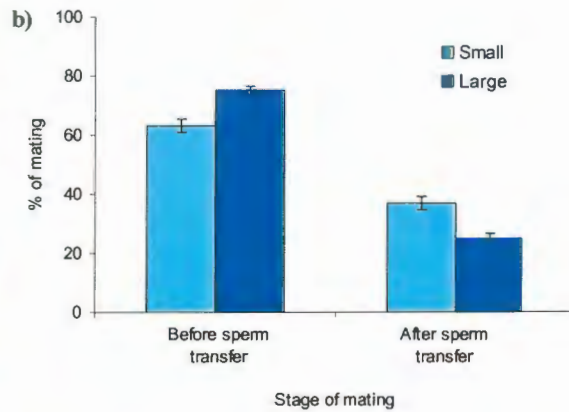


Figure 9.3 (a) Male (on right) and female mating in the head-to-head position with arms intertwined. (b) Percentage of mating time spent before and after sperm transfer by different-sized males ($n = 39$).

Table 9.2 Number of matings, number with two sperm transfers, and average duration of mating with respect to male size

| Male size | Total matings (n) | Successful matings (n) | Mean duration (min \pm SE) | Two sperm transfers (n) |
|-----------|-------------------|------------------------|------------------------------|-------------------------|
| Small | 16 | 11 | 2.1 \pm 0.2 | 2 |
| Large | 23 | 20 | 2.5 \pm 0.2 | 4 |
| Overall | 39 | 31 | 2.4 \pm 0.1 | 6 |

9.4.6 Egg deposition and parental care

The process of depositing an individual egg involved a number of stages that were associated with distinctive female head and arm coloration and postures (Fig. 9.4). After laying an egg, the female spent a short time (mean = 1.3 min; SE = 0.1 min; $n = 8$) jetting water forward through the flared arms and buccal area, which appeared to be an action for cleaning the arms of the gelatinous secretions from the egg. This was followed by a period of “egg preparation” (mean = 4.1 min; SE = 0.03 min; $n = 8$) in which an egg was held in the arms, with all but the fourth arms contracted up into short points with white tips, and the anterior region of the head between the eyes in a “humped” profile (Fig. 9.4a). It is assumed that fertilisation of the egg using sperm from the buccal region occurred during this stage.

Following this, the bulge of the egg gradually moved down the arms until the eight of them formed a conical shape around the egg, which was then ready for deposition.



Figure 9.4 Distinctive head and arm posture of female *S. apama* during "egg preparation" stage. The anterior head between the eyes appears "humped" and all but the fourth arms are contracted and white-tipped arms.

The average time to lay an individual egg was 7.6 min (SE = 0.4 min; n = 10) when a female was allowed to oviposit undisturbed. However, more time elapsed between individual eggs (up to 22 min) when the female was interrupted during the egg-laying process or when time was spent searching for an egg-laying site. Females did not deposit all eggs in one egg-laying site but moved frequently in search of appropriate sites, and often deposited eggs in the vicinity of those from other females. This resulted in egg clusters deposited by different females under the same rock. There was no parental care of the eggs by individuals of either sex, or of the offspring immediately after hatch. In fact most adults left the area by the end of August, well before all eggs had developed and hatched. Hatching did not start until mid-September and continued through to early November.

Egg-laying did not necessarily commence immediately following mating. Females engaged in continuous oviposition prior to mating often resumed egg-laying soon after mating (6-25 min after). Other females kept moving about and accepted multiple matings (up to four in 45 min) before commencing egg-laying. A female was not receptive to mating attempts while an egg was held in the arms, as none of 25 mating attempts at such a time was successful. Two such attempts caused the female to expel the egg while jetting away. In contrast, just after depositing an egg, females were more

receptive to mating with 63% of mating attempts ($n = 8$) on a female immediately following egg deposition as successful.

9.4.7 Male behavioural tactics

Male behaviours were predominantly related to male-male competition for access to females for copulations. The multiple tactics used by a male varied according to its size and whether it was lone or paired at the time. Most large males were paired with a female (65%; $n = 26$). While paired, a large male spent most time guarding the female from other males (both pre- and post-copulation). As many as five lone males interacted with pairs at an average rate of over $2 \cdot \text{min}^{-1}$. The number of interactions per pair depended upon their location within the site and the surrounding density of cuttlefish. Paired males that were approached by another female usually attempted to pair with and defend the new female as well. However, if a male had a choice between a newly acquired female and a previously paired one, the male tended to remain with the latter.

Mate guarding was manifested in several ways. Large paired males hovered directly over females or stationed themselves between the female and other males. When females were under a rock, the paired male guarded the opening. Large male challengers generally drew an agonistic display from the paired male (Fig. 9.5). These contests passed through various stages from moderate visual signalling to physical contact, and were characterised by a dramatic passing cloud display (Fig. 9.5; see also Norman et al. 1999). A total of 250 agonistic contests were recorded. Rarely did the contests escalate to physical biting (3 of 250) as most ended with the retreat of one male (cf., Adamo and Hanlon 1996 for *S. officinalis*). The outcome appeared to relate to the size and status of the male (i.e., challenger vs. paired male); most contests were won by the larger or paired male. Paired males that were slightly smaller than challengers sometimes won contests, whilst challengers smaller than the paired male seldom won. Small males rarely evoked an agonistic display from a large paired male but were repelled by a short lunge in their direction. Lone large males searched for lone females and challenged large paired males to agonistic contests in an attempt to “takeover” the paired female.

Small males were capable of all behaviours used by large males. They paired with and guarded females when possible but were easily displaced by a large male. Small males also challenged other small males (paired or lone) to agonistic contests. However, due to the biased sex ratio and greater success of large males at defending females, most small males were not paired ($n = 117$; 84%) and spent most time searching for lone females and opportunities to sneak extra-pair copulations (EPCs) with paired females.



Figure 9.5 Agonistic contest between two large male *S. apama*; the male on the left is challenging (flared white arms) the paired male, who is responding with a full agonistic display (including unilateral passing cloud body pattern, flared white fourth arms, and arched posture).

Small “sneaker” males switched between various tactics to gain EPCs: (1) “open stealth” which involved hovering and watching a pair (Fig. 9.6a), until the paired male was distracted by other small males or a large challenger, and an overt “sneak mating” attempt on the paired female was possible; (2) “hidden stealth” in which a male remained concealed under an egg-laying rock and attempted a covert “sneak mating” with any female that moved under the rock in search of an egg-laying site (Fig. 9.6b). Another variation was when a small male searched for an unguarded entrance to gain access to a paired female already under an egg-laying rock for a concealed EPC; and (3) “female mimicry”, which occurred when a small male adopted the coloration and posture of an egg-laying female (Fig. 9.6a) to gain unchallenged access to the realm of a large paired male and hence the paired female for a “sneak mating” attempt. Small males also mimicked females to avoid aggression from a large male even in the absence of a paired female. Large males appeared to be deceived by this mimicry, often defending the mimic, along with his paired female, from the approach of other challengers or attempting to mate the mimic. Mimics even appeared to deceive other small males; four of 11 mating attempts on mimics were by other small males. Only the smallest males (≤ 200 mm ML) were observed to mimic females.

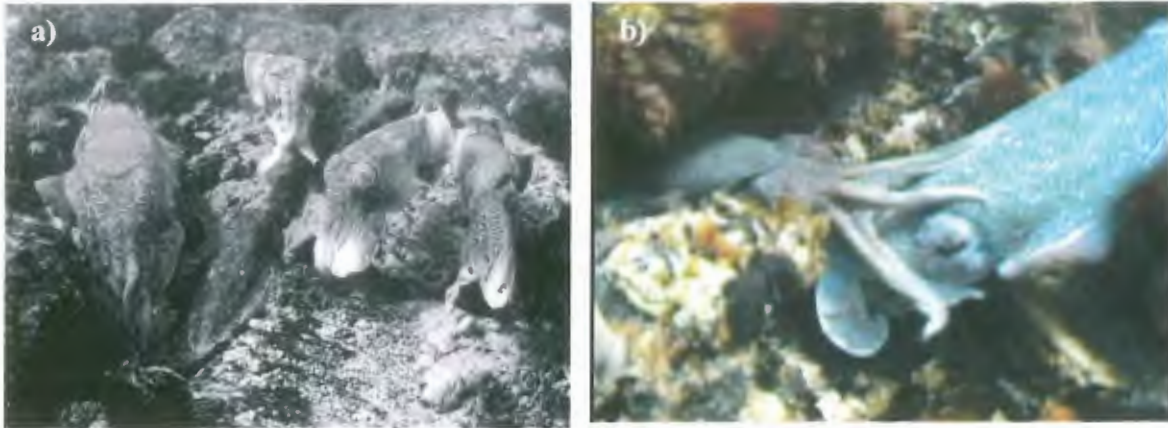


Figure 9.6 Small male behaviours: (a) small mimic male (topmost small male with contracted white-tipped arms) and “open stealth” small males (two on right) hovering near a pair (large paired male to left and female to right); and (b) a small hidden male darting from under a ledge to attempt to mate a paired female approaching an egg-laying site.

9.4.8 Female behavioural tactics

The two most common female behaviours were: (1) moving about the spawning grounds (with or without a paired male) repeatedly looking under rocks; and (2) hovering near or under a rock, usually in the presence of a paired male. Most of this behaviour was related to egg-laying, i.e., either searching for egg-laying sites, or egg deposition for a considerable period at the one site. However, a variety of female behavioural tactics relating to direct and indirect mate choice were also identified. If a male gained access to a female, it did not necessarily guarantee a successful mating.

Females rejected unwanted mating attempts by jetting backwards from the male or forcibly breaking free from a male’s persistent grasp on the head or arms (along with jetting and occasional inking). Of 122 mating attempts, 33 (27%) resulted in a successful mating, 85 (70%) were rejected and four (3%) resulted in a forced copulation. A forced copulation was classified as any mating which followed a prolonged period of the female trying to break free from the persistent grasp of a male, during which the female’s arms remained firmly held together and not open to the mating. Of 85 rejected matings, 25 (29%) were due to the presence of an egg in the female’s arms and 29 (34%) were preceded by a distinct unilateral white stripe (WS) signal along the base of the fin directed towards the male (Fig. 9.7). Only three of 29 mating attempts preceded by a WS signal resulted in successful mating. Although the WS signal usually preceded a mating rejection, many males that received it still

attempted to mate with the female (n = 25). Females with eggs in their arms also used the WS signal towards an approaching male.



Figure 9.7 Female with lateral white stripe along the fin-base approaching a paired male (the approaching female rejected a subsequent mating attempt by the male).

Three other behaviours were also directed at males. Females sometimes exhibited an “arching posture” in which the arms were held together, extended anteriorly and then arched towards the male. This apparent act of aggression often resulted in retreat of the approaching male. One female showed a full challenge display with flared white arms similar to the male challenge display (Fig. 9.5). Finally, there was a “white banner display” in which all of the arms were white, extended anteriorly and waved like banners towards the male. This resulted in a mating attempt from males in every case.

Behaviours relating to indirect mate choice (i.e., behaviours that set up increased male-male competition) included: (1) the fact that females aggregate to spawn at a predetermined location and habitat type, causing males to aggregate in large numbers; (2) there is a highly male-biased sex ratio suggesting females may spawn asynchronously, and be receptive to mating for shorter periods than males; and (3) females not engaged in continuous oviposition moved extensively in search of egg-laying sites and interacted with many males, possibly to increase male-male competition. They readily approached paired males and inspected nearby egg-laying sites, often rejecting the male’s immediate attempts to mate and/or expressing the WS signal as they approached.

After a prolonged period of moving about whilst rejecting numerous mating attempts, of up to 11 in a 14 minute period, a female would suddenly accept a mating. There appeared to be no consistency to the

choice of an acceptable male. Of 22 final mating acceptances, 50% were with small males, and 50% with large ones. Overall, 36% of females mated with a male that had previously been rejected. Females were also observed to reject a large paired male and subsequently mate with a small lone one.

9.5 Discussion

9.5.1 Sperm competition behaviour

Sperm competition can occur whenever a female mates with more than one male, which places their sperm in competition for the fertilisation of a given set of ova (Birkhead and Parker 1997). Evidence of sperm competition for *S. apama* included: (1) the fact that females mated with multiple males, thereby allowing for multiple sources of sperm; (2) the presence of a sperm receptacle that could store sperm from multiple males; (3) the fact that a large percentage of the total mating time of males was potentially associated with sperm removal (the effectiveness of which is yet unknown); (4) the persistent mate-guarding that suggests the possibilities of last male precedence, sperm displacement, or sperm dilution mechanisms; (5) the existence of sneaker male mating tactics to circumvent mate-guarding; and (6) the existence of female choice suggesting variation in male/sperm quality. A similarly high level of sperm competition behaviour was described for *S. officinalis* studied in captivity (Hanlon et al. 1999).

The long interval between the deposition of individual eggs (even when a female was consistently ovipositing) allowed for many interactions with different males. Females mated with up to four different males before laying an individual egg. The absolute fecundity and duration of spawning of individual females remains unclear (Chapter 8). However, tagged females remained within the aggregation area for up to six weeks. Since most pair formations were short (5-10 min) and many EPCs were successful, females evidently mate with many different males over the course of the spawning season, and can potentially store the sperm from many different males (cf., Hanlon et al. 1999).

Males formed temporary pairs with females (maintained by strong pre- and post-copulatory mate guarding) to gain access to copulations and to prevent other males from mating the female before or during oviposition. Most females were paired due to the highly male-biased sex ratio. Therefore, lone males either tried to displace a paired male via a direct challenge (usually resulting in an agonistic contest) or searched for EPC opportunities. Large males usually prevailed in agonistic contests and accounted for most of the pair formations. Small males mostly used alternative tactics to “sneak” EPCs and readily switched tactics. They could also perform all the behaviours manifested by large males,

although with less success, suggesting conditional rather than genetic control over their expression (Gross 1998). Large males, however, rarely used small “sneaker” male tactics and only the smallest males mimicked females.

Small males achieved almost half of the successful matings. Studies of *S. officinalis* in captivity found that large dominant males monopolised matings (Adamo et al. 2000) and successfully prevented small males from accessing females. Small male *S. apama* outnumber large males in the aggregation area, which may contribute to their higher mating success in the field (cf., Widemo and Owens 1995). However, mating success does not necessarily translate directly into fertilisation success due to post-copulatory sperm competition mechanisms (indicated in this system by strong post-copulatory mate guarding). Large paired males seemed to prioritise ridding the contest of large male challengers over small males, even to the extent of spending minutes “locked” in stereotypical agonistic contests leaving the female more exposed to EPC attempts by omnipresent small males. This suggests large males pose a greater threat in terms of sperm competition than small males.

9.5.2 Female mate choice: direct, indirect and cryptic

Mate choice exercised by females ultimately determined which males’ sperm entered into the competition for fertilisation. Females actively rejected unwanted mating attempts: 70% were successfully rejected and only 3% resulted in forced copulations, which indicates that the benefits of rejection outweigh the costs (Reynolds and Gross 1990). Only 29% of the rejections were due to an egg being held in the arms; thus, more than two thirds were due to direct choice by the female. Comparable quantification of direct female choice in other species of the Cephalopoda are not available. Similar rejections were described for female *S. officinalis* in laboratory experiments (Boal et al. 1999), yet the cause was unclear and may have been an artifact of using immature (and hence non-receptive) females in the experiments.

Females used a distinct visual signal – lateral white stripe – directed unilaterally towards males to signal their intention to reject a mating attempt. Similar white-based signals to discourage males have been described for female *Sepioteuthis sepioidea* (“pied display”; Moynihan and Rodaniche 1982) and *S. latimanus* (“near white centre bar pattern”; Corner and Moore 1980).

Indirect choice by females can lead to increased male-male competition (Wiley and Poston 1996). In female *S. apama*, it included: (1) aggregation to spawn at a predetermined location and resource type; (2) an operational sex ratio biased towards males possibly caused by asynchronous spawning, in which females are not all mature at the same time; and (3) movement throughout the aggregation area in

search of good egg-laying sites, leading to interactions with multiple males. Lone females commonly rejected many mating attempts, before eventually accepting a mate. This behaviour may also serve as a tactic to indirectly choose only the most persistent or fittest males: 36% of accepted matings were with a male that the female had previously rejected.

Post-copulatory cryptic female choice of sperm may also be occurring in *S. apama*. There could be two sources of sperm available to a female when fertilising an egg: those from the most recent matings that are present externally in spermatangia around the buccal region, or those stored internally in the sperm receptacle, also in the buccal region. In the latter case, multiple males are likely to be represented. Several factors are worthy of future investigation, including: the anatomy of the sperm receptacle; mechanisms controlling the storage of sperm in the receptacle; properties of ejaculates that may aid in storage; and possible manipulations of spermatangia by females (see Eberhard 1996).

Patterns of female preference or choice criteria could not be discerned from our analysis. There were no obvious secondary sexual characteristics or courtship displays of males shown towards females. It is also probable that the male behavior of brushing the fourth arms over the dorsal mantle of the female is not courtship, because males do not use this behavior when first encountering a female. Females ignored the elaborate agonistic displays, often moving away from males engaged in such contests (contrary to Norman et al. 1999). Nor did females consistently choose one size class of males over the other. Future investigations should include controlled laboratory experiments on female choice (e.g., Boal 1997) and paternity analysis (using DNA fingerprinting) of both laboratory trials and field samples of known mating history to determine the relative fertilisation success of different size males (e.g., Brockman et al. 1994; Hanlon et al. 1997; Poston et al. 1999).

9.5.3 Mating system

A mating system may be considered the outcome of competition between conflicting interests (e.g., male vs. female control of access to mates, large male vs. small male reproductive success) within the constraints and opportunities set by ecological variables (e.g., clumped resources or temperature influenced sexual maturation) (Reynolds 1998; Emlen and Oring 1977). The intensity and direction of mating system evolution will be determined by which competing interest dominates under any given set of ecological variables and the traits which show genetic variation.

The spawning population composition and reproductive behaviour of *S. apama* at the aggregation area indicate a complex mating system with some similarities to a lek (Bradbury 1981). There was a highly skewed sex ratio creating a strong gradient for sexual selection. Male-male competition was intense

and alternative tactics, such as mate-guarding and “sneaker” EPC tactics, were used by different sized males. Females exercised mate choice both directly and indirectly. However, unlike classic leks, males used tactics to defend females rather than habitat defence. These features are very different from the reproductive behaviour described previously for *S. apama* near Edithburg in Gulf St. Vincent, where males were highly territorial, occupying and guarding caves or overhangs in the reef structure and thus “defending” the habitat (egg-laying sites) rather than guarding a female (Rowlings 1994). These tactics may represent plasticity in the mating system according to local ecological conditions (Emlen and Oring 1977). The mating system of *S. apama* at the aggregation area may have evolved in response to the clumped distribution of suitable egg-laying substrate, which led to high densities of cuttlefish. This egg-laying component of the mating system distinguishes it from a classic lek (Bradbury 1981).

Biased fishing or overfishing on a spawning aggregation may alter the density and composition of the spawning population. A prolonged reduction of the density of the aggregated *S. apama* through overfishing may result in a gradual change in the prevailing mating system towards habitat defence territoriality, similar to that currently found in other areas of low-density across the species distribution. A change in the mating system of a population may lead to “artificial” sexual selection (cf., Hewitt and Butlin 1997; Hanlon 1998), potential loss of genetic variation and reproductive output, and a decrease in the ability of the population to evolve in response to environmental change (Caro 1998; Anthony and Blumstein 2000).

10 Population structure

K.S. Kassahn and S.C. Donnellan

10.1 Introduction

Determining population structure is crucial to fisheries management (Carvalho and Hauser 1994; Pierce and Guerra 1994). In fisheries, self-recruiting populations are referred to as stocks, and they represent the appropriate scale at which the fishery should be managed (Begg and Waldman 1999 and references therein). Different methods have been used to identify cephalopod stock/population structure, including: tag-recapture studies (Hamabe and Shimizu 1966); multivariate morphometrics (Kristensen 1982; Pierce et al. 1994; Martínez et al. 2002); life history studies on spawning season, growth types, and migratory patterns (Murata 1989); allozyme electrophoresis (Carvalho and Loney 1989; Carvalho et al. 1992; Brierley et al. 1995); comparison of microsatellite allele frequencies (Shaw *et al.* 1999); and in some cases a combination of these methodologies (Carvalho and Pitcher 1989). Different stock identification techniques may yield non-concordant results (Leslie and Grant 1990), and so to gain a holistic understanding of population structure, it may be best to use a suite of complementary methods that address different aspects of the biology of the species, including at least one phenotypic-based and one genetic approach in stock identification (Begg and Waldman 1999).

For *Sepia apama* there is a need for detailed investigation of both the taxonomy and population structure. The type specimen of this species consists of the cuttlebone only, and its taxonomic description has not accounted for morphological variation across the broad geographic range of the species. Anecdotal evidence suggests that individuals from New South Wales and South Australia differ with respect to colour patterns and morphology of the fourth left arm (A. Reid and M. Norman, pers. comm.). Such differences in morphology may indicate the existence of two subspecies or even species. Species that have a wide geographic range and show unaccountable morphological variation are prime candidates for the existence of cryptic species (Donnellan and Aplin 1989).

There are no data on the population structure of *S. apama*. The lack of a dispersive egg and larval stage, and possible localised movement of adults makes *S. apama* prone to isolation by distance. But morphological differentiation mentioned above may indicate reproductive isolation between populations, e.g. intrinsic or extrinsic barriers to gene flows. As *S. apama* is an important natural

resource as a fishery species and also for eco-tourism, an understanding of its population structure and phylogeography is an important part of this overall study.

10.2 Aims

This study determined the population structure of *S. apama* at different spatial and temporal scales using an array of molecular genetic methods and morphological analyses. Furthermore, the data were used to test the hypothesis originating from anecdotal evidence that the taxon may be divisible into two species. The methods employed included comparison of allele frequencies of allozyme and microsatellite loci, phylogeographic analysis of mitochondrial sequence data, multivariate morphometrics, and analysis of colour patterns. The hypothesis that *S. apama* is a single species was addressed by applying the evolutionary species concept (Wiley 1978). The results are discussed in terms of the management of the *S. apama* fishery.

10.3 Methods

10.3.1 Sampling

This study of population structure was based on samples collected from throughout the species range from Perth, Western Australia to Coffs Harbour in northern New South Wales. A total of 173 animals were collected from 20 locations between 1998 and 2002. Most were collected from commercial fishing vessels, whilst samples from northern Spencer Gulf were collected using the techniques described in Chapter 3 (Table 10.1; Fig. 10.1).

The genomic DNA was extracted from either frozen or alcohol-preserved muscle tissue using DNAzol homogenization buffer (DNAzol) (pH 7.6) (Life Technologies Inc., Rockville, MD, USA) following the manufacturer's instructions or using a salt extraction method (Miller et al. 1988).

10.3.2 Mitochondrial sequence data

A fragment of the mitochondrial *cytochrome oxidase III* gene (*COXIII*) was considered from 44 individual *S. apama* and the outgroup taxon *S. novaehollandiae* Hoyle, 1909. This gene, consisting of approximately 620 bp, was amplified by polymerase chain reaction (PCR). Two primer pairs, forward 5'-AGCCCATGACCTTTAACAGG-3' and reverse 5'-GACTACATCAACAAAATGTCAGTATCA-3'

(Bonnaud *et al.* 1997) and forward 5'-TTTAACAGGATCATTAGGTG-3' and reverse 5'-AGTATCATGCTGCTGCTTC-3', were employed.

Table 10.1 Sampling localities, sampling dates, total number of animals per sample and number of animals per analysis. The bottom row indicates the total number of populations sampled, sampling date range, total number of animals analysed and total number of animals analysed for each technique.

| Code | Locality | Sampling date | Total (n) | mtDNA (n) | Allozymes (n) | Morphometrics and colour patterns (n) | Microsatellites (n) |
|-----------|-----------------------|------------------------|------------|-----------|---------------|---------------------------------------|---------------------|
| PE | Perth, WA | May-02 | 2 | 2 | 2 | 2 | 2 |
| AL | Albany, WA | Jan-98 | 1 | 1 | 1 | - | 1 |
| EC | Esperance, WA | Jan-98 | 1 | 1 | 1 | - | 1 |
| WSA | Western SA | May-02 | 2 | 2 | 2 | 2 | 2 |
| SB | Streaky Bay, SA | May-02 | 10 | 3 | 2 | 10 | 10 |
| SG1 | Whyalla, SA | May-00; May-98 | 50 | - | - | 20 | 30 |
| SG2 | Wallaroo, SA | Feb-99 | 1 | 1 | 1 | - | - |
| SG3 | Spencer Gulf, SA | April-01 | 2 | 2 | 2 | - | - |
| KI | Kangaroo Island, SA | May-02 | 1 | 1 | 1 | 1 | - |
| GSV1 | Glenelg, SA | Feb/Mar/May-98 | 20 | - | - | - | 20 |
| GSV2 | Myponga, SA | Feb/Mar-98 | 28 | 1 | 2 | - | 28 |
| GSV3 | Gulf St Vincent, SA | May-01 | 20 | 2 | 1 | 20 | - |
| MG | Mount Gambier, SA | May-02 | 1 | 1 | - | 1 | 1 |
| PP | Port Phillip Bay, VIC | May-02 | 4 | 4 | 3 | 3 | 3 |
| LE | Lakes Entrance, VIC | May-02 | 10 | 6 | 3 | 10 | 10 |
| JB | Jervis Bay, NSW | May-02 | 2 | 2 | - | - | 2 |
| WG | Wollongong, NSW | May-02 | 3 | 3 | 3 | 2 | 3 |
| NC | Newcastle, NSW | May-02 | 4 | 4 | 3 | 4 | 4 |
| CH | Coffs Harbour, NSW | May-02 | 11 | 11 | 3 | 4 | 11 |
| 20 | | Feb-98 - May-02 | 173 | 47 | 30 | 79 | 128 |

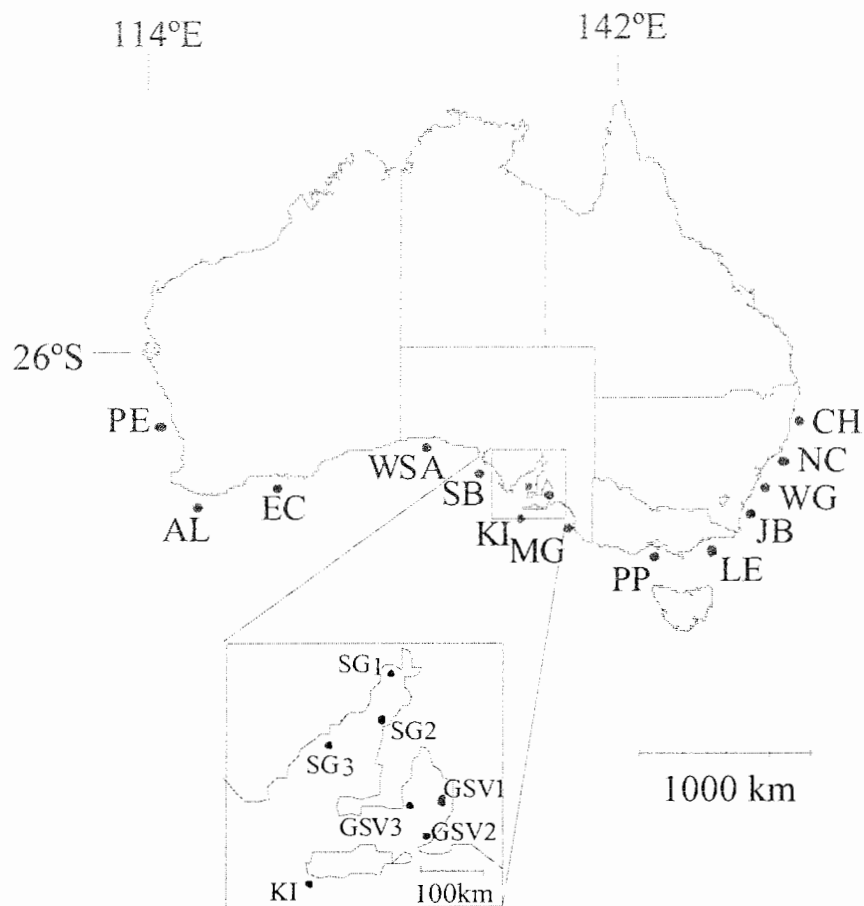


Figure 10.1 Map of Australia showing the locations around the southern coastline where the samples were collected for the genetic and morphometric analyses. The inset shows the detailed map of the South Australian Gulfs showing the collection sites in the two gulfs. Refer to Table 10.1 for site codes.

PCR amplifications were carried out in a final volume of 50 μ l with 10-40 ng DNA, 5 μ l GeneAmp[®] 10x PCR Gold Buffer (Applied Biosystems Inc., Madison, WI, USA), 4 mM MgCl₂, 0.2 mM of each dNTP, 10 pmol of each primer (0.2 μ M), and 0.2 μ l of AmpliTaq Gold[®] Polymerase (Applied Biosystems). The PCR program comprised one cycle at 94°C for 9 min, 48°C for 1 min, 72°C for 1 min, followed by 34 cycles at 94°C for 45 s, 48°C for 45 s, 72°C for 1 min and one final cycle at 72°C for 6 min, 26°C for 10 s run on a Corbett FTS-320 Thermal Sequencer.

PCR products were purified using UltraClean PCR clean-up columns (Mo Bio Laboratories Inc., Carlsbad, CA, USA) following the manufacturer's instructions. Sequencing reactions using the ABI PRISM[®] BigDye[™] Version 3 Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) were carried out in 20 μ l reaction volumes, using 0.2-1.0 μ g of purified PCR product, 6 μ l Ready

Reaction Premix and 5 pmol (0.25 μ M) primer. Reactions were performed on a Corbett FTS-1 Thermal Sequencer running 25 cycles at 94°C for 30 s, 50°C for 15 s, 60°C for 4 min followed by one cycle at 60°C for 4 min, 25°C for 10 s. DNA was precipitated with 80 μ l of 75% isopropanol at room temperature for 15 min, centrifuged at 4°C for 20 min at 11,000 rpm, washed in 300 μ l of 75% isopropanol, dried at 95°C for 1 min, and resuspended in deionised water before being run on an ABI PRISM 3700 DNA analyser (Applied Biosystems).

Sequences were aligned by eye using Se-AI (v2.0) (Rambaut 1996). The following outgroups were also included in the analysis: *S. smithi* Hoyle, 1885 (GenBank Accession Number X97951), *S. elegans* Blainville, 1827 (Y07843), and *S. officinalis* Linnaeus, 1758 (X97954).

Phylogenetic analyses

Phylogenetic analyses at the interspecies level were performed using PAUP* version 4.0b8 (Swofford 1999). Modeltest version 3.06 was used to select a model of sequence evolution (Posada and Crandall 1998). Trees were generated with maximum parsimony (MP) and using the model and parameters suggested by Modeltest by Neighbour-joining (NJ), minimum evolution (ME), and maximum likelihood (ML). Non-parametric bootstrap support was evaluated from 1000 (NJ/ ME/ MP) and 100 (ML) bootstrap pseudoreplicates.

Intraspecific gene genealogy using genealogical networks

A minimum spanning network was generated in order to analyse the *COXIII* intraspecific gene genealogy using the computer program TCS Version 1.06 (Clement *et al.* 2000) and the statistical parsimony criterion of Templeton *et al.* (1992).

Tests for neutrality of sequence evolution and past demographic processes

The mitochondrial sequence data were analysed for signals of past demographic processes. A series of tests for neutrality of sequence evolution, Tajima's D (1989), Fu and Li's (1993) F* and D*, and Fu's (1997) F_s, were performed using the program DnaSP Version 3.51 (Rozas and Rozas 1999). These different tests for neutrality have different sensitivity levels for detecting the genetic traces of population growth, population stability or selection (Fu 1997). Mismatch distributions were also employed to infer population history and to test for the genetic signatures of population expansion. The three parameters needed to model the mismatch distribution for expanding populations θ_0 ($2N_0\mu$ where

N_0 is the initial population size and μ the mutation rate per generation), θ_i ($2N_i\mu$ where N_i is the final population size), and τ (the time since expansion in mutational units, $2\mu t$, where t is the time since expansion in generations) were estimated from the dataset using the method of moments (Rogers 1995) and mismatch analyses were carried out in DnaSP version 3.51 (Rozas and Rozas 1999). The significance of the raggedness statistic r under different population models, e.g. constant population size and 10-, 1000- and 1,000,000- fold population growth, was tested using computer simulations generating 1000 theoretical mismatch distributions (program provided courtesy of H. Harpending).

10.3.3 Allozyme electrophoresis

Tissues for allozyme electrophoresis were homogenised by sonication in two volumes of homogenization solution (deionised water containing 0.2% 2-mercaptoethanol and 0.2mg ml⁻¹ NADP). A total of 30 individuals from across the range of *S. apama* plus one sample of the outgroup taxon *S. novaehollandiae* were stained for 40 enzymes (Table 10.1). The nomenclature for referring to loci and allozymes follows Murphy *et al.* (1996).

Analysis of allozyme allele frequencies

Genotypes at all pairs of loci were tested for linkage disequilibrium and genotype distributions within samples were tested for conformity to Hardy-Weinberg expectations, using exact tests with significance levels determined by Markov Chain simulation using GENEPOP version 3 (Raymond and Rousset 1995b). To determine the genetic affinities of all individuals sampled without *a priori* knowledge of their grouping, principal coordinates analysis (PCoA) was performed on a matrix of Rogers' genetic distance (Rogers 1972) using the computer program PATN (Pattern Analysis Package) (Belbin 1994). Subsequent allozyme analyses were performed on the groups obtained from PCoA.

Allele frequency differentiation between pairs of samples was tested with Fisher's exact test. Multilocus F_{ST} values between pairs of samples were calculated according to Weir and Cockerham (1984) using GENEPOP version 3. (Raymond and Rousset 1995b). Significance values of simultaneous pairwise allele frequency comparisons and multilocus F_{ST} values were adjusted using the sequential Bonferroni correction (Rice 1989).

10.3.4 Morphological analyses

Anecdotal reports suggest that specimens from NSW differ from those in South Australia with respect to colour patterns. To test for a real difference the presence of stripes and dots on the web between the

third and fourth left arms was examined in 79 animals across the range of *S. apama* including 10 individuals from NSW and 53 from South Australia (Table 10.1). Sex was determined by examination of the buccal cavity for the presence of a sperm receptacle (Chapter 5).

For the morphometric analysis, a total of 32 measurements (Fig. 10.2) were taken for a selection of 79 animals from across the whole range (Table 10.2). The measurements included total weight (TW), head weight (HW), head width (HW), eye diameter (ED), anterior mantle to head (AMH), mantle length (ML), mantle width (MW), fin width (FW), cuttlebone length (CbL), cuttlebone width (CbW), last loculus length (LoL), striated zone length (StZ), inner cone width (ICW), outer cone width (OCW), club length (CL), club sucker diameter (CSD), length of third arm (AL3), arm sucker diameter of third arm (ASD3), length of fourth arm (AL4), arm sucker diameter of fourth arm (ASD4), upper hood length (UHL), upper rostral length (URL), upper wing length (UWL), upper lateral wall (ULW), upper height (UH), upper crest (UC), lower hood length (LHL), lower rostral length (LRL), lower rostral gap (LRG), lower wing length (LWL), lower lateral wall (LLW), lower shoulder height (LSH) (Reid 2000; Martínez et al. 2002). Statistical analyses were performed in SPSS version 10.0.5 (SPSS Inc., Chicago, IL, USA). No attempt was made to correct for the effect of size due to theoretical difficulties associated with common size-adjusting procedures. Bookstein *et al.* (1985) argued that size adjustment procedures such as using regression residuals on some size variable or ratio data cannot adjust for size. The authors employ a geometric approach to Mosimann's central theorem (Mosimann 1970) to demonstrate that size and shape are not completely independent factors. Thus, common procedures such as regression residuals and ratio data do not separate the effect of size from shape or remove the "confounding" effects of size. In addition, one may argue that size does not describe a somewhat irrelevant variance but rather a meaningful covariance of the data (Bookstein *et al.* 1985). The data were log-transformed to improve normality and homoscedasticity.

Prior to analysis, the data were screened for outliers. One outlier in the Kangaroo Island sample was identified. This specimen had an extreme Mahalanobis distance ($p < 0.05$) and leverage value higher than the suggested cut-off limit by Lunneborg (1994) due to extreme values in arm length IV (AL4), arm sucker diameter III (ASD3), lower wing length (LWL), lower shoulder height (LSH), and upper wing length (UWL). Unfortunately measurements could not be retaken as the specimen had been fixed in alcohol after processing. This one outlier was removed, leaving 78 cases for analysis.

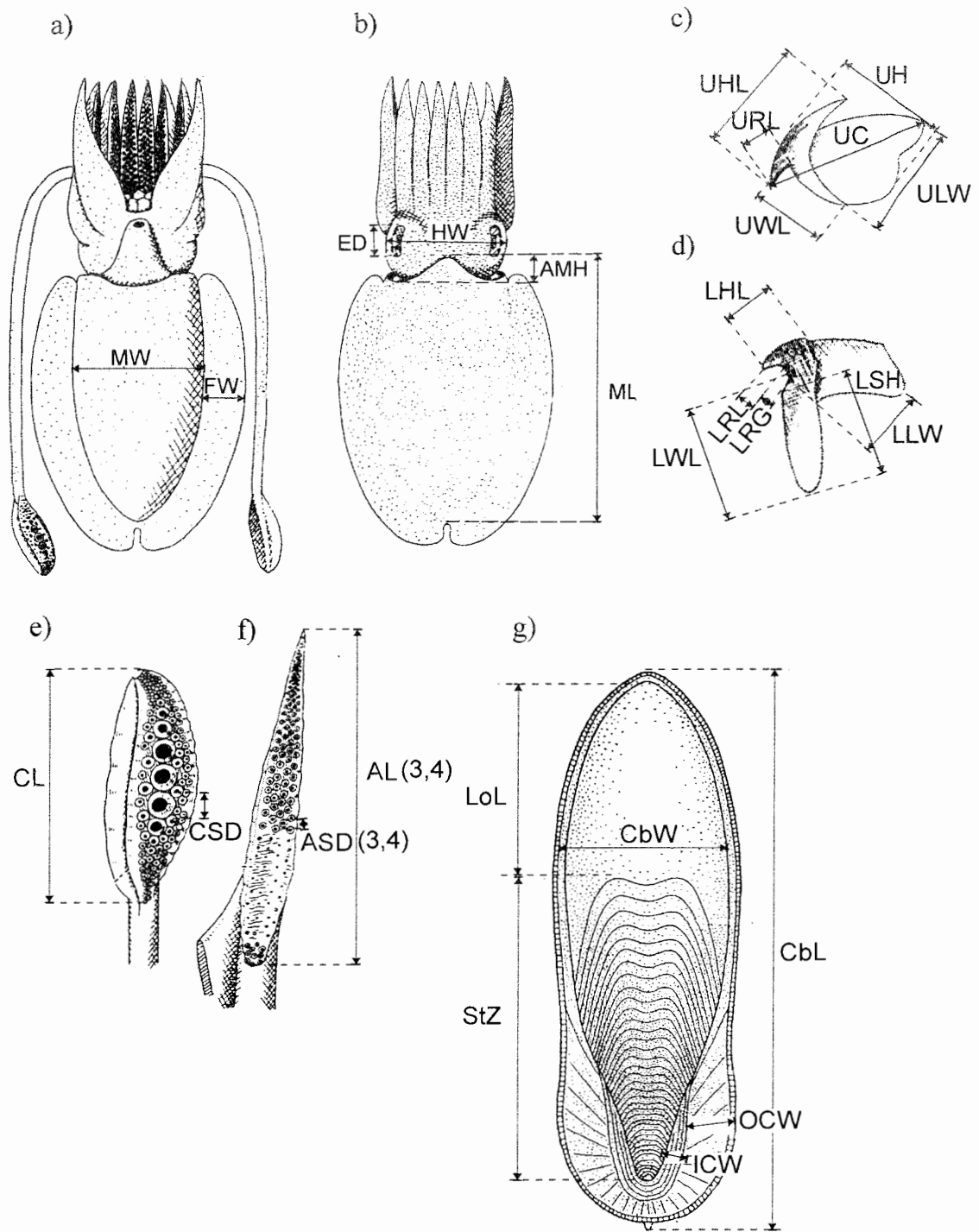


Figure 10.2 Morphometric characteristics measured for the morphological analyses: (a) ventral view; (b) dorsal view; (c) upper beak; (d) lower beak; (e) tentacular club; (f) arm; (g) cuttlebone ventral view; modified from Tompsett (1939).

Discriminant function analysis (DFA) was used to assess whether group membership of individuals was predicted reliably from morphometric measurements. *A priori* groupings for DFA were identified in two ways. One analysis performed DFA on the genetic groupings obtained from allozyme analysis. The second analysis used the exploratory technique of principal components analysis (PCA) to identify morphometric groupings without any *a priori* knowledge of groupings from other analyses.

Stepwise discriminant function analysis was used to identify the most important variables for the discrimination of groups and to reduce the potential problem of overfitting (Tabachnik and Fidell 2001). To ensure entry of important variables a more liberal probability-to-enter criterion of Wilks' Lambda with a $p(\text{entry}) = 0.2$ and $p(\text{removal}) = 0.25$ was chosen following the recommendations by Constanza and Afifi (1979). Cross-validation was performed to test the generality of the results.

Sexual dimorphism, e.g. the difference of physical form or shape between sexes, was assessed using animals from Spencer Gulf, South Australia from where the largest sample sizes were available. Univariate analysis of variance (ANOVA) showed that three variables differed between sexes: fin width (FW) ($p = 0.014$), arm length III (AL3) ($p = 0.041$) and arm length IV (mating arm) (AL4) ($p = 0.002$). After removal of these variables the DFA could still discriminate males and females based on the combination of remaining variables ($p = 0.017$ and 100% correct classification). Therefore, the sexes were analysed separately in subsequent analyses. The regression of each character on mantle length (ML) indicated isometric growth. Thus, juveniles and adults were analysed together.

10.3.5 Microsatellite genotyping

A total of 128 animals from across the species range including Streaky Bay, Whyalla, Glenelg and Myponga in South Australia (Table 10.1), were screened for variation at eight microsatellite loci: Sap191, Sap21, Sap22a, Sap57, Sap65, Sap69, Sap72, and Sap94b. PCR amplifications were performed under the following conditions: one cycle of 9 min at 94°C, 27 to 40 cycles of 45 s at 92°C, 30 s at the primer specific annealing temperature (between 50°C and 56°C), and 10 s at 72°C, followed by a final cooling step of 10 s at 30°C using either Eppendorf Mastercycler[®]gradient or Hybaid Omn-E Thermal Cycler. A 'touchdown' protocol was used in all loci but Sap22a and Sap94b, dropping down over five cycles from an initial temperature that was 5°C above annealing temperature.

Reaction mixes of 15 µl volume contained 10-30 ng template DNA, 1.5 to 4 mM MgCl₂, 0.125 mM of each nucleotide, 0.5 µM of each primer (forward primer fluorescently labelled with either NED, VIC, PET (ABI PRISM[®] BigDye[™] primers, Applied Biosystems) or 6-FAM (Fluorescein; GeneWorks Pty Ltd), 1.5 µl GeneAmp[®] 10x PCR Gold Buffer and 0.06 µl of AmpliTaq Gold[®] DNA Polymerase

(Applied Biosystems). For each individual the PCR products of all eight loci were mixed (multiplex) and run on an ABI PRISM[®]3700 DNA analyser using ABI PRISM[®]3700 POP-6 Polymer gels (Applied Biosystems). Product sizes were determined by comparison with a LIZ[™]500 Size Standard added to each gel lane using the software ABI PRISM[®]GeneScan[™] and ABI PRISM[®]Genotyper Version 3.7 (Applied Biosystems).

Animals from New South Wales (Coffs Harbour, Newcastle, Wollongong, Jervis Bay) and from the rest of the species range had a fixed difference at locus Sap65. To confirm that the same locus had been amplified the PCR product from one NSW specimen and one SA specimen were sequenced and the sequences aligned by eye using Se-Al Version 2.0 (Rambaut 1996).

Analysis of microsatellite allele frequencies

Loci were tested for linkage disequilibrium and genotype distributions within samples, i.e. morphometric groupings, were tested for conformity to Hardy-Weinberg expectations using the program GENEPOP version 3 (Raymond and Rousset 1995b). Differences in allele frequency between pairs of samples were tested with Fisher's exact test using GENEPOP following the test procedure by Raymond and Rousset (1995a), based on allelic frequency distribution. When sampling is unbalanced as in the present study allelic goodness of fit tests are preferred over F_{ST} tests (Goudet *et al.* 1996).

Unbiased R_{ST} values were calculated using the RST-CALC program (Goodman 1997). This unbiased R_{ST} statistic relaxes the assumptions of Slatkin's R_{ST} statistic (Slatkin 1995) of equal sample size across populations and equal variances across loci by standardising the dataset before analysis. Significance values of multilocus R_{ST} values were corrected using the sequential Bonferroni correction (Rice 1989).

As a test for an isolation by distance model of population structure, Mantel's matrix correlation test (Mantel 1967) was used to examine the relationship between geographic distance (measured along the intervening coastline) and R_{ST} values, i.e. genetic divergence among populations, using GENEPOP version 3.

10.4 Results

10.4.1 Mitochondrial DNA sequence diversity in *Sepia apama*

Sequencing of a 620bp fragment of the *COXIII* gene from 44 individual *S. apama* from across the species range yielded 18 haplotypes defined by 43 variable nucleotide sites. Sequences of the 18 haplotypes are deposited with GenBank (accession numbers XXXXX-XXXXX). Sixteen of the 18 haplotypes were singletons. Most substitutions occurred at the third codon position (0.837), but some second and first codon positions were also variable (0.116 and 0.047 respectively). As a result most nucleotide substitutions were silent changes that did not affect the encoded amino acid sequence. Among all haplotypes only four amino acid changes were observed.

The hierarchical likelihood ratio testing with Modeltest suggested a complex model of sequence evolution: the General Time Reversible (GTR) model with 50.26% invariable sites and a gamma distribution shape parameter of 2.0456. Base frequencies were biased towards adenine (A) and thymine (T), which occurred at a frequency of 0.3 and 0.4079, respectively. The 18 haplotypes formed two major, reciprocally monophyletic lineages. Phylogenetic analyses of the relationship of *S. apama* haplotypes yielded concordant results: different tree building methods (NJ, ME, MP) produced the same tree topology with similarly very strong bootstrap values for the two major *S. apama* lineages. The ML analysis (Fig. 10.3a) agreed with the previous analyses. Thus, the data and the model seem to be robust with respect to the choice of tree-building method. The ML analysis in addition found a monophyletic group within the southern lineage that did not include the Western Australian haplotypes (B, C, and D), although bootstrap support values for this sub-lineage was low at 62%.

Minimum spanning network revealed a dumbbell pattern (Avisé 2000) (Fig. 10.3b). The two major lineages, northern and southern, are separated by some 22 to 26 base pair substitutions or 5.8% to 6.3% uncorrected sequence divergence. Within lineages the maximum level of divergence was 0.2% (northern) and 0.8% (southern).

The two major lineages were found to be geographically isolated. The southern lineage, found in 36 of the 44 individuals sequenced, was distributed from Perth in WA to Newcastle in NSW. The northern lineage, found in 11 of the 44 individuals sequenced, was restricted to a single sample site, i.e. Coffs Harbour in northern NSW (Fig. 10.4). Haplotype diversity was highest in the Victorian sample sites Port Phillip Bay and Lakes Entrance, where six of the 18 haplotypes were present (Fig. 10.4).

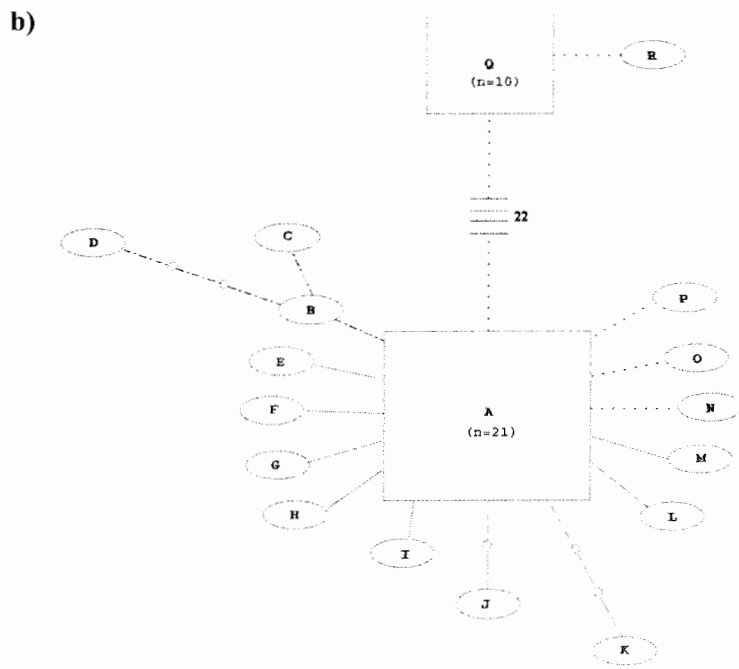
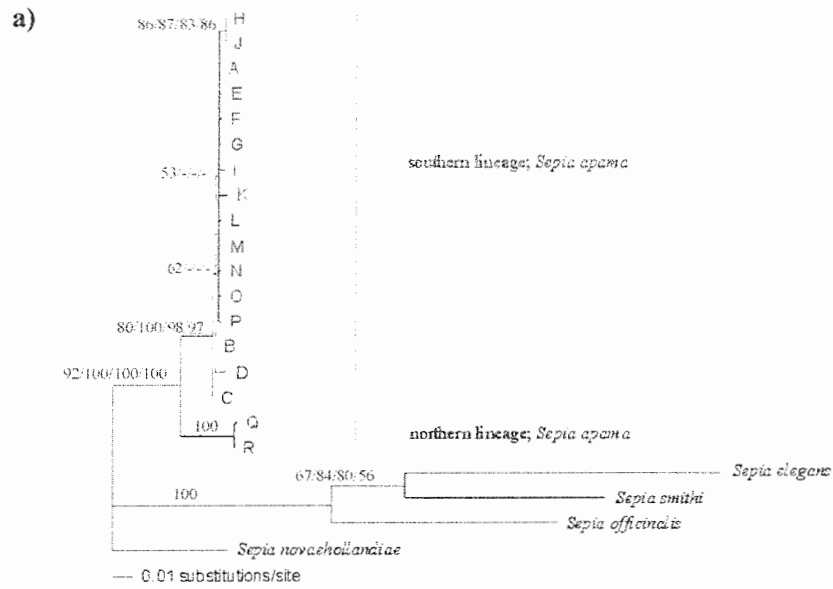


Figure 10.3 Likelihood with bootstrap support values from 100 bootstrap pseudoreplicates and 1000 pseudoreplicates (NJ/ ME/ MP) given above the nodes; - indicates that node was not extracted; where only one number is given bootstrap support was the same for all tree-building methods; (b) Haplotype network showing the relationship among haplotypes detected in a sample of 47 cuttlefish from across southern Australia. Number of individuals sampled carrying each haplotype is one unless otherwise specified. --- NSW samples; - - - WA samples; — SA and VIC samples.

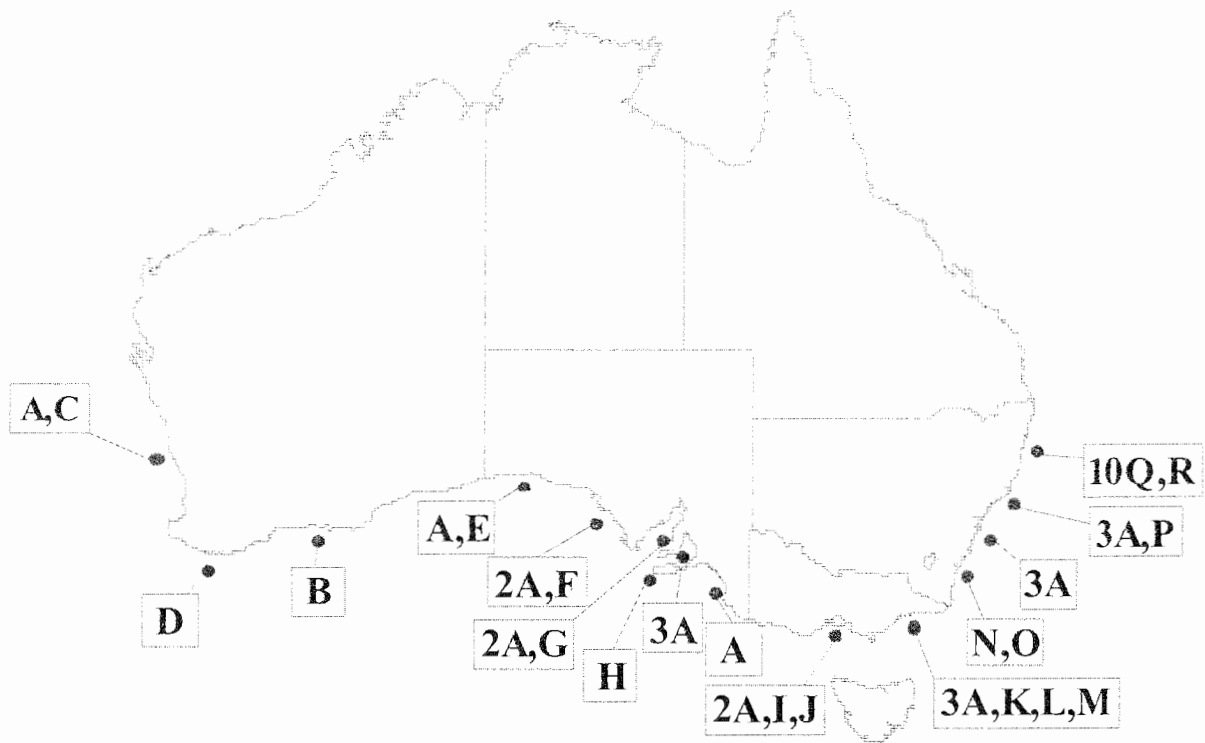


Figure 10.4 Geographic distribution and number of cuttlefish haplotypes based on analysis of the *COXIII* gene by mitochondrial DNA analysis.

Tests for neutrality of sequence evolution and past demographic processes

The test for the null hypothesis of neutral sequence evolution could not be rejected for the northern lineage (Table 1.2). However, the southern lineage showed significant deviation from neutrality in Tajima's D , Fu and Li's (1993) F^* and D^* , and Fu's (1997) F_S test statistics. Table 1.2 presents expected significance levels under the operation of selection and population growth. The results for the southern lineage indicate a combined effect of selection (significant D , D^* and F^*) and population growth (significant F_S).

Table 10.2 Tests of neutrality of sequence evolution for two major haplotype lineages in *S. apama*. Significant values are given in bold (*p < 0.05; **p < 0.01; ***p < 0.001). Expected values under the operation of selection and population growth are given in columns 3 and 4.

| | Southern Lineage (n = 36) | Northern Lineage (n = 11) | Expectation under | |
|----------------------------|------------------------------|------------------------------|-------------------|-------------------|
| | | | Selection | Population growth |
| Number of haplotypes | 16 | 2 | | |
| Nucleotide diversity (%) | 0.00213 | 0.00029 | Low | Low |
| Tajima's D | -2.45512** | -1.1285 | Significant | Significant |
| Fu & Li's (1993) F* | -4.16342* | -1.39919 | Significant | NS |
| Fu & Li's (1993) D* | -4.05518* | -1.28946 | Significant | NS |
| Fu's (1997) F _s | -12.951*** | -0.410 | NS | Significant |
| Raggedness r | 0.0276 | 0.4380 | unknown | Small |

Both, southern and northern lineages had low raggedness indices (0.0276 and 0.438 respectively, Table 1.2). Due to the low sample size for the northern lineage (n = 11) no further analyses were carried out. For the southern lineage θ_0 was estimated as 0.69195 and τ as 0.5900462 using Rogers' method of moments (Rogers 1995). Using simulations to test the significance of the observed raggedness value r , the null hypothesis of constant population size was rejected (p = 0.002), whereas models of population growth (10-, 1000- and 1000000-fold) were borderline significant and could not be rejected at the 1% level (0.025 < p < 0.041).

The distribution of estimates of the raggedness statistic in simulated populations is presented in Fig. 1.5. An arrow at 0.0276 indicates the observed raggedness value in the southern lineage. For example, for the model of constant population size the observed raggedness value is smaller than that of 998 of the 1000 simulated populations. Hence, the probability of the observed raggedness value being the result of constant population size is p = 0.002. This can be regarded as the significance level by which the hypothesis of constant population size is rejected (Harpending 1994).

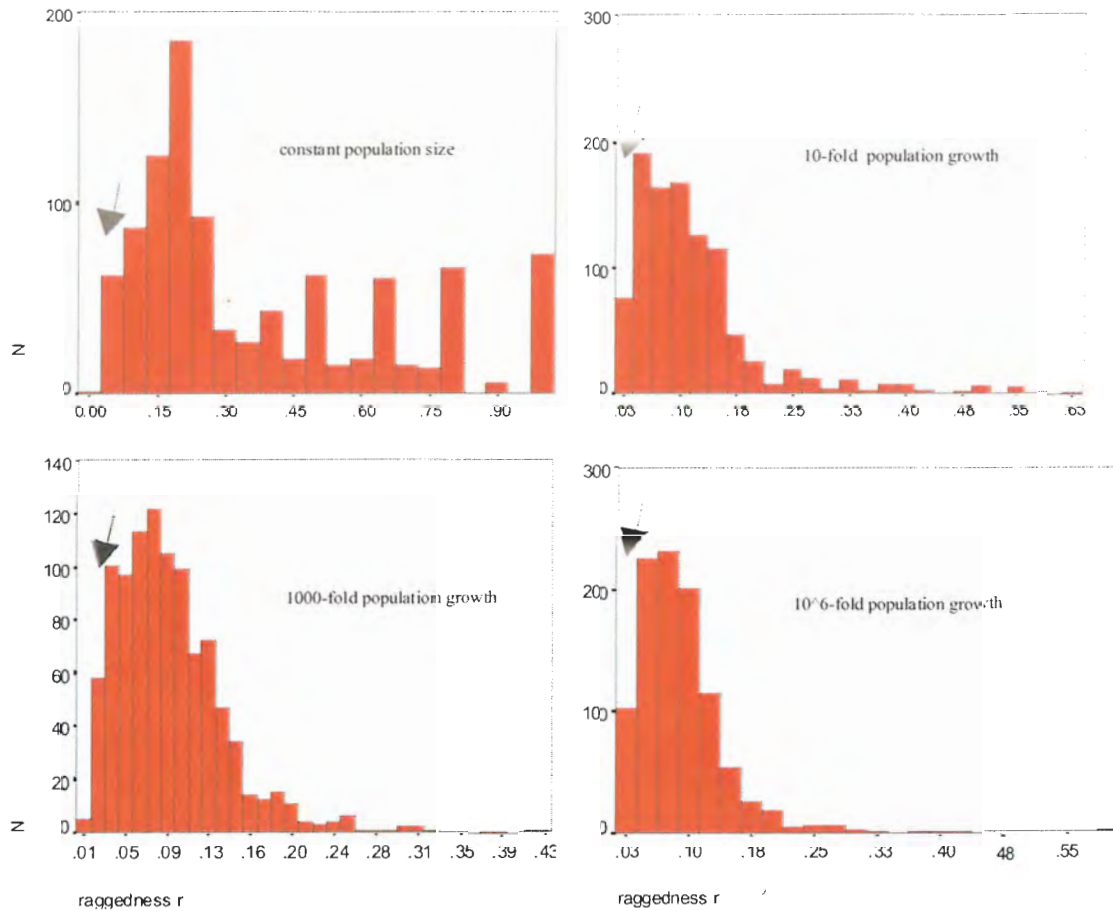


Figure 10.5 Distribution of raggedness statistics in simulated populations. Each bar chart shows the results of 1000 simulations of a sample of 36 haplotypes, $\theta_0 = 0.69195$ and $\tau = 0.5900462$. Arrows show the observed raggedness value for the *S. apama* dataset.

10.4.2 Allozyme variation in *Sepia apama*

The 40 enzymes for which gels were analysed revealed the presence of 51 putative enzyme coding loci, of which 49 displayed interpretable banding patterns. Of these, nine loci, i.e. 18.4% of the total, displayed electrophoretic variation, indicating the existence of two or more alleles at a Mendelian gene. The total number of alleles in these polymorphic loci ranged from two to four.

PCoA on Rogers' genetic distance matrix revealed three distinct genetic clusters that related to the division of the animals from the three regions of Western Australia, South Australia and Victoria, and New South Wales (Fig. 10.6). The first PCoA dimension separated the latter group from the former

two, while the second PCoA dimension separated the WA group from the SA/VIC one. In the following analyses individuals were pooled into these three groups obtained from PCoA.

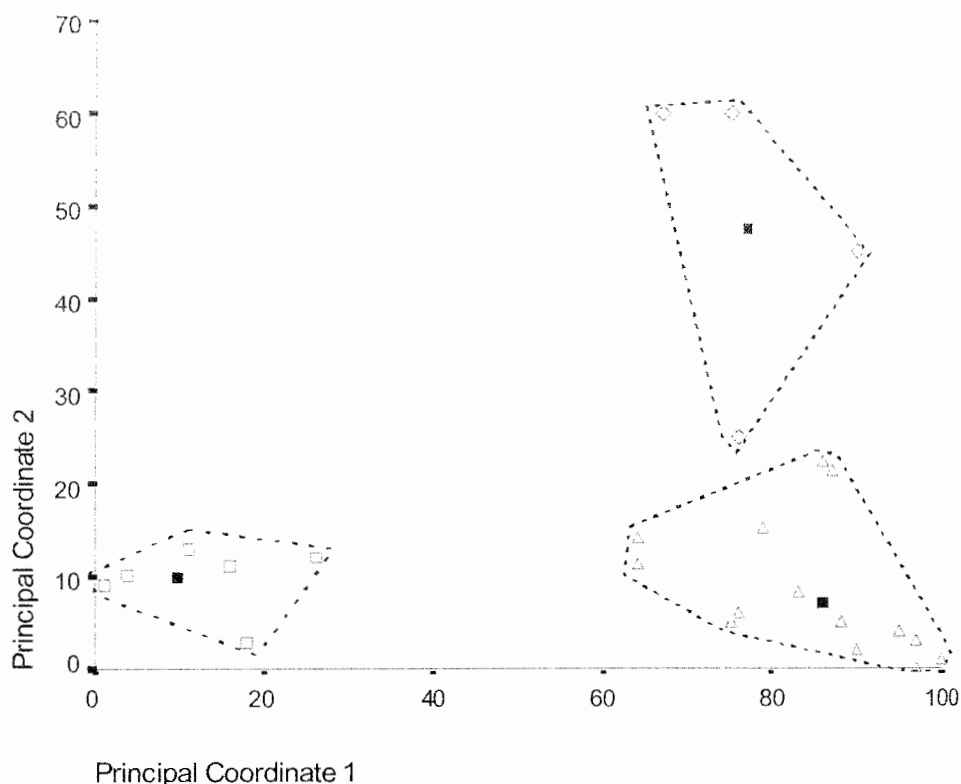


Figure 10.6 Scatterplot of first two principal coordinate scores for allozyme data from 31 specimens. The Principal Coordinate Analysis (PCoA) was performed on Rogers' (1972) genetic distance matrix. □ NSW; △ SA and VIC; ◇ WA; ■ group centroids.

No evidence of linkage disequilibrium was found for genotypes in any pairwise comparison of the nine polymorphic loci. Observed heterozygosities within each pooled sample conformed to Hardy-Weinberg expectations. The *Gda* locus showed highly significant deviations in allele frequencies: individuals from WA, SA and VIC were fixed for allele *Gda^a*, while 8 individuals from NSW were homozygous for allele *Gda^b*, and only one individual from Wollongong in southern NSW was heterozygous carrying the genotype *Gda^{ab}* (Table 10.3). Several other loci were invariant in one or two groups, while allelic polymorphism was maintained in another group (e.g. *Odh1*, *Odh2*, *Pep-A1*, *Pgk*, *Pnp2*) (Table 10.3).

Table 10.3 Allozyme frequencies as percentage in three groups of *S. apama* at nine polymorphic loci (2n in parentheses). "WA" comprises samples from Western Australia, "SA and VIC" from South Australia and Victoria, "NSW" from New South Wales. The following loci were invariant: *Aat*, *Acoh*, *Acyc*, *Ada*, *Adh*, *Ak*, *Alat*, *Aldh*, *Argk*, *Ca*, *Eno*, *Est*, *Fba*, *Fbp*, *Fumh*, *Gapdh*, *Gtdh*, *G3pdh*, *Gpi*, *Guk*, *Hbdh*, *Iddh*, *Lap*, *Lgl*, *Mdh*, *Mdhp*, *Ndpk*, *Pep-De*, *Pgam*, *Pgm*, *Plk*, *Tpi*.

| Locus | Allele | WA (2n=8) | SA and VIC (2n=36) | NSW (2n=18) |
|---------------|----------|-----------|--------------------|-------------|
| <i>Gda</i> | <i>a</i> | 100 | 100 | 6 |
| | <i>b</i> | | | 94 |
| <i>Idh2</i> | <i>a</i> | 50 | 6 | |
| | <i>b</i> | 50 | 94 | 100 |
| <i>Pnp1</i> | <i>a</i> | - | 6 | |
| | <i>b</i> | 25 | 6 | 77 |
| | <i>c</i> | 25 | 3 | 6 |
| | <i>d</i> | 50 | 85 | 17 |
| <i>Pnp2</i> | <i>a</i> | 25 | 100 | 100 |
| | <i>b</i> | 75 | | |
| <i>Odh1</i> | <i>a</i> | 75 | 100 | 100 |
| | <i>b</i> | 25 | | |
| <i>Odh2</i> | <i>a</i> | 33 | 19 | 100 |
| | <i>b</i> | 50 | 62 | - |
| | <i>c</i> | 17 | 19 | - |
| <i>Pep-A1</i> | <i>a</i> | | | 11 |
| | <i>b</i> | | | 11 |
| | <i>c</i> | 100 | 100 | 78 |
| <i>Pgdh</i> | <i>a</i> | 25 | 36 | 6 |
| | <i>b</i> | 62 | 56 | 94 |
| | <i>c</i> | 13 | 8 | |
| <i>Pgk</i> | <i>a</i> | | | 6 |
| | <i>b</i> | 100 | 100 | 94 |

Loci that showed significant differences in allele frequencies between groups including p-values of less than 0.001 after Bonferroni correction, are presented in Table 10.4. All pairwise group comparisons of multilocus F_{ST} values are significant at level $p < 0.05$ indicating large and significant levels of between-group genetic differentiation (Table 10.4).

Table 10.4 Results of exact tests of differences in allozyme allele frequencies (above diagonal) and pairwise estimates of multilocus F_{ST} (below diagonal) between all pairs of samples of *S. apama* (* $p < .05$, ** $p < .01$, *** $p < .001$ after Bonferroni correction).

| | | Individual loci exhibiting significant differences | | |
|----------|------------|--|--------------------------------|---|
| | | WA (2n=8) | SA and VIC (2n=36) | NSW (2n=18) |
| F_{ST} | WA | - | <i>Pnp2</i> ***, <i>ldh2</i> * | <i>Gda</i> ***, <i>Pnp2</i> ***, <i>Odh2</i> **, <i>ldh2</i> * |
| | SA and VIC | .319* | - | <i>Odh2</i> ***, <i>Pnp1</i> ***, <i>Gda</i> ***, <i>Pep-A1</i> *, <i>Pgdh</i> * |
| | NSW | .560* | .597* | - |

10.4.3 Morphological analyses

Stripes on the web between the third and fourth arm were more common in males, while females mostly had dots (Fig. 10.7).

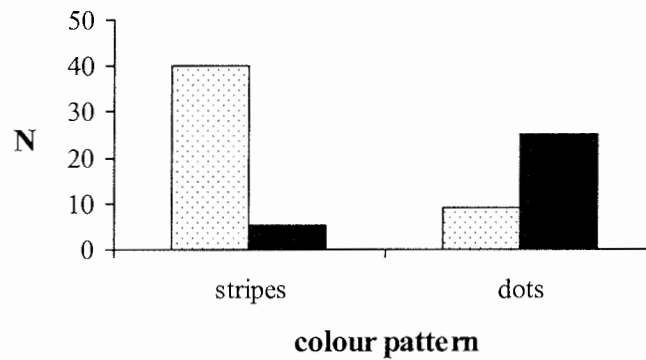


Figure 10.7 Number of individuals carrying stripes and dots on the web between third and fourth arm, separated by sex; □ males; ■ females. Fisher exact test rejected a random association of sex and colour pattern ($p < 0.0001$).

(1) Allozyme groupings

Stepwise discriminant function analysis for 48 *S. apama* males using the groups identified from allozyme analysis (WA, SA/VIC, NSW; Fig. 10.6) extracted ten variables (MW, FW, HW, ED, AL3, ASD3, URL, LHL, LRG, LWL) and resulted in 97.4% correct classification. One animal from Gulf St. Vincent (SA/VIC group) was incorrectly assigned to the WA group, while one animal from Lakes Entrance (SA/VIC group) was incorrectly assigned to the NSW group.

The two discriminant functions accounted for 86.7% and 13.3% of the between-group variability with a combined $\chi^2(20) = 52.206$, $p = 0.000$. The scatterplot of the two discriminant function scores for males and the group centroids are shown in Fig. 10.8a. Discriminant function 1 separated the SA/VIC group from the WA and NSW groups, while discriminant function 2 separated the two latter groups.

Discriminant function analysis for 30 females involved only two groups due to a lack of samples from Western Australia. Stepwise discriminant function analysis reduced the dataset to eight variables (LHL, FW, ML, AL3, ASD4, CBL, CL, ULW), while maintaining 100% classification success. The discriminant function had a high eigenvalue of 12.158 and was significant with $\chi^2(8) = 61.849$, $p = 0.000$. The scatterplot of the discriminant function scores for females plotted against sample order revealed a significant change between the NSW and SA/Vic groups (Fig. 10.8b).

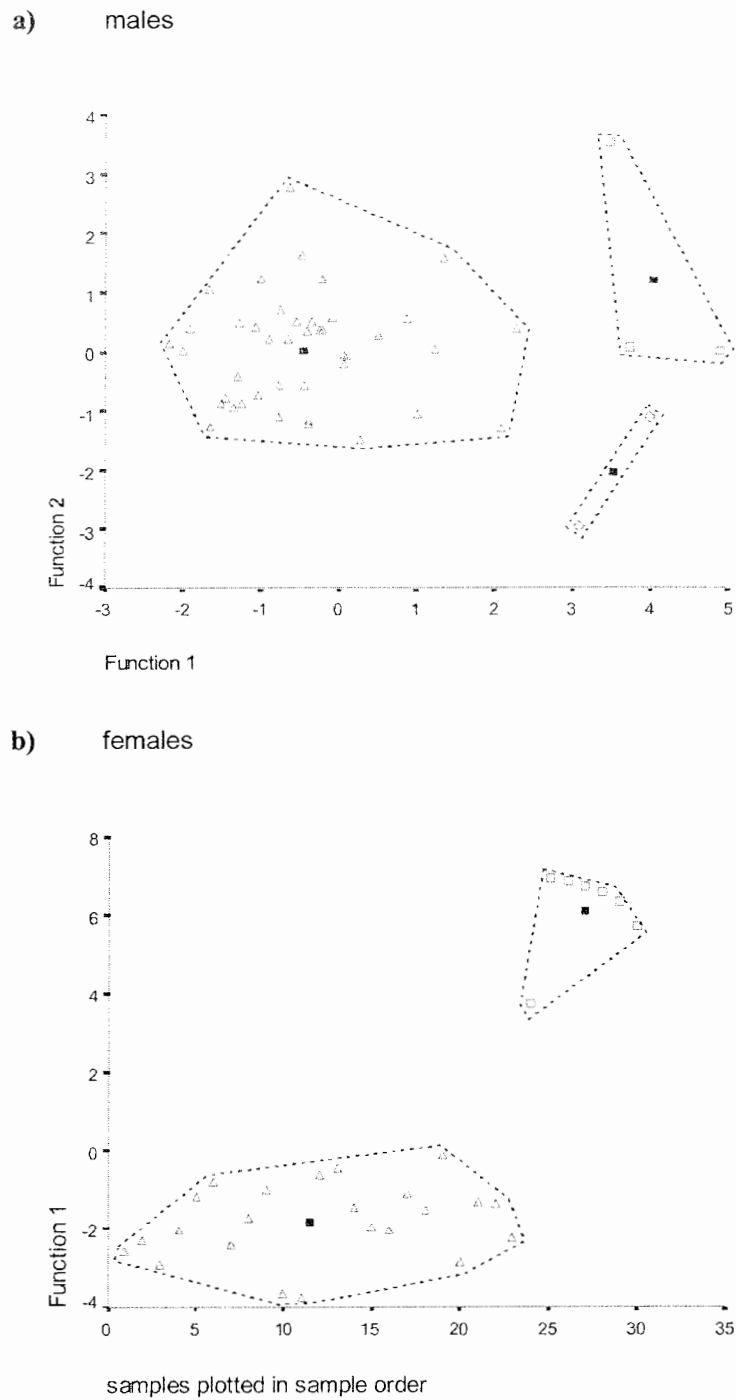


Figure 10.8 Scatterplot of first two discriminant function scores for (a) 48 *S. apama* males using 10 morphometric variables in the analysis. (b) 30 *S. apama* females using eight morphometric variables in the analysis (note that in females discriminant function scores are plotted against sample order). □ NSW; △ SA and VIC; ◇ WA; ■ group centroids.

(2) Groupings from PCA

For males and females, extraction of two principal components accounted for 94% of the variance. PCA revealed six partially overlapping clusters in males: PE/WSA, SB, SG, GSV, MG/PP/LE, WG/NC/CH (Fig. 10.9a). Females were separated into five (no WA samples) clearly separated clusters: SB, SG, GSV, PP/LE, WG/NC/CH (Fig. 10.9b). Individuals from Coffs Harbour (CH) were not identified as a separate group from the other NSW samples, but sample sizes were small.

Stepwise DFA on the PCA groupings reduced the total character space to ten variables for males (LoL, AMH, LRG, LRL, URL, ICW, AL4, UHL, CBL, ED) and resulted in 98% correct classification. One animal from Gulf St. Vincent was incorrectly assigned to Streaky Bay. The five discriminant functions had a combined $\chi^2(50)=210.528$, $p=0.000$ accounting for 53.9%, 28.8%, 9.7%, 5.4% and 2.1% of the between-group variability.

Stepwise DFA on the PCA groupings in females reduced the character set to nine variables (HW, STZ, UHL, CBL, ULW, LRL, TG, HG, and CL) while maintaining 100% classification success. All functions were significant with $p = 0.000$ to $p = 0.036$ and a combined $\chi^2(36) = 162.841$, $p = 0.000$ accounting for 50.6%, 37.3%, 9.5%, and 2.6% of the total variance.

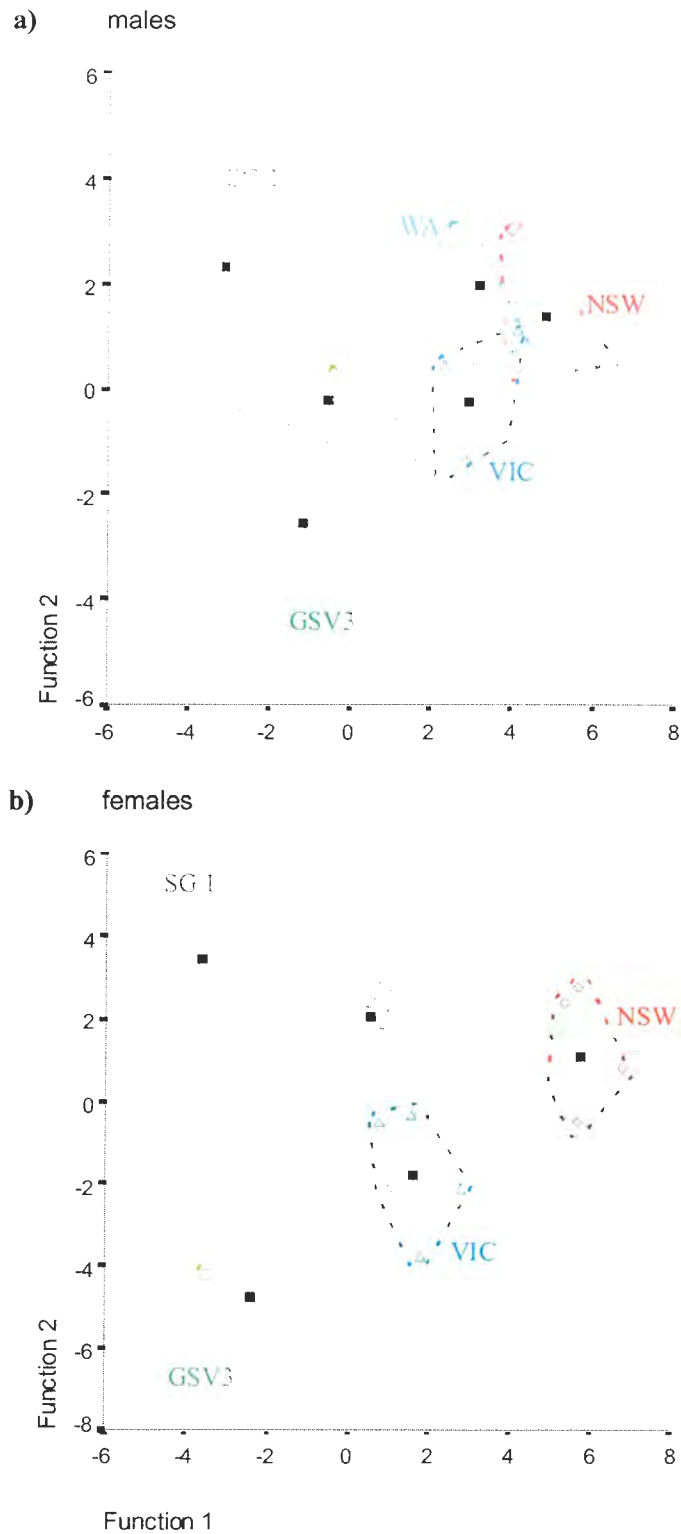


Figure 10.9 *Sepia apama*. Scatterplot of principal component scores for 34 morphometric measurements taken in (a) 48 *S. apama* males, (b) 30 *S. apama* females. □ Gulf St Vincent 3; ◻ Spencer Gulf 1; ◻ Streaky Bay; ◻ Mount Gambier, Port Phillip Bay, Lakes Entrance; ◻ Coffs Harbour, Wollongong, Newcastle; ◻ Western SA, Perth; ◻ group centroids.

10.4.4 Microsatellite variation

Differences in PCR product sizes were consistent with all alleles at all loci stepping up in repeat unit lengths except for three alleles at locus *Sap21* that were one base pair out of stepping-order. However, these fractional alleles only occurred in the samples from Western Australia and New South Wales at frequencies that ranged from 0.03 to 0.13. It is unlikely that these alleles were scored erroneously as their occurrence was too frequent to be a scoring error and they were analysed on different gel runs. Due to the geographic restriction of the fractional alleles to WA and NSW their sizing has minimal impact on the analyses and in particular will not affect the analyses of population differentiation within SA and VIC. In order to avoid distortion of variance-based population comparisons, these few alleles were pooled with the next allele size (e.g. 140 and 141 = 141; 142 and 143 = 143; 144 and 145 = 145).

Sequencing of the 124bp allele from NSW and the 132bp allele from SA of locus *Sap65* showed that the same locus had been amplified and that the two alleles differed by two repeat unit lengths.

Individuals were pooled into the groupings obtained from multivariate morphometric analyses. The WA group contained the Perth, Albany, and Esperance samples, the VIC group contained the one sample from Mount Gambier, SA, and samples from Port Phillip Bay and Lakes Entrance, and the NSW group contained the Jervis Bay, Wollongong, Newcastle and Coffs Harbour samples. Streaky Bay and Spencer Gulf (Whyalla) were treated as separate samples consistent with their distinction in multivariate morphometric analyses. The two samples from Gulf St. Vincent, i.e. Glenelg and Myponga, were also treated separately to test for possible differentiation within the gulf. The Western SA and the Kangaroo Island samples were excluded from subsequent analyses due to their grouping being uncertain.

Most loci had high heterozygosity values with means across samples that ranged from 0.58 to 0.79 and mean number of alleles that ranged from 3.3 to 9.1 (Table 1.5). Locus *Sap94b* presented low heterozygosity values in the SA samples (0 to 0.11) and was fixed in the SA sample from Streaky Bay while polymorphism was maintained in the WA, VIC and NSW samples. Locus *Sap65* was fixed in the NSW samples for an allele smaller than any allele sampled in any of the other populations (124bp in NSW versus 132bp minimum allele size in all other populations). In fact, at four out of eight loci, the NSW population had the smallest alleles, and at two other loci shared the smallest allele with the southern populations. Heterozygosity values within samples conformed with expected values under Hardy-Weinberg equilibrium and there was no evidence for linkage disequilibrium between loci.

Table 10.5 Levels of genetic variation at eight microsatellite loci within seven samples (Repeat motif is indicated next to each locus name, "Allele size" size range of alleles in base pairs, "H_O" observed heterozygosity, "H_E" expected heterozygosity under Hardy-Weinberg equilibrium; the means over all loci and all individuals are given; "Mean n" mean number of individuals genotyped for each sample across all loci).

| Sample Locus | PE/AL/EC (n=4) | SB (n=10) | SG1 (n=30) | GSV1 (n=20) | GSV2 (n=28) | MG/PP/LE (n=15) | JB/WG/NC/CH (n=20) | Locus means |
|---------------------------|----------------|-----------|------------|-------------|-------------|-----------------|--------------------|-------------|
| Sap191 (AAG) _n | | | | | | | | |
| No. of alleles | 4 | 6 | 10 | 7 | 7 | 8 | 9 | 7.3 |
| Allele size | 151-175 | 148-172 | 151-181 | 148-175 | 148-175 | 148-175 | 145-175 | 145-181 |
| H _O | 0.5 | 0.9 | 0.93 | 0.75 | 0.81 | 0.93 | 0.75 | 0.80 |
| H _E | 0.69 | 0.73 | 0.85 | 0.74 | 0.78 | 0.82 | 0.83 | 0.78 |
| Sap21 (complex) | | | | | | | | |
| No. of alleles | 7 | 3 | 9 | 8 | 9 | 7 | 13 | 8 |
| Allele size | 127-177 | 157-171 | 131-179 | 153-181 | 149-179 | 147-177 | 139-167 | 127-181 |
| H _O | 0.75 | 0.6 | 0.55 | 0.37 | 0.58 | 0.46 | 0.75 | 0.58 |
| H _E | 0.84 | 0.59 | 0.51 | 0.37 | 0.55 | 0.54 | 0.9 | 0.61 |
| Sap22a (CA) _n | | | | | | | | |
| No. of alleles | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Allele size | 204-210 | 204-210 | 204-210 | 204-210 | 204-210 | 204-210 | 204-210 | 204-210 |
| H _O | 0.75 | 0.9 | 0.53 | 0.55 | 0.67 | 0.71 | 0.16 | 0.61 |
| H _E | 0.66 | 0.68 | 0.65 | 0.59 | 0.63 | 0.64 | 0.23 | 0.58 |
| Sap57 (AAG) _n | | | | | | | | |
| No. of alleles | 7 | 9 | 11 | 10 | 11 | 7 | 9 | 9.1 |
| Allele size | 209-233 | 200-242 | 215-251 | 215-245 | 194-239 | 218-242 | 194-236 | 194-245 |
| H _O | 1 | 0.78 | 0.86 | 0.7 | 1 | 0.83 | 0.69 | 0.84 |
| H _E | 0.84 | 0.76 | 0.85 | 0.83 | 0.89 | 0.82 | 0.59 | 0.80 |
| Sap65 (GATA) _n | | | | | | | | |
| No. of alleles | 6 | 9 | 9 | 9 | 7 | 9 | 1 | 7.1 |
| Allele size | 132-168 | 132-168 | 132-172 | 132-168 | 132-164 | 132-172 | 124 | 124-171 |
| H _O | 0.5 | 0.75 | 0.69 | 0.74 | 0.62 | 0.85 | 0 | 0.59 |
| H _E | 0.81 | 0.86 | 0.75 | 0.84 | 0.81 | 0.82 | 0 | 0.70 |
| Sap69 (GATA) _n | | | | | | | | |
| No. of alleles | 4 | 7 | 7 | 8 | 10 | 8 | 9 | 7.6 |
| Allele size | 311-331 | 311-339 | 311-335 | 307-335 | 307-343 | 307-335 | 299-331 | 299-339 |
| H _O | 1 | 0.9 | 0.79 | 0.79 | 0.85 | 0.62 | 0.8 | 0.82 |
| H _E | 0.72 | 0.8 | 0.82 | 0.82 | 0.83 | 0.78 | 0.86 | 0.80 |

| | | | | | | | | |
|---------------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| Sap72 (GATA) _n | | | | | | | | |
| No. of alleles | 6 | 7 | 7 | 10 | 11 | 9 | 5 | 7.9 |
| Allele size | 169-189 | 173-197 | 165-193 | 165-201 | 165-209 | 169-201 | 157-173 | 157-201 |
| H _O | 0.75 | 1 | 0.63 | 0.85 | 0.89 | 0.93 | 0.79 | 0.83 |
| H _E | 0.78 | 0.81 | 0.74 | 0.83 | 0.88 | 0.85 | 0.66 | 0.79 |
| Sap94b (AG) _n | | | | | | | | |
| No. of alleles | 3 | 1 | 4 | 2 | 3 | 3 | 7 | 3.3 |
| Allele size | 108-112 | 108 | 100-112 | 106-108 | 106-112 | 106-112 | 108-134 | 100-134 |
| H _O | 0.5 | 0 | 0.1 | 0.1 | 0.11 | 0.43 | 0.82 | 0.29 |
| H _E | 0.53 | 0 | 0.1 | 0.1 | 0.11 | 0.48 | 0.81 | 0.30 |
| Mean <i>n</i> | 3.9 | 9.5 | 29.1 | 19.6 | 25.6 | 13.4 | 18.5 | 17.0 |
| Mean <i>n</i> of alleles | 4.6 | 5 | 6.4 | 6.4 | 6.9 | 5.9 | 6 | 5.9 |
| Mean H _O | 0.66 | 0.62 | 0.52 | 0.51 | 0.59 | 0.6 | 0.5 | 0.57 |
| Mean H _E | 0.65 | 0.56 | 0.55 | 0.55 | 0.59 | 0.62 | 0.51 | 0.57 |

Significant deviations in allele frequencies at individual loci were detected in any pairwise sample comparison except for the two Gulf St Vincent sites. The most significant deviations in allele frequencies were between the SA samples and the NSW and VIC samples, as well as between the NSW and VIC samples (Table 10.6). R_{ST} values for comparisons of SA samples to either VIC or NSW samples were significant at the 0.1% to 5% level. Comparison of allele frequencies among samples from SA showed that individual loci exhibited significant deviations, but R_{ST} values were not significant at the 5% level (Table 10.6).

Mantel's test showed a significant correlation between geographic distance and R_{ST} values, i.e. genetic distinctiveness, for comparisons among samples from South Australia and Victoria ($p = 0.000$) (Fig. 10.10). However, the linear relationship between geographic distance and genetic distinctiveness of populations did not hold when comparing WA or NSW samples with the SA and VIC samples. In particular, the comparison between the Victorian and NSW samples showed a far larger R_{ST} value (0.4972) than expected under isolation by distance (0.1) (Fig. 10.10). This result from microsatellite analysis is concordant with the highly significant differences in allozyme allele frequencies and the fixed difference at one microsatellite locus. Comparisons involving the sample from WA had generally smaller R_{ST} values than expected under the operation of isolation by distance.

Table 10.6 Results of exact tests of differences in microsatellite allele frequencies between all pairs of samples of *S. apama* (above diagonal) and pairwise estimates of R_{ST} with results of permutation testing of significant departures from zero (below diagonal). Abbreviations for loci: "1" Sap191, "2" Sap21, "3" Sap22a, "4" Sap57, "5" Sap65, "6" Sap69, "7" Sap72, "8" Sap94b; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ after Bonferroni correction.

| | PE/AL/EC (n=4) | SB (n=10) | SG1 (n=30) | GSV1 (n=20) | GSV2 (n=28) | MG/PP/ LE (n=15) | JB/WG/NC/CH (n=20) |
|-------------|-------------------|--------------|---------------|----------------|----------------|---------------------|--|
| PE/AL/EC | | 2** | 2***, 5** | 2*** | 1*, 2*** | 2** | 2*, 3**, 4*, 5***, 7***, 8*** |
| SB | 0.0382 | | 2***, 5* | 2***, 4*** | 2***, 6* | 2**, 4**, 8* | All loci *** |
| SG1 | 0.0475 | 0.0568 | | 4* | 5* | 4***, 5***, 8*** | All loci *** |
| GSV1 | -0.0152 | 0.0666 | 0.0404 | | NS | 5*, 8* | All loci but 6 ***, 6* |
| GSV2 | -0.0239 | 0.0283 | 0.0261 | 0.0023 | | 4*, 5*, 8* | All loci but 6 ***, 6* |
| MG/PP/LE | 0.079 | 0.1336* | 0.1097*** | 0.0732* | 0.1043*** | | 2***, 3**, 4***, 5***, 6**, 7***, 8*** |
| JB/WG/NC/CH | 0.3501* | 0.4185*** | 0.4425*** | 0.4224*** | 0.3996*** | 0.4972*** | |

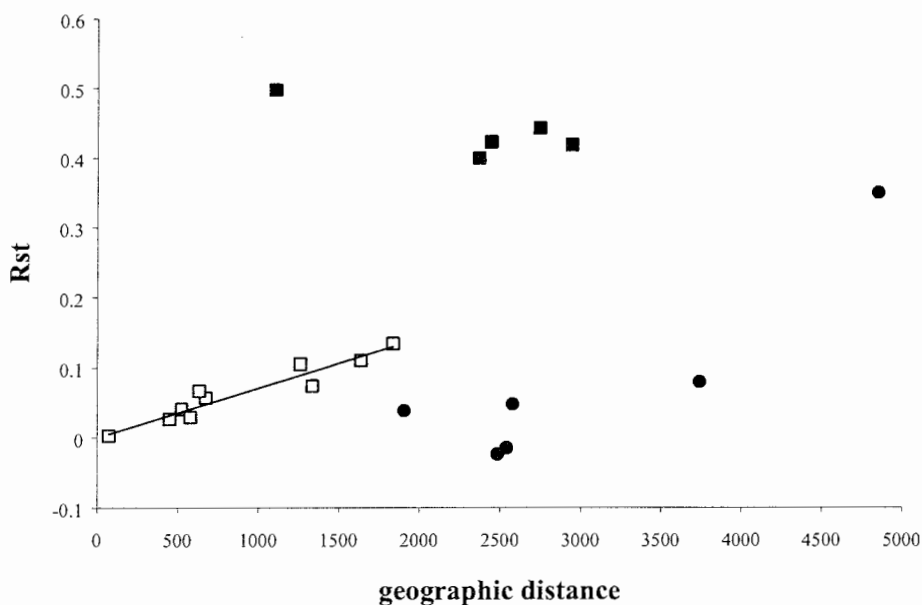


Figure 10.10 Scatterplot of R_{ST} values versus geographic distance for pairwise comparison of all samples. □ comparisons between two South Australian samples or a South Australian and a Victorian sample; ■ comparisons involving NSW samples; ● comparisons involving the WA sample.

10.5 Discussion

10.5.1 Broad-level divergence between eastern and southern populations

Anecdotal evidence suggested that animals from NSW and SA differed in colour pattern. This study determined that the pattern that was previously attributed to a difference between localities was better explained as a sexual dimorphism, where males exhibited stripes while females exhibited dots on the web between the third and fourth left arms.

Analysis of nine polymorphic allozyme loci resulted in the identification of three genetic groups that conformed to three spatially separated regions, i.e. WA, SA/VIC, and NSW. One allozyme locus (*Gda*) presented a fixed difference between animals from NSW and the rest of the range except for a single individual, i.e. a heterozygote in southern NSW. Heterozygotes carrying WA and SA/VIC alleles were also found. Thus, despite significant deviations in allozyme allele frequencies between three groups of *S. apama* (NSW, SA/VIC, WA), and in particular between NSW and the rest of the range, the presence of hybrids or heterozygotes suggests lack of reproductive isolation. Alternatively, NSW may represent a different taxon, which has maintained an ancestral polymorphism at locus *Gda*. However, the fact that the only heterozygote was found in southern NSW, i.e. near the SA/VIC group, favours the hypothesis that there is a low level of gene flow.

Results from eight microsatellite loci support the allozyme data with respect to the divergence between the NSW and the SA/VIC group. All but one locus presented significant deviations in allele frequencies for the comparison of NSW and VIC samples and one locus presented a fixed difference.

Morphometric analyses further corroborate the divergence between NSW and SA/VIC groups. Principal component analysis clearly distinguished between these two groups and classification success in discriminant function analysis was extremely high. Mitochondrial haplotypes formed two reciprocally monophyletic lineages whose geographic distribution did not overlap in the area sampled. However, mitochondrial sequence data did not support the nuclear and morphometric data with respect to the divergence between VIC and NSW populations, but rather placed a genetic break within NSW. The reasons for mitochondrial and nuclear discordance will be discussed further on. The two major mitochondrial lineages presented approximately 6% sequence divergence. Sequence divergence between other congeners in *Sepia* ranges between 10% and 15% (data from Bonnaud *et al.* (1994)). Thus, the level of sequence divergence does not indicate the presence of different species for *S. apama*.

In conclusion, mitochondrial, allozyme, microsatellite, and morphometric data indicate that the NSW population, or parts thereof, has a different evolutionary history from populations from the rest of the range. However, whichever dichotomy one considers, the two groups seem to occur allopatrically (at least there is no conclusive evidence for sympatry). It is difficult to prove the existence of allopatric species. Generally, there is lack of conclusive evidence that two allopatric populations have reached a divergence level where one can deduce that they are following separate evolutionary pathways (Wiley 1978). Thus, it is not clear if these populations represent two different species. Taking a conservative approach, the allopatric distribution of genetic differences is most parsimoniously interpreted as allopatric divergence rather than speciation. Therefore, the hypothesis that *S. apama* from across the range constitute a single species cannot be rejected. Further data from the potential contact zone, northern VIC to NSW, are needed. Specimens from WA also represent a separate genetic unit. However, WA specimens are closer to those from SA/VIC than are NSW specimens.

10.5.2 Origin of the genetic differentiation between eastern and southern populations

During the Pleistocene period Bass Strait was closed by an intermittent land bridge. The geographic locality of genetic and morphometric differentiation is consistent with the explanation that the land bridge across Bass Strait presented a historical barrier to gene flow and led to the divergence of populations. Comparative biogeography can be used to test hypotheses about the origins of a phylogeographic pattern (Avice 1996; Palumbi 1996). Several other species of marine organisms share a biogeographic pattern similar to that of *S. apama*: the Australian pilchard *Sardinops neopilchardus* (Dartnall 1974); the Australian salmon species *Arripis trutta* and *A. truttaceus* (Cappo *et al.* 2000); the limpets *Patelloida latistrigata submarmorata* and *P. l. latistrigata*; two closely related shore crabs *Paragrapsus laevis* and *P. gaimardii* (Poore 1994); and the gemfish *Rexea solandri* (Colgan and Paxton 1997). These provide just a few examples of marine organisms whose population structure or species distribution have been attributed to the closure and re-opening of Bass Strait during the last ice ages. The phylogeographic pattern of *S. apama* is concordant with the biogeography of these co-distributed species. It is, therefore, highly likely that the phylogeographic structure of *S. apama* is also the result of historical vicariance due to the closure of Bass Strait in the Pleistocene.

The data suggest that after subsequent sea level changes, the two previously isolated populations have come into secondary contact. The present data cannot exclude introgression of genes from the SA/VIC group into the NSW group after secondary contact. Tests for neutrality of sequence evolution for the mtDNA data showed that the southern lineage has the genetic signatures characteristic of selection, population expansion or both. The star phylogeny in the haplotype network demonstrated that there are

a lot more recent mutations than there are ancient mutations, indicated by the large negative value of the raggedness index r of the mismatch distribution. Thus, the results of the tests for neutrality of sequence evolution, the star phylogeny and the mismatch distributions suggest that the southern lineage is experiencing a range expansion and may be expanding north.

The fact that the southern mitochondrial lineage appears to be expanding north and that one heterozygote at an otherwise fixed allozyme locus was sampled in southern NSW, suggests a low level of gene flow between the VIC and NSW populations rather than reproductive isolation between these groups. Thus, the null hypothesis for future studies is that there is low level of gene flow in a secondary contact zone of two divergent populations of *S. apama* that diverged due to historical vicariance. As indicated above, further sampling from the contact zone is required to test this hypothesis.

10.5.3 Different resolution for nuclear and mitochondrial markers

Nuclear and mitochondrial data did not agree on the exact locality of the genetic break between NSW and SA/VIC populations. Different resolution for nuclear and mitochondrial markers in respect to the locality and even presence of a genetic break has been observed previously for several species, including *Drosophila simulans* (Ballard *et al.* 2002) and the American oyster *Crassostrea virginica* (Hare and Avise 1996; Hare *et al.* 1996; Hare and Avise 1998).

Hybrid zones have also provided valuable insights into the patterns of introgression of different genetic markers. Hybrid zone studies have shown that differences in mitochondrial DNA may shift away from the set of coincident electrophoretic, chromosomal and morphological differentiation, e.g. the mice *Mus musculus*/*M. domesticus* (Ferris *et al.* 1983), field crickets (Harrison *et al.* 1987; Harrison 1990), and the grasshopper *Caledia captiva* (Moran 1979; Moran *et al.* 1980). This difference has been attributed to the fact that, on average, mitochondrial genes are less closely linked to nuclear genes than nuclear genes are to each other and that, therefore, selection against deleterious alleles in the nuclear background affects mitochondrial haplotypes less severely. To flow past a cline or hybrid zone, a neutral allele must recombine into the new genetic background before it is eliminated by selection against the alleles with which it is initially associated (Barton 1979; Petry 1983). This process would be facilitated for mitochondrial markers as compared to nuclear markers. Thus, barriers to gene flow may be less strong for mitochondrial than for nuclear alleles and as a result mitochondrial alleles may flow across a barrier more rapidly than most nuclear alleles (Barton and Jones 1983).

In the case of *S. apama* mitochondrial haplotypes may have introgressed further than nuclear alleles because of: the smaller effective population size of the mitochondrial genome and thus accelerated

lineage sorting at the mitochondrial *COXIII* locus; the fact that the barrier to gene flow is weaker for the mitochondrial locus than for the nuclear loci analysed; or selection on the mitochondrial *COXIII* gene. The present data are consistent with selection acting on the *COXIII* gene. It cannot be excluded that introgression of mtDNA into NSW was driven and accelerated by selection. Even weakly selected alleles may flow past a barrier to gene flow with a negligible delay of only a few hundred generations, while neutral alleles would be delayed for tens of thousands of generations (Barton 1979).

10.5.4 Complex population structure and isolation by distance revealed by microsatellite markers

Analysis of eight microsatellite loci revealed a complex population structure for *S. apama*. Microsatellite analysis further corroborated the divergence between NSW and VIC/ SA populations. In addition, even within South Australia populations differed in allele frequencies at individual loci. Thus, South Australian specimens of *S. apama* do not represent one panmictic population. Overall, genetic divergence across South Australian and Victorian populations adheres to a model of isolation by distance.

Interestingly, the genetic population structure of *S. officinalis* across the Iberian Peninsula is similar to that of *S. apama* across southern Australia, i.e. significant deviations between samples as little as 300 km apart, isolation by distance, and large level divergence between Mediterranean and Atlantic samples possibly due to historical vicariance (Pérez-Losada *et al.* 2002). These high levels of population structure indicate that cuttlefish are either relatively poor dispersers or they return to their own natal sites to spawn.

10.5.5 Morphometrics illuminates stock structure

The results of PCA, which accounted for 94% of the total variance for both males and females, indicate that there was a clear differentiation among groups (high levels of between-group variability). Multivariate morphometrics using the information from exploratory PCA and DFA differentiated between six groups across the range of *S. apama*. These six groups also presented significant differences in microsatellite allele frequencies at individual loci.

The level of morphometric differentiation among groups was strikingly high. As different localities were sampled in different years (between 2000 and 2002), some morphometric differences may have related to temporal effects. Thus, further sampling across South Australia is needed to eliminate the confounding between spatial and temporal effects. However, because microsatellite and morphometric

groupings are concordant it seems probable that the morphometric groupings are caused by population structuring.

What is the origin of the high level of morphometric differentiation amongst sample localities? Cephalopods have extremely variable growth patterns, and in general water temperature and food regimes significantly affect their development and growth (Forsythe *et al.* 1994; Forsythe *et al.* 2001, Chapter 7). It is possible that these environmental factors explain the differences in morphometric proportions. Even though such a pattern may imply plastic growth more than genetic differentiation, it still indicates that animals from different localities belong to different stocks as they presumably have different life histories and growth patterns.

10.5.6 Management implications

Populations from WA, SA/VIC and NSW showed significant genetic differences. These populations warrant special conservation consideration due to their separate evolutionary histories and likely different evolutionary trajectory. These populations are best managed as evolutionary significant units (Moritz 1994), to preserve the genetic diversity found among them.

Multivariate morphometrics and microsatellite allele frequencies proved promising for the estimation of contemporary levels of gene flow and analysis of stock structure. Microsatellite and morphometric data revealed a complex population structure for *S. apama*. The hypothesis of a panmictic South Australian population has to be rejected. There seem to be at least three distinct populations or stocks: Streaky Bay, Spencer Gulf, and Gulf St Vincent. South-eastern SA (Mount Gambier) seems to be closer to the Victorian populations. The present study exemplifies the importance of employing an array of techniques and a multitude of genetic markers to exclude technique-specific or marker-specific effects and to gain a holistic understanding of the population structure of the species under consideration and the underlying processes.

11 General discussion

K.C. Hall and A.J. Fowler

The overall aim of this study was to provide a better understanding of the life history of *S. apama* and to relate this to the population dynamics of the exploited spawning aggregation at Point Lowly in the northern Spencer Gulf. This was the first detailed study of *S. apama* in southern Australian waters and also the first for a temperate Australian cuttlefish species. Although this provided an exciting opportunity to explore new ground, it meant that there was little empirical basis from which to formulate informed hypotheses and develop appropriate methodologies. Thus, many exploratory and descriptive techniques were used in the first instance, which were often adapted from studies on other northern hemisphere species of *Sepia*. Unfortunately, this ultimately resulted in some unsuccessful outcomes to experiments. Details of these experiments were still included in the report to demonstrate the logical succession of ideas used to address the aims of the study.

In this chapter we review the results of the study and discuss their implications for the life history of *S. apama* and the potential exploitation of the spawning aggregation in northern Spencer Gulf.

11.1 Overview of results

11.1.1 Abundance and biomass

Over 170,000 *S. apama* aggregated over shallow inshore reef at the aggregation area between May and August through each of the years of 1998 to 2001. Due to the small area of reef very high densities of up to 85 cuttlefish.100m⁻² were recorded, although density varied significantly throughout the area. The timing of the spawning aggregation was predictable, occurring within one or two weeks of the same start date each year. Numbers rapidly increased in early May, reached a peak by early June, and then gradually decreased until the end of August. Outside of this distinct aggregation period cuttlefish numbers in the area were very low (less than 1 cuttlefish.100m⁻²), clearly indicating that the cuttlefish were not residents but moved into the area from elsewhere. Individuals of both sexes stayed at the aggregation area for up to 6 weeks, suggesting that there was not a constant turnover of animals during the season. However, movement throughout the aggregation area was substantial and the results of the tagging study did not preclude the repeated movement of individuals in and out of the area.

The aggregation is unique for *S. apama* and indeed for *Sepia* species in general. No such aggregation of comparable density has been reported in the literature, although details of cuttlefish spawning populations in their natural environment are scarce. In general, cuttlefish are considered solitary animals only coming together towards the end of their life cycle to spawn (Hanlon and Messenger 1996). Most species concentrate in inshore waters or bays to spawn, but generally form pairs or small groups over quite extensive areas rather than dense spawning aggregations (Corner and Moore 1980; Hanlon and Messenger 1988; Gutsal 1989; Norman 2000). Recent laboratory studies of sexually mature *S. officinalis* suggest that individuals maximise the distance between conspecifics even while spawning (Boal et al. 1999; Adamo et al. 2000).

On the other hand, many squid species are more social and often form shoals or dense spawning aggregations (Hanlon and Messenger 1998). The densities of *S. apama* at the aggregation area in northern Spencer Gulf are more similar to those reported for dense spawning aggregations of coastal Loliginid squids (Hanlon 1998) than other *Sepia* species. *Loligo* species tend to form very dense aggregations that range in numbers from tens to thousands, concentrated in small distinct areas of around 20 to 50 m² that contain one large or several smaller communal egg beds (McGowan 1954; Griswold and Prezioso 1981; Jefferts et al. 1987; Sauer et al. 1992; Segawa et al. 1993; Sauer 1995). These localised concentrations usually have a patchy distribution over large areas of inshore seabed and their precise location may vary from year to year.

The winter spawning period of *S. apama* was also unusual for cuttlefish. Most *Sepia* species spawn during spring or early summer, a strategy that results in hatching coinciding with the best growing conditions over summer (Mangold 1987). The unusual winter spawning season for *S. apama* may relate to its large size. *S. apama* is the largest cuttlefish species in the world and produces some of the largest known molluscan eggs, which require a long development time. A slow winter development may have evolved as an alternative strategy that still ensures the hatchlings emerge at the start of spring to coincide with optimal conditions for juvenile growth.

11.1.2 Sex and size composition

The highly male-biased sex ratio with an average of five males per female, was another peculiar feature of the spawning aggregation. In contrast, the sex ratios of samples collected from the wider Gulf were close to unity. This suggests the biased sex ratios of the spawning population arise subsequent to initial sexual differentiation at birth but the mechanism responsible remains unresolved. The biased sex ratios had serious implications for the estimates of spawning biomass, as only 16-25% of the total spawning

biomass consisted of reproductive females that contributed to egg production, and suggested a strong gradient for sexual selection.

No comparable sex ratios have been reported for any other cephalopod spawning aggregations. The sex ratios reported for *Loligo* aggregations are generally male-biased, but tend to be only between 1:1 to 3:1 males per female (Augustyn 1990; Hanlon 1998; Hanlon et. al. in press). Some highly male-biased sex ratios (up to 12M:1F) have been reported for *L. vulgaris reynaudii* in trawl surveys off the coast of South Africa, but these were generally in deeper offshore waters (Augustyn 1991), and probably related to the sex-segregation of offshore squid shoals rather than spawning dynamics. Sex ratios quoted for other *Sepia* species are generally from catch data as opposed to sampling in specific spawning locations and are close to unity in most instances or have even been biased toward females (e.g. Silas et al. 1985b).

The broad size distribution of males in both the aggregation area and the NSG population suggested the presence of multiple age classes. In comparison, females had a much narrower size distribution with only one distinct size mode. For both sexes there were more large individuals at the start of the spawning season than toward the end. The existence of multiple size classes within a single population has been described for many other cephalopod species. Based on analysis of statoliths, these have often been interpreted for some squid species as multiple "micro-cohorts" within a single year class resulting from different hatch dates and/or plasticity in growth rates, rather than multiple year classes (e.g. Hatfield 1996; Brodziak and Macy 1996). Alternatively, multiple size classes for cuttlefish species have usually been interpreted as different year classes (e.g. Boletzky 1983). Clarification of time scales using independent age estimation techniques has been slower for cuttlefish species due to the poor resolution of growth increments in the statoliths (Bettencourt and Guerra 2000).

11.1.3 Age estimation

The microstructure of statoliths of *S. apama* could not be reliably interpreted. Thus, an alternative ageing technique based on the internal microstructure of the cuttlebones was developed. Results suggested the presence of two year classes for both sexes in the aggregation population, and that size was related to age. The different patterns in the juvenile sections of the bones suggested two alternative life cycles: the first was characterised by juveniles that grew slowly in their first year, delayed maturity and did not return to the aggregation area to spawn until they were much larger and in their second year; whereas, in the second life cycle juveniles grew rapidly during the first summer after hatching and returned to spawn in the following winter at 6 to 7 months old, as the smaller size class.

Individuals representing both life cycles, regardless of the age at spawning, only participated in one reproductive season. None returned in the following year.

The interpretation of the microstructure of the cuttlebone relied on the assumption that growth increments of different widths were deposited during periods of different growth rates, as determined by environmental conditions such as temperature and food availability. The ages assigned to individuals assumed that faster growth rates were achieved during summer. Evidence to support these assumptions was provided by juvenile growth experiments in aquaria in which temperature and feeding regimes were manipulated.

11.1.4 Egg development and juvenile growth

Egg deposition in the aggregation area occurred throughout the spawning season from May to August, during which time water temperatures varied considerably. The time taken for eggs to develop in aquaria and the field varied depending on the date of deposition, with those laid later in the season taking only 2 months compared to 5 months, due to increasing water temperatures. Hatching began in late September and was completed by early November. During this period, water temperature increased dramatically such that the later hatchlings experienced much higher temperatures at hatch than those that hatched earlier in the season.

Aquarium experiments in which juveniles were subjected to different regimes of water temperature and food availability resulted in large differences in growth rate. These results verified the plasticity of growth under different environmental conditions that could be manifested as the two hypothesised life cycle types. Such different life history traits, as a consequence of environmental variation, have been proposed for many cephalopod species (Forsythe 1993), but few studies have demonstrated the phenomenon in the wild (Perez and O'Dor 1998).

11.1.5 Reproductive biology

All cuttlefish at the aggregation area were sexually mature irrespective of size or sex, whereas most captured in NSG away from the aggregation area were immature or maturing and were also actively feeding. This verified the existence of a discrete winter spawning period and the probable absence of spawning at other times of the year. There was a significant decrease in both reproductive and somatic condition over the course of the spawning season, consistent with a semelparous reproductive strategy for both life cycle types. An intermittent terminal spawning mode was proposed, similar to that described for *S. officinalis* (Boletzky 1987).

Representatives of the different life cycle types varied in their “reproductive quality”. Larger and older individuals of both sexes had larger gonads and gametes than smaller, younger individuals, although the gonad weight relative to total body weight was greater in smaller individuals of both sexes. This suggests that by delaying maturity, the potential reproductive output of an individual is increased such that any loss in fitness resulting from increased risk of mortality may be offset by an increase in fecundity related to larger size.

11.1.6 Mating system

A complex mating system was evident at the aggregation area. The highly male-biased sex ratio resulted in strong intra-sexual competition for access to females. Males of different sizes representing the different year classes, used different suites of behavioural tactics to achieve matings. Large males were more successful at defending females, whereas small males searched for opportunities for "sneak" mating attempts. Both size groups achieved successful matings with these tactics; however, fertilisation success may differ from mating success due to sperm competition and female cryptic choice mechanisms operating after copulation. Preliminary results from the paternity analysis of eggs and adult tissues collected from the field suggest that sperm from both small and large males successfully fertilise eggs (Naud et al. unpub. data). No consistent differences in reproductive behaviour between females of different sizes were noted, although only one size mode of females was evident. Females displayed behaviours that related to direct and indirect mate choice, which ultimately determined which males successfully mated. No clear pattern to female choice was evident.

There was no assortative mating with respect to size, i.e. small and large males mated with the full size range of females and vice versa. Although mean size decreased during the spawning season, some individuals of both size classes were present simultaneously throughout most of the spawning season. Thus, there was significant interbreeding between the two year classes and it is unlikely that the two life cycle types were reproductively isolated.

Similar mating systems have been described for the dense spawning aggregations of some *Loligo* squid species, where males of two distinct size classes co-occur and compete for access to females using different behavioural tactics (Hanlon 1996; Hanlon 1998; Sauer et al. 1997). In such systems, however the different sized males have generally been considered to be of similar age, with the bimodality in size arising from plasticity in growth rates rather than distinct differences in age. Here the largest males were older and had delayed maturity to spawn in their second year, possibly as an alternative reproductive tactic (discussed in more detail below).

11.1.7 Population structure based on molecular and morphological analyses

The population structure and species status of *S. apama* across its geographical distribution was assessed using data from allozyme electrophoresis, microsatellite loci, nucleotide sequences of the mitochondrial *COXIII* gene, multivariate morphometrics and colour patterns. Analyses of allozyme and microsatellite allele frequencies revealed two very divergent but geographically separated populations consisting of specimens from the east coast and southern Australia. However, the presence of a heterozygote in a putative contact zone between the east coast and southern Australia suggested that these populations were not reproductively isolated. Mitochondrial haplotypes seem to have introgressed further north into the contact zone than have nuclear alleles. Differences in colour patterns that were anecdotally associated with populations from different geographic regions were, in fact, correlated with sexual dimorphism. These data are most consistent with the hypothesis that populations were geographically isolated in the past (historical vicariance) and which have come into secondary contact. Comparison of microsatellite allele frequencies among four South Australian samples indicated significant deviations from panmixia. The South Australian samples were also reliably diagnosed by means of multivariate morphometrics.

11.2 Life history of *S. apama*

The life history traits of an individual are thought to vary under the influences of natural and sexual selection towards optimising combinations or strategies, that maximise the fitness of an individual in a particular environment (Stearns 1976), where fitness is considered to be the genetic contribution of an individual to the gene pool of the next generation (Daan and Tinbergen 1997). Since there is usually a finite quantity of resources (time and energy) available for distribution between traits, an increase in the value of one trait will often be at the expense of another (Lessells 1997). Thus the life history strategy can be considered the optimal outcome of many trade-offs between different traits within the constraints imposed by the developmental, physiological and morphological limitations of the species to maximise fitness (Calow 1987; Kozłowski 1992). In theory, there should be one optimal strategy in any environment for a given species, but for many different taxa there are cases of intraspecific diversity in life history strategies (Stearns 1977; Sibly and Calow 1983).

Two principal mechanisms have been proposed to account for this intraspecific diversity in life history traits: (1) genetic determination, via life history traits that are heritable and show genetic variation, such that two life history strategies with equal fitness and distinctive genotypes evolve and become evolutionarily stable through frequency-dependent disruptive selection; or (2) environmental

determination, via phenotypic plasticity in the life history traits expressed from a single genotype in response to variation in environmental conditions (Gross 1996). Nevertheless, it can be difficult to separate the influences of each factor due to the complex relationship between genetics, development, environment and evolution on phenotypic expression (Fig. 11.1). For example, traits that may appear discontinuous and under genetic determination may have an underlying continuous distribution of genetic effects but an environmental threshold for phenotypic expression that results in two distinctive phenotypes (Scheiner 1993).

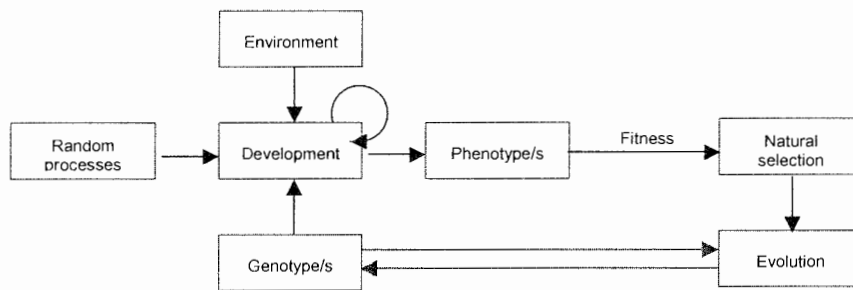


Figure 11.1 Schematic diagram of the relationship between genetics, development, environment and evolution. Reproduced from Scheiner 1993.

The results of this study suggest the existence of two life cycle types for both sexes of *S. apama*. The first is characterised by a short time to first reproduction, a small size at maturity and lower potential fecundity. The second has a longer time to first reproduction, a larger size at maturity and higher potential fecundity. Presumably, the survival rate to reproduction of individuals that adopt the first life cycle would be higher than those that adopt the second, because of the shorter time required to reach reproduction (Lessells 1997). The two types differed in their growth patterns during the juvenile stage, as evident in the incremental pattern in the juvenile section of the cuttlebones. This difference in growth could be controlled genetically or result from phenotypic plasticity in response to different environmental conditions as a result of variation in hatch dates, or as a combination of both factors. The plausibility of these and other mechanisms that may contribute to the existence of the two life cycles of *S. apama* is discussed below in relation to the results of this study.

(1) Genetic determination of life cycle type

If determination of life cycle was under genetic control, the hatchlings of large females should show slow initial growth, irrespective of hatch date and environmental conditions at that time. Therefore, from the experiments done as part of this study that manipulated temperature and food levels,

significant differences in growth rates of juveniles derived from eggs of different aged females would be expected regardless of treatment. Such a result was not obtained. Rather, no significant differences were found between juveniles from the two maternal groups, other than those that related to the initial variation in egg weight and hatchling size. Also, food regime and temperature had a much greater influence on juvenile growth rate than initial hatchling size. It should be recognised, however, that the experiments only covered the initial phase of juvenile growth and hence would not reflect any genetic differences that would be manifested later in life, such as different genetically controlled threshold criteria for life history traits. Furthermore, the study did not consider genetic variation between paternal groups.

Age at maturity for some salmon species is at least partly under genetic control, as parental age at maturity strongly influenced the age at maturity of the progeny in breeding experiments (Hankin et al. 1993). Many salmon species display alternative intraspecific life history strategies, which often serve as alternative reproductive tactics (Gross 1985; Bohlin et al. 1990; Fleming 1996; Parker et al. 2001). There are some similarities with the life history traits of *S. apama* determined in this study. Some male salmon mature at a much younger age and smaller size, whilst others delay maturity until larger (Bohlin et al. 1990). Also, the different-sized male salmon use different tactics to gain access to females (Gross 1985). The two tactics are more successful when rare in the population, thus fitness is frequency-dependent and theoretically no single tactic is evolutionary stable, resulting in evolution of a mixture of different strategies that maximise fitness.

The two life cycle types of *S. apama* may have similarly evolved under frequency-dependent selection as alternative reproductive tactics. The different age classes differed in their potential reproductive output and there appeared to be a competitive advantage of being of large size for dominating access to females through aggression. Therefore, the potential losses in fitness caused by delaying maturation and increasing the chances of mortality before reproduction might be countered by an increase in potential reproductive output through increased access to females. Furthermore, the biased sex ratio and the high level of sperm competition and mate choice behaviour for the mating system suggest that sexual selection pressures on the spawning population are high. Thus, it is plausible that the different life history strategies of *S. apama* have evolved in response to sexual selection pressure as alternative reproductive tactics. However, this explanation does not account for why females also showed two different life cycles as no consistent differences in reproductive behaviour between different sized females were noted.

(2) Environmental determination, fixed according to conditions at time of hatching

The conventional view of the effect of temperature on the time to sexual maturation and life span is that low temperatures at hatching produce slow growth, preventing the attainment of sexual maturity in the first spawning season, which is delayed until a larger size is attained (Berrigan and Charnov 1994). This results in an extended life span. In contrast, high temperatures at hatching accelerate growth allowing maturity to be reached earlier at a smaller size, thus resulting in a shorter life span. Due to the unusual winter spawning period of *S. apama*, eggs develop through the coldest months of winter, such that the first hatchlings experience relatively low temperatures compared to those that hatch later. These early hatchlings also presumably experience the later warmer temperatures of spring and summer at a larger size than later hatchlings. Therefore, this mechanism implies that early hatchlings that have low early growth rates are not able to increase these later in summer when conditions are more favourable.

(3) Different migration patterns, genetically or environmentally controlled

An alternative explanation is that early hatchlings may migrate into more southerly cool waters after hatching and hence do not experience the warm summer waters in the northern Gulf that the later hatchlings experience. The different life histories of some salmon species are often associated with different migration patterns (Parker et al. 2001). Males that return to spawn in the following season as small spawners often remain in the freshwater stream where they hatched, whereas those that return in later seasons as large individuals, migrate to oceanic waters after hatching. This niche shift is associated with better food resources available in the open ocean (Noakes et al. 1989). Similarly, Type 2 cuttlefish may remain and grow in the northern Gulf region in close proximity to the aggregation area, whereas those with Type 1 bones may migrate further away. Migration early in the life cycle might also account for the slower growth of the first part of Type 1 bones compared to Type 2 bones.

Evidence in support of this hypothesis is found in the relative frequency of different bone types in the NSG samples. Type 2 individuals far outnumbered Type 1 and Type 3 individuals, but the proportions of each were relatively equal in the aggregation area in May. A low proportion of Type 3 individuals might be expected due to the longer time for which they are vulnerable to natural mortality. But the fact that less Type 1 bones were also noted in the population in February and April, suggests that either less Type 1/Type 3 individuals are produced at the time of hatching, or that not all remain in the trawlable areas of the Gulf during the summer months. Thus, it is possible that Type1/Type 3

individuals do migrate out of the NSG area, in response to either genetic programming or environmental gradients or a combination of both, and that this may ultimately result in the two life cycle types proposed. However, it should be noted that selective sampling could account for the relative abundances of the different types. Type 1 individuals were smaller than Type 2 individuals and if they were the late hatchers only the largest ones would have been susceptible to trawling by February and April. Video analysis of the response of *S. pharaonis* to bottom trawls suggested larger individuals either swam in front of the trawl gear or actively tried to avoid capture (Gustal 1989). Thus, it is possible that the large Type 3 individuals were more effective at avoiding the trawl than the other two smaller types.

(4) Environmental determination, according to conditions at a cut-off point

Van Heukelum (1979) proposed that cephalopod life cycles involve a pre-programmed sequence of stages, with the duration of each stage determined by prevailing environmental conditions such as light, temperature and nutrition, and size rather than chronological age. Results from rearing experiments using juvenile *Sepioteuthis lessoniana* supported this theory, as the duration of the juvenile growth phase and subsequent timing of the onset of sexual maturation related to size rather than age (Forsythe et al. 2001). In addition, Durchon and Richard (1967; cited in Van Heukelum 1979) provided evidence that the onset of sexual maturation of *S. officinalis* was stimulated by light through the day length regime, via hormones produced by the optic glands, and that the gonad tissue had to be developed to some minimal level of competence before it would fully respond to the optic gland hormone and continue maturation. Thus, if gonad development had not reached this minimum level by a certain date, maturation would not commence and somatic growth would continue until the same conditions of day length regime were encountered again in the following year (Durchon and Richard, as cited in Van Heukelum 1979).

The seasonal spawning period of *S. apama* suggests that sexual maturation may be under environmental control according to a similar mechanism to that proposed above. Given the dichotomous rather than continuous nature of the two life cycle types, a cut-off mechanism appears to be the most likely mechanism of life cycle determination. But which individuals would be unable to reach the cut-off point and are destined to become the two year old age class? There are two possibilities: (1) the late hatchers which initially experience high temperatures and fast growth at the time of hatching but have less time to reach a certain size before the cut-off point; or (2) the early hatchers which initially experience low temperatures and growth rates after hatching and presumably never achieve sufficient growth to meet the cut-off point.

The first alternative would potentially result in alternating generations, as has been proposed for *S. officinalis* (Boletzky 1983), in which the large females are first to spawn in the season, lay large eggs which produce larger offspring that hatch first and consequently have more time to grow through summer and reach maturity in time for the next spawning season. Hence, the offspring from large females become the small adults in the following year (Boletzky 1983). The second alternative would require that the eggs of small females hatch later in the season, achieve exceptionally fast growth rates after hatching and reach the first spawning season where they spawn again as the small individuals. This latter alternative, however, once again begs the question as to why the early hatchlings, although experiencing early slow growth after hatching don't increase in growth rate later in the spring when more favourable growth conditions occur?

Clearly, further research is required to identify the mechanisms that determine life cycle type for *S. apama*. However, this study should provide a foundation from which to formulate appropriate questions and to generate hypotheses for further studies. The species should serve as an ideal candidate for studies that test models about life history determination.

11.3 Implications for exploitation and management

Many fisheries biology models have been developed for relatively long-lived finfish species, whose populations are comprised of multiple age classes and where individuals participate in multiple reproductive events during their lifetime (Fig. 11.2a). The models presuppose a carry-over of spawning biomass from one year to the next, which provides a reserve of reproductive potential and genetic variability (O'Dor 1998). In contrast, many cephalopod species have very short life spans, often less than one year, and individuals experience only one reproductive period at the end of their lives. Thus, there is no accumulation of spawning biomass from one generation to the next, and little buffer against years of poor recruitment or overfishing (Fig. 11.2b) (O'Dor 1998). From the results of this study, it appears *S. apama* is characterised by two life cycles that have two different life spans, one annual and one biennial, but that both still only have one reproductive event at the end of their lifetime (Fig. 11.2c). Therefore, although there is no spawning biomass carried over from one year to the next, the recruits from any single year are split, with some spawning in the next year, and the remainder in the following year (Fig. 11.2c). The advantage of such a life history is that the population should be more robust to isolated years of poor recruitment that result from adverse environmental conditions, in comparison with a strictly annual population. As such, the two life cycle types may serve as a risk-spreading strategy for survival in an unpredictable environment by spreading breeding effort over two

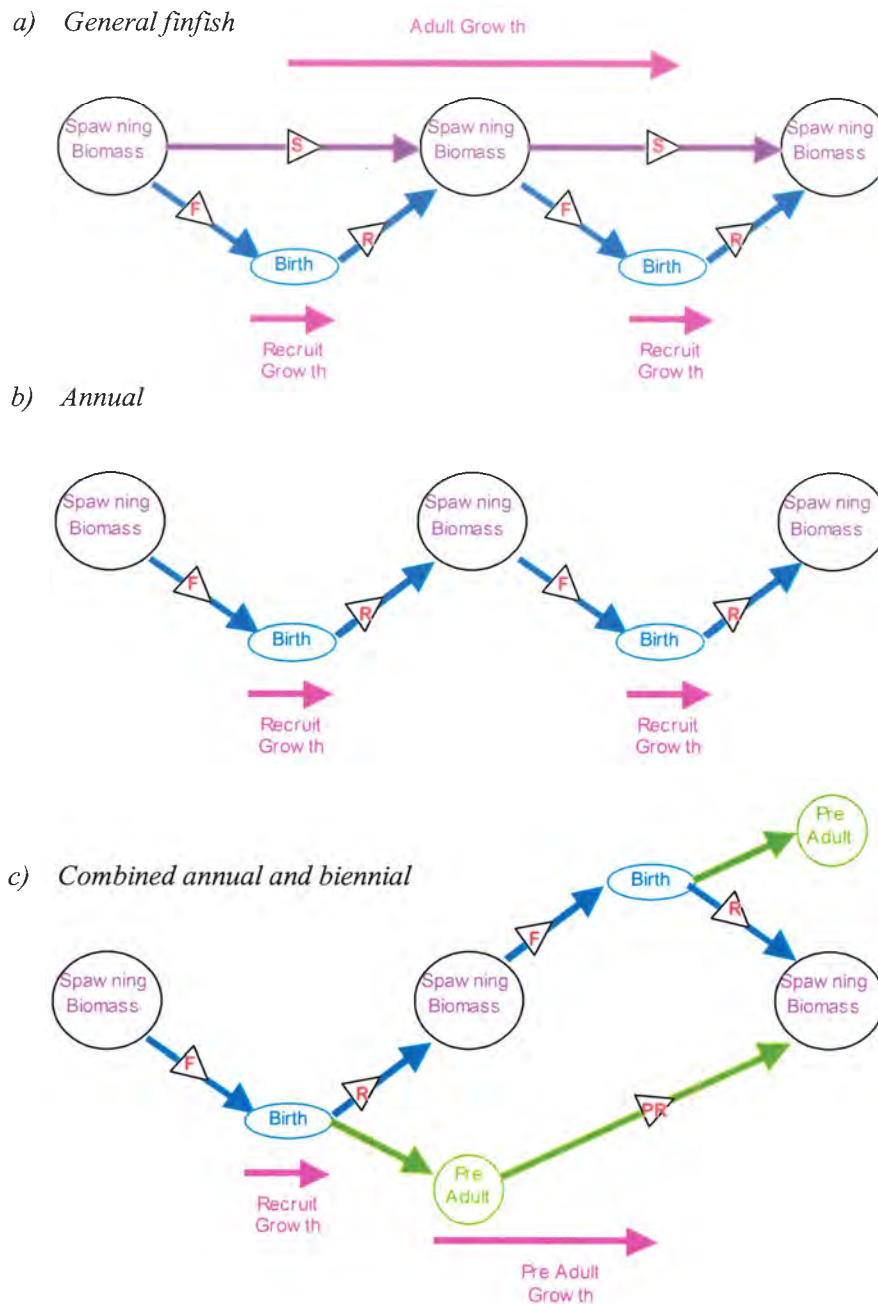


Figure 11.2 Schematic representation of different life history strategies. (a) A general long-lived finfish species with a proportion of the adult population surviving from one spawning season to the next (as indicated by solid line between spawning biomass circles); (b) an annual species with all adults dying following spawning and the spawning biomass in the following year is completely reliant upon recruitment from reproduction; and (c) the hypothesised alternative life cycles of *S. apama*, a combination of annual and biennial life cycles. S = proportion of spawning biomass surviving, F = fecundity of spawning biomass, R = proportion of eggs surviving to maturity, PR = proportion of eggs surviving to maturity that undergo biennial life cycle.

years instead of one, whilst still ensuring that populations are flexible enough to take advantage of interannual fluctuations in environmental conditions.

This might be taken to suggest that the spawning aggregation should be more robust to exploitation than a strictly annual cycle. However, the apparent reduction in spawning biomass between 1999 and 2001 suggests that the intense levels of fishing effort between 1996 and 1998 were not sustainable over the long-term. Certainly the population is vulnerable to within-season declines as in 1998 just 32 days of unrestricted fishing accounted for approximately 50% of the estimated biomass in the area, and in the following years after the fishing closure, the biomass in the previously fished areas increased by 150%. Clearly, any future exploitation of the aggregation would need to be carefully managed. However, given the uncertainty surrounding our understanding of many important life history and population parameters, it would be wise to maintain the current precautionary approach toward exploitation and management of the spawning aggregation until further research can resolve some of the outstanding issues.

To ensure the sustainable management of *S. apama* in South Australia and in particular the spawning aggregation in the northern Spencer Gulf, future research and management decisions should aim to address the following issues:

- (1) to determine the level of spawning biomass that is required to reproduce each year to sustain the population biomass over time. This would require a detailed knowledge of stock-recruitment relationships and other population and life history parameters that currently remain unresolved. Special consideration should be given to the extremely biased sex ratios of the spawning population and efforts made to elucidate the mechanisms responsible for them, as these may strongly influence the potential egg production levels of the population;
- (2) to account for the variation in the composition of the spawning population at the aggregation area throughout the season, the uneven distribution of cuttlefish in the area and the movement of cuttlefish within the aggregation area, when introducing any future time or area closures to protect spawning biomass;
- (3) to ensure that all components of the complex mating system are adequately protected to avoid imposing artificial sexual selection on the population through fishing activities (Hanlon 1998; Rodhouse et al. 1998);
- (4) to address the fundamental gap in our knowledge relating to the stock structure of *S. apama* populations in Spencer Gulf. The population structure study based on a diversity of techniques

provided evidence for a complex population structure for *S. apama* within South Australia. Multivariate morphometrics and microsatellite allele frequency analysis suggested there were at least four distinct populations in South Australia: Spencer Gulf, Gulf St Vincent, west coast (Streaky Bay) and south-east (Mt Gambier). Sample coverage in the study was low and more comprehensive sampling within Spencer Gulf will likely reveal even finer levels of stock structure;

- (5) to determine the level of gene flow between the aggregation population and others in Spencer Gulf is of particular importance. The unique mating system and exceptional density at the aggregation area suggests that this may be a separate population for management purposes. If cuttlefish “homed” to their natal spawning sites, different spawning populations may be reproductively isolated (e.g. Fig. 11.3). Therefore, overexploitation of individual populations would result in local extinctions. The movement and migration patterns of adults and juveniles outside of the aggregation area and the level of mixing of populations within the Gulf remain unknown. One possible method to resolve these important questions regarding fine scale stock structure and movement patterns within Spencer Gulf is the microchemical analysis of the pre-hatch and adult portions of either the cuttlebone or statolith (Campana 1999);

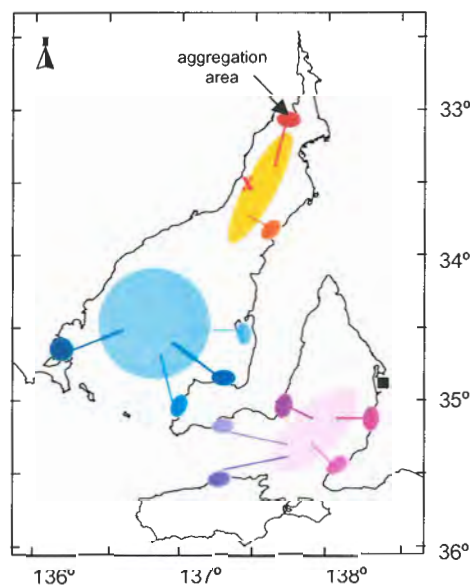


Figure 11.3 Hypothetical population structure of *S. apama* in the SA gulf system if individuals homed to natal spawning sites, resulting in mixed feeding but separate spawning populations. The large ovals represent possible areas of mixing of individuals from different spawning locations during non-spawning times. The small ovals represent areas where anecdotal evidence suggests *S. apama* spawning occurs. The red "x" marks the release site of the tagged cuttlefish recaptured at the aggregation area during the 2000-spawning season.

- (6) to determine the extent of dependence of other *S. apama* populations in the Gulf or around the State on recruitment from the aggregation area. Given the large size of the spawning aggregation and the low numbers of spawning cuttlefish reported elsewhere around the State, the aggregation is clearly an area of high reproductive output for the species and a potential source of recruitment for other areas;
- (7) to consider the considerable scientific and cultural value of the spawning aggregation in an unexploited condition. The unique nature of the spawning aggregation - high densities, complex mating system, multiple life history strategies within a single species and habitat, and its accessible location for field studies – make it particularly attractive as an eco-tourism resource and for scientific research;
- (8) to investigate the potentially important role of the spawning aggregation in the trophodynamics of the northern Spencer Gulf ecosystem. *S. apama* is preyed upon by many marine mammals, in particular dolphins, and also sea birds and large fish species such as snapper (T. Bramley pers. comm. 2000; Gales et al. 1993; Anonymous 1993) but the role of the aggregation in the local food chain is still unknown.

The study documented in this report has revealed numerous unusual features of the spawning aggregation and life history of *S. apama* in northern Spencer Gulf. The results provide a good contrast to the biology of *S. officinalis*, a northern hemisphere species that has been considered in detail (Boletzky 1983; Le Goff and Daguzan 1991). The study revealed that many life history traits were similar between the two species, including the complex nature of the multiple life cycle types evident within a single population. Although the evolutionary basis of this complexity can only be speculated upon, it appears to be a bet-hedging strategy against years of poor recruitment. Nevertheless, because of the short life span of cuttlefish, the strategy is only effective for a single year. In this way cuttlefish differ from squid, that tend to use other strategies such as prolonged or year-round spawning and migration, to minimise vulnerability associated with their annual life cycle (O'Dor 1998).

12 Benefits and Staff

A.J. Fowler

12.1 Benefits

This was the first comprehensive study of the fishery and biology of the giant Australian cuttlefish *Sepia apama*. The study has made a significant contribution to understanding the population biology and life-history of this species, particularly with respect to the spawning aggregation that occurs annually in northern Spencer Gulf of South Australia. The specific contributions include: annual estimates of abundance and biomass of the aggregation; an understanding of the population structure in terms of sex ratio, and size and age structures; and aspects of the reproductive behaviour and potential. Furthermore, the study indicated the possibility of a relatively fine scale of stock structure, indicating separated regional populations. The regular sampling revealed a considerable reduction in biomass through the course of the study between 1998 and 2001, which may be a consequence of the high fishery catches taken through 1996, 1997 and early 1998.

It was largely on the basis of the findings of this study that fishing on the aggregation was partly and then completely closed to fishing in 1998, and has remained closed since. Thus, the project findings provided substantial assistance to the fishery managers and community representatives who were concerned about the long-term effects of the intensive, localised fishing on the population once the cuttlefish were densely aggregated for spawning. Clearly, this management outcome cannot be considered immediately beneficial for the commercial and recreational fisheries for the species in northern Spencer Gulf. Nevertheless, it is likely that given the rate at which commercial fishing effort was growing each year through the 1990's, that an unregulated fishery would not have been sustainable for long. The closing of the fishery has quite likely prevented the collapse of the population through over-fishing, which presents several benefits:

- (1) the closure of the fishery is reconsidered each year, thus providing the opportunity for the fishery to be reopened if a regulated fishery management regime can be developed that would allow some catch without threatening the sustainability of the resource;
- (2) since the cuttlefish spawning aggregation has now become a popular scuba dive event that is recognised both nationally and internationally, the community of Whyalla benefits through the attraction of tourists to the region;

- (3) as the spawning aggregation is the only one known for any cuttlefish species in the world it has considerable scientific and natural history interest. As such scientists, photographers and film makers are attracted to work on the aggregation from around the world;
- (4) the ecosystem of northern Spencer Gulf must benefit from the closure of the fishery since *S. apama* constitutes an important component of the trophic system of the region, being eaten by snapper, dolphins and seabirds.

The above factors must be considered when the question about re-opening the fishery is considered by fishery and natural resource managers. The results of this study, as presented in this report, constitute the basis of any such discussions.

12.2 Staff

| | |
|--|---|
| Dr Anthony Fowler (SARDI) | Principal Investigator, PhD & Honours supervisor |
| Karina Hall (FRDC / SARDI / Adelaide Uni) | PhD student |
| Karin Kassahn (Adelaide Uni / SA Museum) | Honours student |
| Dr Stephen Donnellan (SA Museum) | Honours supervisor |
| Ass. Professor Michael Geddes (Adelaide Uni) | PhD supervisor |
| David McGlennon (SARDI) | original Principal Investigator |
| Val Boxall | technical support |
| David Fleer (SARDI) | technical support |
| Bruce Jackson (SARDI) | technical support |
| Paul Jennings (SARDI) | technical support |
| David Short (SARDI) | technical support |
| Sangeeta Taylor (FRDC) | technical support |
| Sonia Venema (FRDC) | technical support |

References

- Adamo S.A. and Hanlon R.T. (1996). Do cuttlefish (Cephalopoda) signal their intentions to conspecifics during agonistic encounters? *Anim. Behav.* 52: 73-81.
- Adamo S.A., Brown W.M., King A.J., Mather D.L., Mather J.A., Shoemaker K.L. and Wood J.B. (2000). Agonistic and reproductive behaviours of the cuttlefish *Sepia officinalis* in a semi-natural environment. *J. Molluscan Stud.* 66: 417-419.
- Alcock J. (1998). *Animal behaviour: an evolutionary approach*. Sinauer Associates Inc., Sunderland, M.A., U.S.A.
- Andersson M. (1994). *Sexual selection*. Princeton University Press, Princeton, N.J., U.S.A.
- Andersson M. and Iwasa Y. (1996). Sexual selection. *Trends Ecol. Evol.* 11: 53-58.
- Andrew N.L. and Mapstone B.D. (1987). Sampling and the description of spatial pattern in marine ecology. *Oceanogr. Mar. Biol. Ann. Rev.* 25: 39-90.
- Andrewartha H.G. and Birch L.C. (1954). *The distribution and abundance of animals*. The University of Chicago Press, Chicago, U.S.A., 782pp.
- Andrewartha H.G. and Birch L.C. (1984). *The ecological web: more on the distribution and abundance of animals*. The University of Chicago Press, Chicago, U.S.A., 506pp.
- Anonymous (2001). *Fisheries Victoria commercial fish production information bulletin 2001*. Marine and Freshwater Resource Institute, Queenscliff, Vic., 29pp.
- Anthony L.L. and Blumstein D.T. (2000). Integrating behaviour into wildlife conservation: the multiple ways that behaviour can reduce N_e . *Biol. Conserv.* 95: 303-315.
- Aoyama T. and Nguyen T. (1989). Stock assessment of cuttlefish (*Sepia pharaonis*) off the coast of the People's Democratic Republic of Yemen. *J. Shimonoseki Univ. Fish.* 37: 61-112.
- Arkipkin A.I. (1991). Methods for cephalopods age and growth studies with emphasis on statolith ageing techniques. In: Jereb P., Ragonese S. and Boletzky S.v. (eds). *Squid age determination using statoliths*. Proceedings of the International Workshop held in the Istituto di Tecnologia della Pesca e del Pescato. NTR - ITPP Spec. Publ. 1, pp. 11-18.
- Arnold J.M. and Williams-Arnold L.D. (1977). Cephalopoda: Decapoda. In: Giese A.C. and Pearse J.S. (eds). *Reproduction of marine invertebrates*, Vol. IV. Molluscs: Gastropods and cephalopods. Academic Press, London, U.K., pp. 243-290.
- Augustyn C.J. (1990). Biological studies on the chokker squid *Loligo vulgaris reynaudii* (Cephalopoda: Myopsida) on spawning grounds off the south-east coast of South Africa. *S. Afr. J. mar. Sci.* 9: 11-26.
- Augustyn C.J. (1991). The biomass and ecology of chokka squid *Loligo vulgaris reynaudii* off the west coast of South Africa. *S. Afr. J. Zool.* 26: 164-181.

- Augustyn C.J., Roel B.A. and Cochrane K.L. (1993). Stock assessment in the Chokka squid *Loligo vulgaris reynaudii* fishery off the coast of South Africa. In: Okutani T., O'Dor R.K. and Kubodera T. (eds). Recent Advances in Fisheries Biology. Tokai University Press, Tokyo, Japan, pp. 3-14.
- Awise J.C. (1996). Toward a regional conservation genetics perspective: phylogeography of faunas in the southeastern United States. In: Hamrick J.L. (ed.) Conservation genetics: case histories from nature. Chapman & Hall, New York, pp. 431-470.
- Awise J.C. (2000). Phylogeography. The History and Formation of Species. Harvard University Press, Cambridge, MA, U.S.A.
- Bakhayokho M. (1983). Biology of the cuttlefish *Sepia officinalis hierredda* off the Senegalese coast. In: Caddy J.F. (ed.). The Cephalopods: factors relevant to their population dynamics and to the assessment and management of stocks. FAO Fish. Tech. Pap. 231: 204-263.
- Bakhayokho M. (1991). From the competition and the complementary between traditional and industrial fisheries of cuttlefish in Senegal to a new fishery management policy. In: Boucaud-Camou E. (ed.). La seiche, The cuttlefish. 1st International Symposium on the Cuttlefish *Sepia*, Caen, June 1989. Centre de Publications de L'Université de Caen, Caen, France, pp. 335-341.
- Bakhayokho M. and Ito K. (1991). New gear and bait for cuttlefish fishing trap. In: Boucaud-Camou E. (ed.). La seiche, The cuttlefish. 1st International Symposium on the Cuttlefish *Sepia*, Caen, June 1989. Centre de Publications de L'Université de Caen, Caen, France, pp. 325-333.
- Ballard J.W.O., Chernoff B. and James A.C. (2002). Divergence of mitochondrial DNA is not corroborated by nuclear DNA, morphology, or behavior in *Drosophila simulans*. Evolution 56: 527-545
- Bandel K. and von Boletzky S. (1979). A comparative study of the structure, development and morphological relationship of chambered cephalopod shells. Veliger 21: 313-354.
- Barton N.H. (1979). Gene flow past a cline. Heredity 43: 333-339
- Barton N.H. and Jones J.S. (1983). Mitochondrial DNA: new clues about evolution. Nature 306: 317-318
- Beamish R.J. and McFarlane G.A. (1983). The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112: 735-743.
- Beddington J.R., Rosenberg A.A., Crombie J.A. and Kirkwood G.P. (1990). Stock assessment and the provision of management advice for the short-fin squid fishery in Falkland Islands waters. Fish. Res. 8: 351-365.
- Beets J. and Friedlander A. (1998). Evaluation of a conservation strategy: a spawning aggregation closure for red hind, *Epinephelus guttatus*, in the US Virgin Islands. Environ. Biol. Fishes 33: 91-98.
- Begg G.A. and Waldman J.R. (1999). An holistic approach to fish stock identification. Fish. Res. 43: 35-44
- Begon M. and Mortimer M. (1986). Population ecology: a unified study of animals and plants, 2nd Edition. Blackwell Scientific Publications, Oxford, U.K., 220pp.

- Belbin L. (1994). PATN: Pattern Analysis Package. Reference Manual. CSIRO Division of Wildlife and Ecology: Gungahlin, Canberra, Australia.
- Bellido J.M., Pierce G.J. and Wang J. (2001). Modelling intra-annual variation in abundance of squid *Loligo forbesi* in Scottish waters using generalised additive models. *Fish. Res.* 52: 23-39.
- Berrigan D. and Charnov E.L. (1994). Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. *Oikos* 70: 474-478.
- Bettencourt V. and Guerra A. (2000). Growth increments and biomineralization process in cephalopod statoliths. *J. Exp. Mar. Biol. Ecol.* 248: 191-205.
- Bettencourt V. and Guerra A. (2001). Age studies based on daily growth increments in statoliths and growth lamellae in cuttlebone of cultured *Sepia officinalis*. *Mar. Biol.* 139: 327-334.
- Beverton R.J.H. and Holt S.J. (1957). On the dynamics of exploited fish populations. *Fish. Invest. Ser.* 2, Vol. 19.
- Birkhead T.R. and Parker G.A. (1997). Sperm competition and mating systems. In: Krebs J.R. and Davies N.B. (eds). *Behavioural ecology: an evolutionary approach*, 4th Edition. Blackwell Science Ltd., Oxford, U.K., pp 121-145.
- Blanchier B. and Boucaud-Camou E. (1984). Lipids in the digestive gland and the gonad of immature and mature *Sepia officinalis* (Mollusca: Cephalopoda). *Mar. Biol.* 80: 39-43.
- Boal, J.G. (1996). Absence of social recognition in laboratory-reared cuttlefish, *Sepia officinalis* L. (Mollusca: Cephalopoda). *Anim. Behav.* 52: 529-537.
- Boal J.G. (1997). Female choice of males in cuttlefish (Mollusca: Cephalopoda). *Behaviour* 134: 975-988.
- Boal J.G. and Marsh S.E. (1998). Social recognition using chemical cues in cuttlefish (*Sepia officinalis* Linnaeus, 1758). *J. Exp. Mar. Biol. Ecol.* 230: 183-192.
- Boal J.G., Hylton R.A., Gonzalez S.A. and Hanlon R.T. (1999). Effects of crowding on the social behaviour of cuttlefish (*Sepia officinalis*). *Behaviour* 134: 975-988.
- Bohlin T., Dellefors C. and Faremo U. (1990). Large or small at maturity – theories on the choice of alternative male strategies in anadromous salmonids. *Ann. Zool. Fenn.* 27: 139-147.
- Boletzky S.v. (1974a). Effets de la sous-nutrition prolongée sur le développement de la coquille de *Sepia officinalis* L. (Mollusca, Cephalopoda). *Bull. Soc. Zool. Fr.* 99: 667-673.
- Boletzky S.v. (1974b). The “larvae” of Cephalopoda - a review. *Thalassia jugosl.* 10: 45-76.
- Boletzky S.v. (1983). *Sepia officinalis*. In: Boyle P.R. (ed.). *Cephalopod Life Cycles, Vol. I: Species Accounts*. Academic Press, London, U.K., pp. 31-52.
- Boletzky S.v. (1987). Fecundity variation in relation to intermittent or chronic spawning in the cuttlefish, *Sepia officinalis* L. (Mollusca, Cephalopoda). *Bull. Mar. Sci.* 40: 382-388.

- Boletzky S.v. (1989). Recent studies on spawning, embryonic development and hatching in Cephalopoda. *Adv. Mar. Biol.* 25: 86-109.
- Boletzky S.v. and Hanlon R.T. (1983). A review of the laboratory maintenance, rearing and culture of cephalopod molluscs. *Mem. Mus. Vic.* 44: 147-187.
- Boletzky S.v. and Overath H. (1991). Shell fracture and repair in the cuttlefish *Sepia officinalis*. In: Boucaud-Camou E. (ed.). *La seiche, The cuttlefish*. 1st International Symposium on the Cuttlefish, *Sepia*, Caen, 1-3 June 1989. Centre de Publications de L'Université de Caen, Caen, France, pp. 69-78.
- Bonnaud L, Boucherrodoni R, Monnerot M (1994) Phylogeny of decapod cephalopods based on partial 16S rDNA nucleotide sequences. *C. R. Acad. Sci. Serie III Sci. Vie* 317: 581-588.
- Bonnaud L., Boucherrodoni R. and Monnerot M. (1997). Phylogeny of cephalopods inferred from mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* 7: 44-54.
- Bookstein F.L., Chernoff B., Elder R.L., Humphries Jr J.M., Smith G.R. and Strauss R.E. (1985). *Morphometrics in evolutionary biology. The geometry of size and shape change, with examples from fishes.* The Academy of Natural Sciences of Philadelphia.
- Bott R.v. (1938). Kopula und Eiablage von *Sepia officinalis* L. *Z. Morphol. Oekol. Tiere* 34: 150-160.
- Boucaud-Camou E. and Boismery J. (1991). The migrations of the cuttlefish (*Sepia officinalis* L) in the English Channel. In: Boucaud-Camou E. (ed.). *La seiche, The cuttlefish*. 1st International Symposium on the Cuttlefish *Sepia*, Caen, June 1989. Centre de Publications de L'Université de Caen, Caen, France, pp. 179-189.
- Bouchaud O. (1991). Energy consumption of the cuttlefish *Sepia officinalis* L. (Mollusca: Cephalopoda) during embryonic development, preliminary results. *Bull. Mar. Sci.* 49: 333-340.
- Bouchaud O. and Daguzan J. (1989). Etude du développement de l'oeuf de *Sepia officinalis* L. (Céphalopode, Sepiidae) en conditions expérimentales. *Haliotis* 19: 189-200.
- Boycott B.B. (1958). The cuttlefish - *Sepia*. *New Biol.* 25: 98-118.
- Boyle P.R. (1990). Cephalopod biology in a fisheries context. *Fish. Res.* 8: 303-321.
- Boyle P.R. and Boletzky S.v. (1996). Cephalopod populations: definitions and dynamics. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351: 985-1002.
- Boyle P.R., Pierce G.J. and Hastie L.C. (1995). Flexible reproductive strategies in the squid *Loligo forbesi*. *Mar. Biol.* 121: 501-508.
- Bradbury J.W. (1981). The evolution of leks. In: Alexander R.D. and Tinkle D. (eds). *Natural selection and social behaviour: recent research and theory.* Chiron Press, New York, U.S.A., pp. 138-169.
- Bradley J.E.S. and Jones R. (1969). *Theory of fish population dynamics as the biological background for rational exploitation and management of fishery resources.* Oliver and Boyd, Edinburgh, U.K., 323pp. (Translation of original monograph by Nikolskii G.V.)

- Brierley A.S., Thorpe J.P., Pierce G.J., Clarke M.R. and Boyle P.R. (1995). Genetic variation in the neritic squid *Loligo forbesi* (Myopsida, Loliginidae) in the Northeast Atlantic Ocean. *Mar. Biol.* 122: 79-86.
- Brockington S. and Clarke A. (2001). The relative influence of temperature and food on the metabolism of a marine invertebrate. *J. Exp. Mar. Biol. Ecol.* 258: 87-99.
- Brockman H.J., Colson T. and Potts W. (1994) Sperm competition in horseshoe crabs (*Limulus polyphemus*). *Behav. Ecol. Sociobiol.* 35: 153-160.
- Brodziak J.K.T. and Macy W.K. III. (1996). Growth of long-finned squid, *Loligo pealei*, in the northwest Atlantic. *Fish. Bull.* 94: 212-236.
- Brooks R.C., Heidinger R.C. and Kohler C.C. (1994). Mass-marking otoliths of larval and juvenile walleyes by immersion in oxytetracycline, calcein or calcein blue. *N. Am. J. Fish. Manag.* 14: 143-150.
- Bumgardner B.W. and King T.L. (1996). Toxicity of oxytetracycline and calcein to juvenile stripe bass. *Trans. Am. Fish. Soc.* 125: 143-145.
- Buresch K.M., Hanlon R.T., Maxwell M.R. and Ring S. (2001). Microsatellite DNA markers indicate a high frequency of multiple paternity within individual field-collected egg capsules of the squid *Loligo pealeii*. *Mar. Ecol. Prog. Ser.* 210: 161-165.
- Burkett R.D. and Jackson W.B. (1971). The eye lens as an age indicator in freshwater drum. *Am. Midl. Nat.* 85: 222-225.
- Caddy J.F. (1991). Death rates and time intervals: is there an alternative to the constant natural mortality axiom? *Rev. Fish. Biol. Fish.* 1: 109-138.
- Caddy J.F. and Rodhouse P.G. (1998). Cephalopod and groundfish landings: evidence for ecological change in global fisheries? *Rev. Fish. Biol. Fish.* 8: 431-444.
- Calow P. (1987). Fact and theory - an overview. In: Boyle P.R. (ed.). *Cephalopod life cycles*, Vol. II: Comparative reviews. Academic Press, London, U.K., pp. 351-365.
- Campana S.E. (1999). Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar. Ecol. Prog. Ser.* 188: 263-297.
- Campana S.E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J. Fish Biol.* 59: 197-242.
- Campana S.E. and Neilson J.D. (1985). Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.* 42: 1014-1032.
- Cappo M. and Brown I.W. (1996). Evaluation of sampling methods for reef fish populations of commercial and recreational interest. C.R.C. Research Centre Technical Report 6, Townsville, 72pp.
- Cappo M., Walters C.J. and Lenanton R.C. (2000). Estimation of rates of migration, exploitation and survival using tag recovery data for Western Australian "salmon" (*Arripis truttaceus* : Arripidae : Percoidae). *Fish. Res.* 44: 207-217.
- Carlton W.G. and Jackson W.B. (1968). The eye lens as an age indicator in carp. *Copeia* 3: 633-636.

- Caro T. (1998). The significance of behavioural ecology for conservation biology. In: Caro T. (ed.). Behavioural ecology and conservation biology. Oxford University Press, New York, U.S.A., pp. 3-26.
- Carrick N.A. (1997). A preliminary assessment of the by-catch from the Spencer Gulf prawn fishery. South Australian Fisheries Assessment Series 97/02, South Australian Research and Development Institute, Adelaide, S.A.
- Carvalho G.R. and Loney K.H. (1989). Biochemical genetic studies on the Patagonian squid *Loligo gahi* d'Orbigny. I. Electrophoretic survey of genetic variability. J. Exp. Mar. Biol. Ecol. 126: 231-241.
- Carvalho G.R. and Pitcher T.J. (1989). Biochemical genetic studies on the Patagonian squid *Loligo gahi* d'Orbigny. II. Population structure in Falkland waters using isozymes, morphometrics and life history data. J. Exp. Mar. Biol. Ecol. 126: 243-258.
- Carvalho G.R., Thompson A. and Stoner A.L. (1992). Genetic diversity and population differentiation of the shortfin squid *Illex argentinus* in the south-west Atlantic. J. Exp. Mar. Biol. Ecol. 158: 105-121.
- Castro B.G., Garrido J.L. and Sotelo C.G. (1992). Changes in composition of digestive gland and mantle muscle of the cuttlefish *Sepia officinalis* during starvation. Mar. Biol. 114: 11-20.
- Choe S. (1963). Daily age markings on the shell of cuttlefishes. Nature 197: 306-307.
- Chotiyaputta C. (1993). Cephalopod resources of Thailand. In: Okutani T., O'Dor R.K. and Kubodera T. (eds). Recent Advances in Fisheries Biology. Tokai University Press, Tokyo, Japan, pp. 71-80.
- Clarke M.R. (1965). "Growth rings" in the beaks of the squid *Moroteuthis ingens* (Oegopsida, Onychoteuthidae). Malacologia 3: 297-307.
- Clarke M.R. (1978). The cephalopod statolith: an introduction to its form. J. Mar. Biol. Assoc. U.K. 58: 701-712.
- Clement M., Posada D., Crandall K.A. (2000). TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9: 1657-1660.
- Cochran W.G. (1977). Sampling Techniques, 3rd Edition. John Wiley and Sons, New York, U.S.A., 428pp.
- Colgan D.J. and Paxton J.R. (1997). Biochemical genetics and recognition of a western stock of the common gemfish, *Rexea solandri* (Scombroidea, Gempylidae), in Australia. Mar. Freshw. Res. 48: 103-118.
- Collins M.A., Boyle P.R., Pierce G.J., Key L.N., Hughes S.E. and Murphy J. (1999). Resolution of multiple cohorts in the *Loligo forbesi* population from the west of Scotland. ICES J. Mar. Sci. 56: 500-509.
- Collins M.A., Burnell G.M. and Rodhouse P.G. (1995a). Recruitment, maturation, and spawning of *Loligo forbesi* Streenstrup (Cephalopoda: Loliginidae). ICES J. Mar. Sci. 52: 127-137.
- Collins M.A., Burnell G.M. and Rodhouse P.G. (1995b). Reproductive strategies of male and female *Loligo forbesi* (Cephalopoda: Loliginidae). J. Mar. Biol. Assoc. U.K. 75: 621-634.

- Corner B.D. and Moore H.T. (1980). Field observations on the reproductive behaviour of *Sepia latimanus*. *Micronesica* 16: 235-260.
- Cortez T., Castro B.G. and Guerra A. (1995). Reproduction and condition of female *Octopus mimus* (Mollusca: Cephalopoda). *Mar. Biol.* 123: 505-510.
- Constanza M.C. and Afifi A.A. (1979). Comparison of stopping rules in forward stepwise discriminant analysis. *J. Am. Stat. Assoc.* 74: 777-785.
- Crivelli A. (1980). The eye lens weight and age in the common carp, *Cyprinus carpio* L. *J. Fish Biol.* 16: 469-473.
- Cronin E.R. (2000). Morphology, respiration and energetics of the eggs of the giant cuttlefish, *Sepia apama*. PhD Thesis, University of Adelaide, Adelaide, S.A., 142pp.
- Cronin E.R. and Seymour R.S. (2000). Respiration of the eggs of the giant cuttlefish *Sepia apama*. *Mar. Biol.* 136: 863-870.
- Daan S. and Tindbergen J.M. (1997). Adaptation of life histories. In: Krebs J.R. and Davies N.B. (eds). *Behavioural ecology: an evolutionary approach*, 4th Edition. Blackwell Science Ltd., Oxford, U.K., pp. 311-333.
- Dare E.G. (1981). Forecasting inshore abundance of squid *Illex illecebrosus* from a preseason biomass survey. *J. Shellfish Res.* 1: 112.
- Dartnall A.J. (1974). Littoral biogeography. In: Williams WD (ed.), *Biogeography and Ecology in Tasmania*. Dr W. Junk, The Hague, pp. 171-194.
- Davies N.B. (1991). Mating systems. In: Krebs J.R. and Davies N.B. (eds). *Behavioural ecology: an evolutionary approach*, 3rd Edition. Blackwell Scientific Publications, Oxford, U.K., pp. 263-294.
- Day R.W., Williams M.C. and Hawkes G.P. (1995). A comparison of flourochromes for marking abalone shells. *Mar. Freshw. Res.* 46: 599-605.
- Denis V. and Robin J.P. (2001). Present status of the French Atlantic fishery for cuttlefish (*Sepia officinalis*). *Fish. Res.* 52: 11-22.
- Denton E.J. and Gilpin-Brown J.B. (1961). The buoyancy of the cuttlefish, *Sepia officinalis* (L.). *J. Mar. Biol. Ass. U.K.* 41: 319-342.
- Dobzhansky T.H. (1950). Mendelian populations and their evolution. *Am. Nat.* 84: 401-418.
- Domeier M.L. and Colin P.L. (1997). Tropical reef fish spawning aggregations: defined and reviewed. *Bull. Mar. Sci.* 60: 698-726.
- Domingues P.M., Kingston T., Sykes A. and Andrade J.P. (2001). Growth of young cuttlefish, *Sepia officinalis* (Linnaeus 1758) at the upper end of the biological distribution temperature range. *Aquac. Res.* 32: 923-930.
- D'Onghia G., Matarrese A., Tursi A. and Maiorano P. (1996). Cephalopods collected by bottom trawling in the north Aegean Sea (Eastern Mediterranean). *Oebalia* 22: 33-46.

- Donnellan S.C. and Aplin K.P. (1989). Resolution of cryptic species in the New Guinean Lizard, *Sphenomorphus jobiensis* (Scincidae) by electrophoresis. *Copeia* 1989: 81-88.
- Douglas R.H. (1987). Ocular lens diameter as an indicator of age in brown trout, *Salmo trutta*. *J. Fish Biol.* 31: 835-836.
- Dudzinski M.L. and Mykytowycz R. (1961). The eye lens as an indicator of age in the wild rabbit in Australia. *CSIRO Wildl. Res.* 6: 156-159.
- Dunn M.R. (1999). Aspects of the stock dynamics and exploitation of cuttlefish, *Sepia officinalis* (Linnaeus, 1758), in the English Channel. *Fish. Res.* 40: 277-293.
- Durholtz M.D. and Lipinski M.R. (2000). Influence of temperature on the microstructure of statoliths of the thumstall squid *Lolliguncula brevis*. *Mar. Biol.* 136: 1029-1037.
- Eberhard W.G. (1996). *Female control: sexual selection by cryptic female choice*. Princeton University Press, Princeton, N.J., U.S.A.
- Edyvane K.S. (1999). Conserving marine biodiversity in South Australia - Part 2 - Identification of areas of high conservation value in South Australia. South Australian Research and Development Institute, Adelaide, S.A.
- Emlen S.T. and Oring L.W. (1977) Ecology, sexual selection, and the evolution of mating systems. *Science* 197: 215-223.
- English K.K., Bocking R.C. and Irvine J.R. (1992). A robust procedure for estimating salmon escapement based on the area-under-the-curve method. *Can. J. Fish. Aquat. Sci.* 49: 1982-1989.
- Escribano R., Iribarren C. and Rodriguez L. (1997). Influence of food quantity and temperature on development and growth of the marine copepod *Calanus chilensis* from northern Chile. *Mar. Biol.* 128: 281-288.
- FAO (2000). *The state of the world fisheries and aquaculture*. Fisheries Department, Food and Agriculture Organization of the United Nations, Rome, Italy, 142pp.
- FAO EASTFISH (1997). *Commodity Update: Cephalopods*. GLOBEFISH, Food and Agriculture Organization of the United Nations, Copenhagen, Denmark, 34pp.
- Fenton G.E. (1992). Population dynamics of *Tenagomysis tasmaniae* Fenton, *Anisomysis mixta australis* (Zimmer) and *Paramesopodopsis rufa* Fenton from south-eastern Tasmania (Crustacea: Mysidacea). *Hydrobiologia* 246: 173-193.
- Ferris S.D., Sage R.D., Huan C.M., Nielson J.T., Ritte U. and Wilson A.C. (1983). Flow of mitochondrial DNA across a species boundary. *Proc. Natl. Acad. Sci. U.S.A.* 80: 2290-2294.
- Fields W.G. (1965). The structure, development, food relations, reproduction, and life history of the squid *Loligo opalescens* Berry. *Calif. Fish Game* 131: 1-108.
- Fisher R.A. (1930). *The genetical theory of natural selection*. Clarendon Press, Oxford, U.K., 272 pp.
- Fleming I.A. (1996) Reproductive strategies of Atlantic salmon: ecology and evolution. *Rev. Fish. Biol. Fish.* 6: 379-416.

- Footo C.J., Brown G.S., Wood C.C. (1997) Spawning success of males using alternative mating tactics in sockeye salmon, *Onchorhynchus nerka*. Can. J. Fish. Aquat. Sci. 54: 1785-1795.
- Forsythe J.W. (1993). A working hypothesis of how seasonal temperature change may impact the field growth of young cephalopods. In: Okutani T., O'Dor R.K. and Kubodera T. (eds). Recent advances in fisheries biology. Tokai University Press, Tokyo, Japan, pp. 133-143.
- Forsythe J.W. and Hanlon R.T. (1988). Effect of temperature on laboratory growth, reproduction and life span of *Octopus bimaculoides*. Mar. Biol. 98: 369-379.
- Forsythe J.M. and Van Heukelem W.F. (1987). Growth. In: Boyle P.R. (ed.). Cephalopod life cycles, Vol II: Comparative reviews. Academic Press, London, U.K., pp. 135-156.
- Forsythe J.W., DeRusha R.H., Hanlon R.T. (1994). Growth, reproduction and life span of *Sepia officinalis* (Cephalopoda: Mollusca) cultured through seven consecutive generations. J. Zool. 233: 175-192.
- Forsythe J.W., Walsh L.S., Turk P.E. and Lee P.G. (2001). Impact of temperature on juvenile growth and age at first egg-laying of the Pacific reef squid *Sepioteuthis lessoniana* reared in captivity. Mar. Biol. 138: 103-112.
- Friend M. (1967). A review of research concerning eye-lens weight as a criterion of age in animals. N.Y. Fish Game J. 14: 152-165.
- Fu Y.X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915-925.
- Gabr H.R., Hanlon R.T., Hanafy M.H. and El-Etreby S.G. (1998). Maturation, fecundity and seasonality of reproduction of two commercially valuable cuttlefish, *Sepia pharaonis* and *S. dollfusi*, in the Suez Canal. Fish. Res. 36: 99-115.
- Gales R., Pemberton D., Lu C.C. and Clarke M.R. (1993). Cephalopod diet of the Australian fur seal: variation due to location, season and sample time. Aust. J. Mar. Freshw. Res. 44: 657-671.
- Gauvrit E., Pinczone Dul Sel G., Blanc A. and Daguzan J. (1998). Detection of cross-mating between two generations of the cuttlefish *Sepia officinalis* by sperm reservoir analysis. J. Moll. Stud. 64: 1-9.
- Gelsleichter J., Cortés E., Manire C.A., Hueter R.E. and Musick J.A. (1997). Use of calcein as a fluorescent marker for elasmobranch vertebral cartilage. Trans. Am. Fish. Soc. 126: 862-865.
- Godfray H.C.J. and Werren J.H. (1996). Recent developments in sex ratio studies. Trends Ecol. Evol. 11: 59-63.
- Goodman S.J. (1997). RST CALC - a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Mol. Ecol. 6: 881-885.
- Gostin V.A., Hails J.R. and Belperio A.P. (1984). The sedimentary framework of northern Spencer Gulf, South Australia. Mar. Geol. 61: 111-138.
- Goudet J., Raymond M., Demeueus T. and Rousset F. (1996). Testing differentiation in diploid populations. Genetics 144: 1933-1940.

- Green H.S. (1984). Fluid transport processes in upper Spencer Gulf. *Mar. Geol.* 61: 181-195.
- Griswold C.A. and Prezioso J. (1981). In situ observations on reproductive behaviour of the long-finned squid, *Loligo pealei*. *Fish. Bull.* 78: 945-947.
- Gross M.R. (1985). Disruptive selection for alternative life histories in salmon. *Nature* 313: 47-48.
- Gross M.R. (1996). Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol. Evol.* 11: 92-98.
- Guerra A. and Castro B.G. (1988). On the life cycle of *Sepia officinalis* (Cephalopoda, Sepioidea) in the ria Vigo (NW Spain). *Cah. Biol. Mar.* 29: 395-405.
- Guerra A. and Castro B.G. (1989). Some aspects of the biology of *Sepia elegans* (Cephalopoda, Sepioidea) from the Ria de Vigo, NW Spain. *Vie Milieu* 39: 213-218.
- Guerra A. and Castro B.G. (1994). Reproductive-somatic relationships in *Loligo gahi* (Cephalopoda: Loliginidae) from the Falkland Islands. *Antarct. Sci.* 6: 175-178.
- Gulland J.A. (1983). *Fish stock assessment: a manual of basic methods*. John Wiley and Sons, New York, U.S.A., 223pp.
- Gutsal D.K. (1989). Underwater observations of distribution and behaviour of the cuttlefish *Sepia pharaonis* in the western part of the Arabian Sea. *Sov. J. Mar. Biol.* 15: 43-49.
- Hails J.R. and Gostin V.A. (1978). Stranded shingle beach ridges, upper Spencer Gulf, South Australia: evidence for high wave energy dissipation during the late Pleistocene. *Trans. R. Soc. Aust.* 102: 169-173.
- Hall K. (1998) The flamboyant and fascinating lifecycle of the giant cuttlefish. *South. Fish.* 6: 20-25.
- Hall KC (2000). Cuttlefish (*Sepia apama*). South Australian Fisheries Assessment Series No. 00/09. South Australian Research and Development Institute, Adelaide, SA, 33pp.
- Hall KC (2002). Cuttlefish (*Sepia apama*). South Australian Fisheries Assessment Series No. 01/09. South Australian Research and Development Institute, Adelaide, SA, 35pp.
- Hamabe M. and Shimizu T. (1966). Ecological studies on the common squid *Todarodes pacificus* Steenstrup mainly in southwestern waters of the Sea of Japan. *Bull. Jpn Sea Fish. Res. Lab.* 16: 13-55.
- Hankin D.G., Nicholas J.W. and Downey T.W. (1993). Evidence for inheritance of age of maturity in chinook salmon (*Oncorhynchus tshawytscha*). *Can. J. Fish. Aquat. Sci.* 50: 347-358.
- Hanlon R.T. (1996). Evolutionary games that squid play: fighting, courting, sneaking, and mating behaviours used for sexual selection in *Loligo pealei*. *Biol. Bull.* 191: 309-310.
- Hanlon R.T. (1998). Mating systems and sexual selection in the squid *Loligo*: how might commercial fishing on spawning squids affect them? *CalCOFI Rep.* 39: 92-100.
- Hanlon R.T. and Messenger J.B. (1988). Adaptive coloration in young cuttlefish (*Sepia officinalis* L.): the morphology and development of body patterns and their relation to behaviour. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 320: 437-487.

- Hanlon R.T. and Messenger J.B. (1996). *Cephalopod Behaviour*. Cambridge University Press, Cambridge, U.K., 232pp.
- Hanlon R.T., Ament S.A. and Gabr H. (1999). Behavioural aspects of sperm competition in cuttlefish, *Sepia officinalis* (Sepioidea: Cephalopoda). *Mar. Biol.* 134: 719-728.
- Hanlon R.T., Maxwell M.R. and Shashar N. (1997). Behavioural dynamics that would lead to multiple paternity within egg capsules of the squid *Loligo pealei*. *Biol. Bull.* 193: 212-214.
- Hanlon R.T., Smale M.J. and Sauer W.H.H. (in press). The mating system of the squid *Loligo vulgaris reynaudii* (Cephalopoda, Mollusca) off South Africa: fighting, guarding, sneaking, mating and egg laying behaviour. *Bull. Mar. Sci.* (in press).
- Hare M.P. and Avise J.C. (1996). Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). *Evolution* 50: 2305-2315.
- Hare M.P. and Avise J.C. (1998). Population structure in the American oyster as inferred by nuclear gene genealogies. *Mol. Biol. Evol.* 15: 119-128.
- Hare M.P., Karl S.A. and Avise J.C. (1996). Anonymous nuclear DNA markers in the American oyster and their implications for the heterozygote deficiency phenomenon in marine bivalves. *Mol. Biol. Evol.* 13: 334-345.
- Harley S.J., Myers R.A. and Dunn A. (2001). Is catch-per-unit-effort proportional to abundance? *Can. J. Fish. Aquat. Sci.* 58: 1760-1772.
- Harman R.F., Young R.E., Reid S.B., Mangold K.M., Suzuki T. and Hixon R.F. (1989). Evidence for multiple spawning in the tropical oceanic squid *Stenoteuthis aualanensis* (Teuthoidea: Ommastrephidae). *Mar. Biol.* 101: 513-519.
- Harpending H.C. (1994). Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum. Biol.* 66: 591-600.
- Harrison R.G. (1990). Hybrid zones: windows on evolutionary process. *Oxf. Surv. Evol. Biol.* 7: 69-128.
- Harrison R.G., Rand D.M. and Wheeler W.C. (1987). Mitochondrial DNA variation in field crickets across a narrow hybrid zone. *Mol. Biol. Evol.* 4: 144-158.
- Hatfield E.M.C. (1996). Towards resolving multiple recruitment into loliginid fisheries: *Loligo gahi* in the Falkland Islands fishery. *ICES J. Mar. Sci.* 53: 565-575.
- Hatfield E.M.C. (2000). Do some like it hot? Temperature as a possible determinant of variability in the growth of the Patagonian squid, *Loligo gahi* (Cephalopoda: Loliginidae). *Fish. Res.* 47: 27-40.
- Hatfield E.M.C. and Rodhouse P.G. (1994). Migration as a source of bias in the measurement of cephalopod growth. *Antarct. Sci.* 6: 179-184.
- Hatfield E.M.C., Hanlon R.T., Forsythe J.W. and Grist E.P.M. (2001). Laboratory testing of a growth hypothesis for juvenile squid *Loligo pealeii* (Cephalopoda: Loliginidae). *Can. J. Fish. Aquat. Sci.* 58: 845-857.

- Hensen S.A. and Warner R.R. (1997). Male and female alternative reproductive behaviours in fishes: a new approach using intersexual dynamics. *Annu. Rev. Ecol. Syst.* 28: 571-592.
- Hewitt G.M. and Butlin R.K. (1997). Causes and consequences of population structure. In: Krebs J.R. and Davies N.B. (eds). *Behavioural ecology: an evolutionary approach*, 4th Edition. Blackwell Science Ltd, Oxford, U.K., pp. 350-372.
- Hewitt R.A. and Stait B. (1988). Seasonal variation in septal spacing of *Sepia officinalis* and some Ordovician actinocerid nautiloids. *Lethaia* 21: 383-394.
- Hilborn R. and Walters C.J. (1992). *Quantitative Fisheries Stock Assessment*. Chapman and Hall, New York, U.S.A., 570pp.
- Hilborn R., Bue B.G. and Sharr S. (1999). Estimating spawning escapements from periodic counts: a comparison of methods. *Can. J. Fish. Aquat. Sci.* 56: 888-896.
- Jackson G.D. (1994). Statolith age estimates of the loliginid squid *Loligo opalescens* (Mollusca: Cephalopoda): corroboration with culture data. *Bull. Mar. Sci.* 54: 554-557.
- Jackson G.D., Alford R.A. and Choat J.H. (2000). Can length frequency analysis be used to determine squid growth? – An assessment of ELEFAN. *ICES J. Mar. Sci.* 57: 948-954.
- Jefferts K., Burczynski J. and Percy W.G. (1987). Acoustical assessment of squid (*Loligo opalescens*) off the central Oregon coast. *Can. J. Fish. Aquat. Sci.* 44: 1261-1267.
- Jenkins J. (1993). Flight of the wandering albatross. *Aust. Fish.* 52: 24-25.
- Jereb P. and Ragonese S. (1991). *Sepia elegans* and *Sepia orbignyana* first studies on the presence of the elegant and pink cuttlefish in the Sicilian channel. In: Boucaud-Camou E. (ed.). *La Seiche, The Cuttlefish*. 1st International Symposium on the cuttlefish, *Sepia*, Caen, 1-3 June 1989. Centre de Publications de L'Universite de Caen, Caen, France, pp. 17-29.
- Jereb P., Ragonese S. and von Boletzky S. (1991). Squid age determination using statoliths. Proceedings of the International Workshop held in the Istituto di Tecnologia della Pesca e del Pescato. NTR - ITPP Spec. Publ. 1, 124pp.
- Kassahn K.S. (2002). Phylogeography of the giant Australian cuttlefish *Sepia apama* (Cephalopoda: Mollusca) and implications for the management of its fishery. Hons. Thesis, University of Adelaide, Adelaide, S.A., 65pp.
- Kennelly S. and McVea T. (eds) (2001). Status of fisheries resources 2000/2001 NSW fisheries. N.S.W. Fisheries, Cronulla, N.S.W.
- Kim Z.G. and Hong B.Q. (1991). Study on possible group separation of cuttlefish *Sepia exculenta* Hoyle in Korean waters. *Bull. Natl. Fish. Res. Dev. Agency (Korea)* 45: 71-84.
- King M. (1995). *Fisheries biology, assessment and management*. Oxford Fishing News Books, Oxford, U.K., 341pp.
- Kirkendall L.R. and Stenseth N.C. (1985). On defining “breeding once”. *Am. Nat.* 125: 189-204.

- Klingenberg C.P. (1996). Multivariate allometry. In: Marcus L.F., Corti M., Loy A., Naylor G.J.P. and Slice D.E. (eds). *Advances in morphometrics*. Plenum Press, New York, U.S.A., pp. 23-49.
- Kolenosky G.B. and Miller R.S. (1962). Growth of the lens of the pronghorn antelope. *J. Wildl. Manag.* 26: 112-113.
- Koueta N. and Boucaud-Camou E. (1999). Food intake and growth in reared early juvenile cuttlefish *Sepia officinalis* L. (Mollusca Cephalopoda). *J. Exp. Mar. Biol. Ecol.* 240: 93-109.
- Koueta N. and Boucaud-Camou E. (2001). Basic growth relations in experimental rearing of early juvenile cuttlefish *Sepia officinalis* L. (Mollusca: Cephalopoda). *J. Exp. Mar. Biol. Ecol.* 265: 75-87.
- Kozlowski J. (1992). Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. *Trends. Ecol. Evol.* 7: 15-19.
- Kramer D.L. and Chapman M.R. (1999). Implications for fish home range size and relocation for marine reserve function. *Environ. Biol. Fishes* 55: 65-79.
- Krebs C.J. (1994). *Ecology: the experimental analysis of distribution and abundance*, 4th Edition. Harper Collins College Publishers, New York, U.S.A., 801pp.
- Kristensen T.K. (1982). Multivariate statistical analysis of geographic variation in the squid *Gonatus fabricii* Lichtenstein 1818 (Mollusca: Cephalopoda). *Malacologia* 22: 581-586.
- Kvarnemo C. and Ahnesjö I. (1996). The dynamics of operational sex ratios and competition for mates. *Trends Ecol. Evol.* 11: 404-408.
- Lange A.M. (1991). Alternative survey indices for predicting availability of longfin squid to seasonal northwest Atlantic fisheries. *N. Am. J. Fish. Manag.* 11: 443-450.
- Lawrence J.M. and Sammaros P.W. (1982). Effects of feeding on the environment: Echinoidea. In: Jangoux M. and Lawrence J.M. (eds). *Echinodermata nutrition*. A.A. Balkema, Rotterdam, Netherlands, pp. 499-519.
- Le Goff R. and Daguzan J. (1991). Growth and life cycles of the cuttlefish *Sepia officinalis* L. (Mollusca: Cephlopoda) in South Brittany (France). *Bull. Mar. Sci.* 49: 341-348.
- Le Goff R., Gauvrit E., Pinczon du Sel G. and Daguzan J. (1998). Age group determination by analysis of the cuttlebone of the cuttlefish *Sepia officinalis* L. in reproduction in the Bay of Biscay. *J. Molluscan Stud.* 64: 183-193.
- Leslie R.W. and Grant W.S. (1990). Lack of congruence between genetic and morphological stock structure of the Southern African anglerfish *Lophius vomerinus*. *S. Afr. J. mar. Sci.* 9: 379-398.
- Lessells C.M. (1991). The evolution of life histories. In: Krebs J.R. and Davies N.B. (eds). *Behavioural ecology: an evolutionary approach*, 3rd Edition. Blackwell Science Ltd, Oxford, U.K., pp. 32-67.
- Lewis A.R. and Choat J.H. (1993). Spawning mode and reproductive output of the tropical cephalopod *Idiosepius pygmaeus*. *Can. J. Fish. Aquat. Sci.* 50: 20-28.

- Lipinski M.R. (1994). Differences among basic biological parameters in a population of chokka squid *Loligo vulgaris reynaudii* (Cephalopoda: Loliginidae) sampled by three methods. *S. Afr. J. mar. Sci.* 14: 281-286.
- Lipinski M.R. (1998). Cephalopod life cycles: patterns and exceptions. In: Payne A.I.L., Lipinski M.R., Clarke M.R. and Roeleveld M.A.C. (eds). *Cephalopod biodiversity, ecology and evolution*. *S. Afr. J. mar. Sci.* 20: 439-447.
- Lipinski M.R. and Underhill L.G. (1995). Sexual maturation in squid: quantum or continuum? *S. Afr. J. mar. Sci.* 15: 207-223.
- Lipinski M., Dawe E. and Natsukari Y. (1991). Introduction. In: Jereb P., Ragonese S. and Boletzky S.v. (eds). *Squid age determination using statoliths. Proceedings of the International Workshop held in the Istituto di Tecnologia della Pesca e del Pescato. NTR - ITPP Spec. Publ. 1*, pp. 77-82.
- Lord R.D. (1959). The lens as an indicator of age in cottontail rabbits. *J. Wildl. Manag.* 23: 358-360.
- Lu C.C. (1998a). A synopsis of Sepiidae in Australian waters (Cephalopoda: Sepioidea). In: Voss N.A., Vecchione M., Toll R.B. and Sweeney M.J. (eds). *Systematics and Biogeography of Cephalopods, Vol. I. Smithsonian. Contrib. Zool.* 586, pp. 159-190.
- Lu C.C. (1998b). Order Sepioidea. pp. 504-514. In: Beesley P.L., Ross G.J.B. and Wells A. (eds). *Mollusca: The Southern Synthesis. Fauna of Australia, Vol. 5, Part A. CSIRO Publishing, Melbourne, Vic.*, 563pp.
- Lunneborg C.E. (1994). *Modeling experimental and observational data*. Duxbury Press, Belmont, U.S.A.
- Macy W.K. III. (1995). The application of digital image processing to the aging of long-finned squid, *Loligo pealeii*, using the statolith. In: Secor D.H., Dean J.M. and Campana S.E. (eds). *Recent developments in fish otolith research*. University of South Carolina Press, U.S.A., pp. 283-302.
- Macy W.K. and Brodziak J.K.T. (2001). Seasonal maturity and size at age of *Loligo pealeii* in waters of southern New England. *ICES J. Mar. Sci.* 58: 852-864.
- Mangold K. (1987). Reproduction. In: Boyle P.R. (ed.). *Cephalopod life cycles, Vol. II: Comparative reviews*. Academic Press, London, U.K., pp. 157-200.
- Mangold K., Froesch D., Boucher-Rodoni R. and Rowe V.L. (1975). Factors affecting sexual maturation in cephalopods. *Pubbl. Staz. Zool. Napoli.* 39(Suppl.): 259-266.
- Mangold K.M., Young R.E. and Nixon M. (1993). Growth versus maturation in cephalopods. In: Okutani T., O'Dor R.K. and Kubodera T. (eds). *Recent Advances in Fisheries Biology*. Tokai University Press, Tokyo, Japan, pp. 697-703.
- Mantel N. (1967). The detection of disease clustering and a generalised regression approach. *Cancer Res.* 27: 209-220.
- Martin P. and Bateson P. (1986). *Measuring behaviour: an introductory guide*. Cambridge University Press, Cambridge, U.K., 200pp.

- Martínez P., Bettencourt V., Guerra Á. and Moltschaniwskyj N.A. (2000). How temperature influences muscle and cuttlebone growth in juvenile cuttlefish (*Sepia elliptica*) (Mollusca: Cephalopoda) under conditions of food stress. *Can. J. Zool.* 78: 1855-1861.
- Martínez P., Sanjuan A. and Guerra A. (2002). Identification of *Illex coindetii*, *I. illecebrosus* and *I. argentinus* (Cephalopoda: Ommastrephidae) throughout the Atlantic Ocean; by body and beak characters. *Mar. Biol.* 141: 131-143.
- Maxwell M.R. and Hanlon R.T. (2000). Female reproductive output in the squid *Loligo pealeii*: multiple egg clutches and implications for a spawning strategy. *Mar. Ecol. Prog. Ser.* 199: 159-170.
- McCormick M.I. and Choat J.H. (1987). Estimating total abundance of a large temperate reef fish using visual strip-transects. *Mar. Biol.* 96: 469-478.
- McGlennon D. and Hall K.C. (1997). Cuttlefish (*Sepia apama*). South Australian Fisheries Assessment Series No. 97/10. South Australian Research and Development Institute, Adelaide, S.A., 15pp.
- McGoodwin J.R. (1990). Crisis in the world's fisheries: people, problems, and policies. Stanford University Press, Stanford, C.A., U.S.A.
- McGowan J.A. (1954). Observations on the sexual behaviour and spawning of the squid, *Loligo opalescens*, at La Jolla, California. *Calif. Fish Game* 40: 47-54.
- Mesnil B. (1977a). Growth and life cycle of squid, *Loligo pealei* and *Illex illecebrosus*, from the northwest Atlantic. *ICNAF Sel.* 2: 55-69.
- Miller S.A., Dykes D.D. and Polesky H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16: 1215.
- Mittwoch U. (1996). Sex-determining mechanisms in animals. *Trends Ecol. Evol.* 11: 63-67.
- Moltschaniwskyj N.A. and Jackson G.D. (2000). Growth and tissue composition as a function of feeding history in juvenile cephalopods. *J. Exp. Mar. Biol. Ecol.* 253: 229-241.
- Moltschaniwskyj N.A. and Martínez P. (1998). Effect of temperature and food levels on the growth and condition of juvenile *Sepia elliptica* (Hoyle 1885): an experimental approach. *J. Exp. Mar. Biol. Ecol.* 229: 289-302.
- Monaghan J.P. Jr. (1993). Comparison of calcein and tetracycline as chemical markers in summer flounder. *Trans. Am. Fish. Soc.* 122: 298-301.
- Moran C. (1979). The structure of the hybrid zone in *Caledia captiva*. *Heredity* 42.
- Moran C., Wilkinson P. and Shaw D.D. (1980). Allozyme variation across a narrow hybrid zone in the grasshopper, *Caledia captiva*. *Heredity* 44.
- Moritz C. (1994). Applications of mitochondrial DNA analysis in conservation - a critical review. *Mol. Ecol.* 3: 401-411.
- Mosimann J.E. (1970). Size allometry: size and shape variables with characterizations of the log-normal and generalized gamma distributions. *J. Am. Stat. Assoc.* 65: 930-945.

Moynihan M. and Rodaniche A.F. (1982). The behaviour and natural history of the Caribbean reef squid *Sepioteuthis sepioidea*: with a consideration of social, signal and defensive patterns for difficult and dangerous environments. *Adv. Ethol.* 25: 1-151.

Murata M. (1989). Population assessment, management and fishery forecasting for Japanese common squid, *Todares pacificus*. In: Caddy J.F. (ed.). *Marine invertebrate fisheries: their assessment and management*. John Wiley, New York, U.S.A., pp. 613-636.

Murphy R.W., Sites J.W., Buth D.G. and Haufler C.H. (1996). Proteins: Isozyme Electrophoresis. In: Mable BK (ed.). *Molecular Systematics*. Sinauer Associates, Sunderland, U.S.A., pp. 51-120.

Nair K.P. (1985). Hand-jigging for cuttlefish at Vizhinjam with a note on modern squid jigging. *CMFRI Bull.* 37: 152-156.

Nair K.P., Srinath M., Meiyappan M.M., Rao K.S., Sarvesan R., Vidyasagar K., Sundaram K.S., Rao G.S., Lipton A.P., Natarajan P., Radhakrishnan G., Mohamed K.S., Narasimham K.A., Balan K., Kripa V. and Sathianandan T.V. (1993). Stock assessment of the pharaoh cuttlefish *Sepia pharaonis* Ehrenberg. *Indian J. Fish.* 40: 85-94.

Nakata J. (1993). Long-term changes in catch and biological features of Japanese common squid (*Todares pacificus*) in waters off the east coast of Hokkaido. In: Okutani T., O'Dor R.K. and Kubodera T. (eds). *Recent Advances in Fisheries Biology*. Tokai University Press, Tokyo, Japan, pp. 343-350.

Narasimham K.A., Kripa V. and Balan K. (1993). Molluscan shellfish resources of India - An overview. *Indian J. Fish.* 40: 112-124.

Natsukari Y. and Tashiro M. (1991). Neritic squid resources and cuttlefish resources in Japan. *Mar. Behav. Physiol.* 18: 149-226.

Natsukari Y., Hirata S. and Washizake M. (1991). Growth and seasonal change of the cuttlebone characters of *Sepia esculenta*. In: Boucaud-Camou E. (ed.). *La seiche, The cuttlefish*. 1st International Symposium on the Cuttlefish, *Sepia*, Caen, 1-3 June 1989. Centre de Publications de L'Université de Caen, Caen, France, pp. 49-67.

Nixon M. and Mangold K. (1998). The early life of *Sepia officinalis*, and the contrast with that of *Octopus vulgaris* (Cephalopoda). *J. Zool.* 245: 407-421.

Noakes D.L.G., Skúlason S. and Snorrason S.S. (1989). Alternative life-history styles in salmonine fishes with emphasis on arctic charr, *Salvelinus alpinus*. In: Bruton M.N. (ed.). *Alternative life-history styles of animals*. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 329-346.

Norman M.D. (2000). *Cephalopods, a world guide*. ConchBooks, Hackenheim, Germany, 319pp.

Norman M.D., Finn J. and Tregenza T. (1999). Female impersonation as an alternative reproductive strategy in giant cuttlefish. *Proc. R. Soc. Lond. B Biol. Sci.* 266: 1347-1349.

Noye J. (1984). Physical processes and pollution in the waters of Spencer Gulf. *Mar. Geol.* 61: 197-220.

Nunes Vaz R.A. and Lennon G.W. (1986). Physical property distributions and seasonal trends in Spencer Gulf, South Australia: an inverse estuary. *Aust. J. Mar. Freshw. Res.* 37: 39-53.

- Nunes Vaz R.A., Lennon G.W. and Bowers D.G. (1990). Physical behaviour of a large, negative or inverse estuary. *Continent. Shelf Res.* 10: 277-304.
- O'Dor R.K. (1998). Can understanding squid life-history strategies and recruitment improve management? In: Payne A.I.L., Lipinski M.R., Clarke M.R. and Roeleveld M.A.C. (eds). *Cephalopod biodiversity, ecology and evolution*. S. Afr. J. mar. Sci. 20: 193-206.
- O'Dor R.K. and Webber D.M. (1986). The constraints on cephalopods: why squid aren't fish. *Can. J. Zool.* 64: 1591-1605.
- O'Dor R.K. and Wells M.J. (1987). Energy and nutrient flow. In: Boyle P.R. (ed.). *Cephalopod life cycles, Vol II: Comparative reviews*. Academic Press, London, U.K., pp. 109-133.
- Oestmann D.J., Scimeca J.M., Forsythe J., Hanlon R.T. and Lee P. (1997). Special considerations for keeping cephalopods in laboratory facilities. *Contemp. Top. Lab. Anim. Sci.* 36: 89-93.
- Packard A. (1972). Cephalopods and fish: the limits of convergence. *Biol. Rev.* 47: 241-307.
- Packard G.C. and Boardman T.J. (1987). The misuse of ratios to scale physiological data that vary allometrically with body size. In: Feder M.E., Bennett A.F., Burggren W.W. and Huey R.B. (eds). *New directions in ecological physiology*. Cambridge University Press, Cambridge, U.K., pp. 216-236.
- Palumbi S.R. (1996). What can molecular genetics contribute to marine biogeography - an urchins tale. *J. Exp. Mar. Biol. Ecol.* 203: 75-92.
- Pannella G. (1971). Fish otoliths: daily growth layers and periodical patterns. *Science* 173: 1124-1127.
- Parker H.H., Noonburg E.G. and Nisbet R.M. (2001). Models of alternative life-history strategies, population structure and potential speciation in salmonid fish stocks. *J. Anim. Ecol.* 70: 260-272.
- Pauly D., Christensen V., Dalsgaard J., Froese R. and Torres F. Jr. (1998). Fishing down marine food webs. *Science* 279: 860-863.
- Pecl G. (2001). Flexible reproductive strategies in tropical and temperate *Sepioteuthis* squids. *Mar. Biol.* 138: 93-101.
- Penn J.W. (ed) (2001). *State of the fisheries report 2000-2001*. Fisheries Research Division, Department of Fisheries, Perth, W.A.
- Perez J.A.A. and O'Dor R.K. (1998). The impact of environmental gradients on the early life inshore migration of the short-finned squid *Illex illecebrosus*. In: Payne A.I.L., Lipinski M.R., Clarke M.R. and Roeleveld M.A.C. (eds). *Cephalopod biodiversity, ecology and evolution*. S. Afr. J. mar. Sci. 20: 293-303.
- Pérez-Losada M., Guerra A., Carvalho G.R., Sanjuan A. and Shaw P.W. (2002). Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) around the Iberian Peninsula indicated by microsatellite DNA variation. *Heredity* 88: *in press*
- Petry D. (1983). The effect on neutral gene flow of selection at a linked locus. *Theor. Popul. Biol.* 23: 300-313.

- Piatkowski U., Pierce G.J. and Morais da Cunha M. (2001). Impact on cephalopods in the food chain and their interaction with the environment and fisheries: an overview. *Fish. Res.* 52: 5-10.
- Pierce G.J. and Guerra A. (1994). Stock assessment methods used for cephalopod fisheries. *Fish. Res.* 21: 255-285.
- Pierce G.J., Boyle P.R., Hastie L.C. and Shanks A.M. (1994). Distribution and abundance of the fished population of *Loligo forbesi* in U.K. waters: analysis of fisheries data. *Fish. Res.* 21: 193-216.
- Pierce G.J., Thorpe R.S., Hastie L.C., Brierley A.S., Guerra A., Boyle P.R., Jamieson R. and Avila P. (1994). Geographic variation in *Loligo forbesi* in the Northeast Atlantic Ocean - Analysis of morphometric data and tests of causal hypotheses. *Mar. Biol.* 119: 541-547.
- PIRSA (1999). Marine scalefish fishery restructure: synopsis of the South Australian marine scalefish fishery. Fisheries and Aquaculture, Primary Industries and Resources South Australia, Adelaide, S.A., 115pp.
- Poore G.C.B. (1994). Marine biogeography of Australia. In: Synnot RN (ed.). *Marine Biology*. Longman Cheshire, Melbourne, pp. 189-212.
- Posada D. and Crandall K.A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- Poston J.P., Wiley R.H. and Westneat D.F. (1999). Male rank, female breeding synchrony, and patterns of paternity in the boat-tailed grackle. *Behav. Ecol.* 10: 444-451.
- Radtke R.L. (1983). Chemical and structural characteristics of statoliths from the short-finned squid *Illex illecebrosus*. *Mar. Biol.* 76: 47-54.
- Rambaut A. (1996). Se-Al: Sequence Alignment Editor. Version 1.0 alpha 1. University of Oxford, Oxford, U.K.
- Rao K.S. (1997). Aspects of biology and exploitation of *Sepia aculeata* Orbigny from Mangalore area, Karnataka. *Indian J. Fish.* 44: 247-254.
- Rao K.S., Srinath M., Meiyappan M.M., Nair K.P., Sarvesan R., Rao G.S., Natarajan P., Vidyasagar K., Sundaram K.S., Lipton A.P., Radhakrishnan G., Narasimha K.A., Mohamed K.S., Balan K., Kripa V. and Sathianandan T.V. (1993). Stock assessment of the needle cuttlefish *Sepia aculeata* Orbigny. *Indian J. Fish.* 40: 95-103.
- Raya C.P. and Hernández-González C.L. (1998). Growth lines within the beak microstructure of the octopus *Octopus vulgaris* Cuvier, 1797. In: Payne A.I.L., Lipinski M.R., Clarke M.R. and Roeleveld M.A.C. (eds). *Cephalopod Biodiversity, Ecology and Evolution*. S. Afr. J. mar. Sci. 20: 135-142.
- Raya C.P., Fernández-Núñez M., Balguerías E., Hernández-González C.L. (1994). Progress towards ageing cuttlefish *Sepia hierredda* from the northwestern African coast using statoliths. *Mar. Ecol. Prog. Ser.* 114: 139-147.
- Raymond M. and Rousset F. (1995a). An exact test for population differentiation. *Evolution* 49: 1280-1283.

- Raymond M. and Rousset F. (1995b). GENEPOP (Version 1.2) - Populations genetics software for exact tests and ecumenicism. *J. Hered.* 86.
- Ré P. and Narciso L. (1994). Growth and cuttlebone microstructure of juvenile cuttlefish, *Sepia officinalis* L., under controlled conditions. *J. Exp. Mar. Biol. Ecol.* 177: 73-78.
- Reid A.L. (2000). Australian cuttlefishes (Cephalopoda : Sepiidae): the 'doratosepion' species complex. *Invertebr. Taxon.* 14: 1-76.
- Reynolds J.D. (1998). Animal breeding systems. *Trends Ecol. Evol.* 11: 68-72.
- Reynolds J.D. and Gross M.R. (1990). Costs and benefits of female mate choice: is there a lek paradox? *Am. Nat.* 136: 230-243.
- Rice W.R. (1989). Analyzing tables of statistical tests. *Evolution* 43: 223-225.
- Richard A. (1967). Influence de la température et de la nutrition sur la forme et la striation de la coquille de *Sepia officinalis* L. (Mollusque: Céphalopode). *C. R. Soc. Biol.* 161: 620-624.
- Richard A. (1969). The part played by temperature in the rhythm of formation of markings on the shell of cuttlefish (*Sepia officinalis* L.) (Cephalopoda, Mollusca). *Experientia* 25: 1051-1052.
- Richard M.A. and Lemaire J. (1975). Détermination et différenciation sexuelles chez la seiche *Sepia officinalis* L. (Mollusque, Céphalopode). *Pubbl. Staz. Zool. Napoli* 39(Suppl.): 574-594.
- Robin J.P. and Denis V. (1999). Squid stock fluctuations and water temperature: temporal analysis of English Channel Loliginidae. *J. Appl. Ecol.* 36: 101-110.
- Rocha F., Guerra A. and Gonzalez A.F. (2001). A review of reproductive strategies of cephalopods. *Biol. Rev.* 76: 291-304.
- Rodhouse P.G. (2001). Managing and forecasting squid fisheries in variable environments. *Fish. Res.* 54: 3-8.
- Rodhouse P.G. and Hatfield E.M.C. (1990). Age determination in squid using statolith growth increment. *Fish. Res.* 8: 323-334.
- Rodhouse P.G., Murphy E.J. and Coelho M.L. (1998). Impact of fishing on life histories. In: Rodhouse P.G., Dawe E.G. and O'Dor R.K. (eds). Squid recruitment dynamics: the genus *Illex* as a model, the commercial *Illex* species and influences on variability. *FAO Fish. Tech. Pap.* 376: 255-268.
- Roel B.A. and Butterworth D.S. (2000). Assessment of the South African chokka squid *Loligo vulgaris reynaudii* – is disturbance of aggregations by the recent jig fishery having a negative impact on recruitment? *Fish. Res.* 48: 213-228.
- Roel B.A., Cochrane K.L. and Field J.G. (2000). Investigation into the declining trend in chokka squid *Loligo vulgaris reynaudii* catches made by South African trawlers. *S. Afr. J. mar. Sci.* 22: 121-135.
- Rogers A.R. (1995). Genetic evidence for a Pleistocene population explosion. *Evolution* 49: 608-615.
- Rogers J.S. (1972). Measures of genetic similarity and genetic distance. *Studies in Genetics VII – Univ. Texas Publ.* 7213: 145-153.

Roper C.F.E., Sweeney M.J. and Nauen C.E. (1984). FAO Species Catalogue, Vol. 3. Cephalopods of the World: an annotated and illustrated catalogue of species of interest to fisheries. FAO Fish. Synop. 125, Vol 3, 277pp.

Rosenberg A.A., Kirkwood G.P., Crombie J.A. and Beddington J.R. (1990). The assessment of stocks of annual squid species. Fish. Res. 8: 335-350.

Rowlings K.P. (1994). Den ecology and behavioural interactions of the Australian giant cuttlefish *Sepia apama*. Hons. Thesis, Flinders University, Adelaide, S.A., 37pp.

Rozas J. and Rozas R. (1999). DnaSP version 3: An integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15: 174-175.

Ryan M.J. (1997) Sexual selection and mate choice. In: Krebs J.R. and Davies N.B. (eds). Behavioural ecology: an evolutionary approach, 4th Edition. Blackwell Science Ltd., Oxford, U.K., pp 179-202.

Sakurai Y., Kiyofuji H., Saitoh S., Goto T. and Hiyama Y. (2000). Changes in inferred spawning areas of *Todares pacificus* (Cephalopoda: Ommastrephidae) due to changing environmental conditions. ICES J. Mar. Sci. 57: 24-30.

Sala E., Ballesteros E. and Starr R.M. (2001). Rapid decline of Nassau grouper spawning aggregations in Belize: fishery management and conservation needs. Fisheries 26: 23-30

Sale P.F. and Sharp B.J. (1983). Corrections for bias in visual transect census of coral reef fishes. Coral Reefs 1: 37-42.

Sánchez P. and Martín P. (1993). Population dynamics of the exploited cephalopod species of the Catalan Sea (NW Mediterranean). Sci. Mar. 57: 153-159.

SANTOS Ltd. (1985). Port Bonython environmental review, Vol. 4. Adelaide, S.A.

Sato T. and Hatanaka H. (1983). A review of assessment of Japanese distant-water fisheries for cephalopods. In: Caddy J.F. (ed.). The Cephalopods: factors relevant to their population dynamics and to the assessment and management of stocks. FAO Fish. Tech. Pap. 231: 145-180.

Sauer W.H.H. (1995). The impact of fishing on chokka squid *Loligo vulgaris reynaudii* concentrations on inshore spawning grounds in the south-eastern cape, South Africa. S. Afr. J. mar. Sci. 16: 185-193.

Sauer W.H.H., McCarthy C., Smale M.J. and Koorts A.S. (1993). An investigation of the egg distribution of the chokka squid, *Loligo vulgaris reynaudii*, in Krom Bay, South Africa. Bull. Mar. Sci. 53: 1066-1077.

Sauer W.H.H., Roberts M.J., Lipinski M.R., Smale M.J., Hanlon R.T., Webber D.M. and O'Dor R.K. (1997). Choreography of the squid's "nuptial dance". Biol. Bull. 192: 203-207.

Sauer W.H.H., Smale M.J. and Lipinski M.R. (1992). The location of spawning grounds, spawning and schooling behaviour of the squid *Loligo vulgaris reynaudii* (Cephalopoda: Myopsida) off the Eastern Cape Coast, South Africa. Mar. Biol. 114: 97-107.

Scheiner S. M. (1993). Genetics and evolution of phenotypic plasticity. Annu. Rev. Ecol. Syst. 24: 35-68.

- Schoener T.W. (1971). Theory of feeding strategies. *Annu. Rev. Ecol. Syst.* 2: 369-404.
- Seber G.A.F. (1982). Estimation of animal abundance and related parameters, 2nd Edition. Charles Griffin and Company Ltd., High Wycombe, U.K., 654 pp.
- Segawa S., Izuka T., Tamashiro T. and Okutani T. (1993). A note on mating and egg deposition by *Sepioteuthis lessoniana* in Ishigaki Island, Okinawa, southwestern Japan. *Venus* 52: 101-108.
- Shaw P.W., Pierce G.J. and Boyle P.R. (1999). Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. *Mol. Ecol.* 8: 407-417.
- Sheldon B.C. (1998). Recent studies in avian sex ratios. *Heredity* 80: 397-402.
- Sibly R. and Calow P. (1983). An integrated approach to life-cycle evolution using selective landscapes. *J. theor. Biol.* 102: 527-547.
- Silas E.G., Nair K.P., Meiyappan M.M., Rao K.S., Sarvesan R. and Sreenivasan P.V. (1985a). Utilisation and export of cephalopods. *CMFRI Bull.* 37: 165-171.
- Silas E.G., Sarvesan R., Nair K.P., Sastri Y.A., Sreenivasan P.V., Meiyappan M.M., Vidyasagar K. and Rao B.N. (1985b). Some aspects of the biology of cuttlefishes. *CMFRI Bull.* 37: 49-70.
- Sivak J.G. (1991). Shape and focal properties of the cephalopod ocular lens. *Can. J. Zool.* 69: 2501-2506.
- Slatkin M. (1995). A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139.
- Smith A. and Bruton J. (1977). A colour atlas of histological staining techniques. Wolfe Medical, London, U.K., 192pp.
- Smith J. (1999). Whyalla, South Australia. Cuttlefish capital of the world
- Starr R.M. and Thorne R.E. (1998). Acoustic assessment of squid stocks. In: Rodhouse P.G., Dawe E.G. and O'Dor R.K. (eds). Squid recruitment dynamics. The genus *Illex* as a model, the commercial *Illex* species and influences on variability. *FAO Fish. Tech. Pap.* 376: 181-198.
- Stearns S.C. (1976). Life-history tactics: a review of the ideas. *Q. Rev. Biol.* 51: 3-47.
- Stearns S.C. (1977). The evolution of life history traits: a critique of the theory and a review of the data. *Annu. Rev. Ecol. Syst.* 8: 145-71.
- Summers W.C. (1971). Age and growth of *Loligo pealei*, a population study of the common Atlantic coast squid. *Biol. Bull.* 141: 189-201.
- Sweeney M.J. and Roper C.F.E. (1998). Classification, type localities, and type repositories of Recent Cephalopoda. In: Voss N.A., Vecchione M., Toll R.B. and Sweeney M.J. (eds). Systematics and biogeography of cephalopods, Vol II. *Smithson. Contrib. Zool.* 586: 561-595.
- Swofford D.L. (1999). PAUP*: Phylogenetic Analysis using Parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, U.S.A.

- Tabachnick B.G. and Fidell L.S. (2001). Using multivariate statistics, 4th Edition. Allyn & Bacon, Needham Heights, M.A., U.S.A., 966pp.
- Taylor J.R. (1982). Introduction to error analysis: the study of uncertainties in physical measurements, 2nd Edition. University Science Books, Mill Valley, C.A., U.S.A., 270pp.
- Templeton A.R., Crandall K.A. and Sing C.F. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* 132: 619-633.
- Tinbergen L. (1939). Zur Fortpflanzungsethologie von *Sepia officinalis* L. *Arch. Néerl. Zool.* 3: 323-365.
- Tompsett D.H. (1939). *Sepia*. Williams and Norgate, London, U.K.
- Ueta Y., Tokai T. and Segawa S. (1999). Relationship between year-class abundance of the oval squid *Sepioteuthis lessoniana* and environmental factors off Tokushima Prefecture, Japan. *Fish. Sci.* 65: 424-431.
- Underwood A.J. and Kennelly S.J. (1990). Ecology of marine algae on rocky shores and subtidal reefs in temperate Australia. *Hydrobiologia* 192: 3-20.
- Van der Veer H.K., Berghan R. and Rijnsdorp A.D. (1994). Impact of juvenile growth on recruitment in flatfish. *Neth. J. Sea Res.* 32: 153-173.
- Van Heukelem W.F. (1979). Environmental control of reproduction and life span in *Octopus*: an hypothesis. In: Stancyk S.E. (ed.). Reproductive ecology of marine invertebrates. Carolina Press, U.S.A., pp. 123-133.
- Vaughan D.L. and Recksiek C.W. (1978). An acoustic investigation of market squid, *Loligo opalescens*. In: Recksiek C.W. and Frey H.W. (eds). Biological, oceanographic, and acoustic aspects of the market squid, *Loligo opalescens* Berry. *Calif. Fish Game* 169: 135-147.
- Villanueva R. (2000). Effect of temperature on statolith growth of the European squid *Loligo vulgaris* during early life. *Mar. Biol.* 136: 449-460.
- Voss G.L. (1983). A review of cephalopod fisheries biology. *Mem. Mus. Vic.* 44: 229-241.
- Wahl M. and Hay M.E. (1995). Associational resistance and shared doom: effects of epibiosis on herbivory. *Oecologia* 102: 329-340.
- Waluda C.M., Rodhouse P.G., Trathan P.N. and Pierce G.J. (2001). Remotely sensed mesoscale oceanography and the distribution of *Illex argentinus* in the South Atlantic. *Fish. Oceanogr.* 10: 207-216.
- Watanuki N., Iwashita T. and Kawamura G. (1993a). Sex composition and sexual maturity of *Sepia esculenta* captured in cuttlefish basket traps. *Bull. Jpn. Soc. Sci. Fish.* 59: 919-924.
- Watanuki N., Rodriguez E., Blanco R. and Badan R. (1993b). Introduction of cuttlefish basket traps in Palawan, Philippines. pp. 627-631. In: Okutani T., O'Dor R.K. and Kubodera T. (eds). Recent Advances in Fisheries Biology. Tokai University Press, Tokyo, Japan, pp. 627-631.

- Wells M.J. and Clarke A. (1996). Energetics: the costs of living and reproducing for an individual cephalopod. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 351: 1083-1104.
- Weir B.S. and Cockerham C.C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.
- Werren J.H. and Beukeboom L.W. (1998). Sex determination, sex ratios, and genetic conflict. *Annu. Rev. Ecol. Syst.* 29: 233-261.
- White M.J.D. (1973). *Animal cytology and evolution*. Cambridge University Press, Cambridge, U.K., 961pp.
- Widemo F. and Owens P.F. (1995). Lek size, male mating skew and the evolution of lekking. *Nature* 373: 148-151.
- Wiley E.O. (1978). The evolutionary species concept reconsidered. *Syst. Zool.* 27: 17-26.
- Wiley R.H. and Poston J. (1996). Indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution* 50: 1371-1381.
- Willis T.J. (2001). Visual census methods underestimate density and diversity of cryptic reef fishes. *J. Fish Biol.* 59: 1408-1411.
- Willmer P., Stone G. and Johnston I. (2000). *Environmental physiology of animals*. Blackwell Science, Oxford, U.K., 644pp.
- Wilson C.A., Beckman D.W. and Dean J.M. (1987). Calcein as a fluorescent marker of otoliths of larval and juvenile fish. *Trans. Am. Fish. Soc.* 116: 668-670.
- Wirtz P. and Morato T. (2001). Unequal sex ratios in longline catches. *J. Mar. Biol. Ass. U.K.* 81: 187-188.
- Wurtz M., Matricardi G. and Rebori F. (1991). Abundance estimates and distribution of *Sepia elegans* and *Sepia orbignyana* in two fishing areas of the central Mediterranean. In: Boucaud-Camou E. (ed.). *La Seiche, The Cuttlefish*. 1st International Symposium on the cuttlefish, *Sepia*, Caen, 1-3 June 1989. Centre de Publications de L'Universite de Caen, Caen, France, pp. 301-311.
- Yatsu A., Watanabe T., Mori J., Nagasawa K., Ishida Y., Meguro T., Kamei Y. and Sakurai Y. (2000). Interannual variability in stock abundance of the neon flying squid, *Ommastrephes bartramii*, in the North Pacific Ocean during 1979-1998: impact of driftnet fishing and oceanographic conditions. *Fish. Oceanogr.* 9: 163-170.
- Zar J.H. (1999). *Biostatistical analysis*, 4th Edition. Prentice-Hall Inc., Upper Saddle River, N.J., U.S.A., 663pp.
- Zeller D.C. (1998). Spawning aggregations: patterns of movement of the coral trout *Plectropomus leopardus* (Serranidae) as determined by ultrasonic telemetry. *Mar. Ecol. Prog. Ser.* 162: 253-263.

***APPENDIX I: Statistical analysis for
Chapter 4***

**PROJECT REPORT
2001 / P190**

**Seasonal Variation in
Cuttlefish**

Prepared by Julian Taylor

For Karina Hall



BiometricsSA

BiometricsSA is a co-operative group, established in late 1997 to provide statistical support for research in the South Australian Research and Development Institute (SARDI) and the Faculty of Agricultural and Natural Resource Sciences (FANRS) of The University of Adelaide.

BiometricsSA provides general statistical advice to the public and private sectors in Australia. The unit specialises in statistics for the Agricultural, Aquatic, Biological, Environmental, Food and Wine Sciences, but is also able to provide its statistical expertise in other fields.

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INTRODUCTION

This report details the comparisons of *Sepia apama* (cuttlefish) within defined sampled areas. The three aggregation areas chosen were defined according to the amount of commercial fishing present. In this case closed-closed (no commercial fishing), open-closed (commercial fishing allowed for half of first year), open-open (commercial fishing allowed in all years). For each of these areas, specific sites were chosen and are summarized in Table 1.

| CLOSED-CLOSED | OPEN-CLOSED | OPEN-OPEN |
|--------------------|-----------------------|--------------------|
| Black Point (U, A) | Stony Point (U, A) | Pt. lowly East (R) |
| 3rd Dip. (U, A) | Santos Tanks (U, A) | Fitz Bay (R) |
| WOSBF (U, A) | Pt. Lowly West (U, A) | |
| False Bay (R) | BHP Wall (B) | |

Table 1: Table of Sites inside each Area (Closed, Open, Open-Open)

Inside each of these sites the cuttlefish inhabited four different habitats. These habitats were Urchins (U), Algal (A), Reef (R) and Boulders (B). Each site represented in Table 1 describes the habitats contained inside it. For example at Black Point there was Urchin and an Algal habitat.

The sampling scheme was attempted over four years from 1998-2001. In each year the sites were sampled at varying times over the spawning season. One sampling time was representative of a sampling period of many days required to complete the task over all the sites. After an appropriate amount of time had passed the next sampling period was recorded as the next sampling time. Due to this inconsistency within sample times and between sample times the intervals are very irregular. For each time within each habitat within each site four transects were attempted and considered to be four replicates. The number of cuttlefish was recorded for each transect.

Due to the nature of the observational study some habitats were not sampled in some years. This information becomes irrelevant in the proceeding sections.

From this study two independent data sets have arisen.

- 1) **1998 Sampling:** The analysis on the first data set attempts to understand the behavior of the cuttlefish over the spawning season and whether the patterns over time change depending on the aggregation area. The data used for this will be generated from the year 1998.

It is clear that from aggregation areas the sites chosen must contain all habitats. Therefore from Table 1 Open-Closed and Closed-Closed contain all information about the Urchin and the Algal habitat. As Open-Open does not it is disregarded for further analysis. Also, in False Bay and BHP Wall, the Reef and Boulder habitats respectively must also be omitted.

Both of these omissions are due to the inference required for analysis. For this particular set of data the sites are nested within each aggregation area and each habitat must be nested within site. As a final difficulty the time is nested within each of the habitats. At the habitat level, Pt Lowly West is also missing eight observations from the Closed-Closed area within the algal habitat at time 4 and 5. It has been suggested that these be replaced with zeroes due to the certainty of this measurement at this particular time.

- 2) **Peak Time Sampling:** The second data set involves observations from the years 1998, 1999, 2000. In these years the observations have been generated from the peak of the season. Similar to the previous data set the Open-Open area is not considered and the sites False Bay, BHP Wall are disregarded.

The peak time each year for this sampling has been objectively judged by the observer and therefore will be possibly biased. This bias will not be able to accurately determined and may infer results that are not necessarily true.

MATERIALS

The experiments will be handled in stages.

1998 Sampling

Figure 1 describes the cuttlefish numbers over the spawning season of 1998. The missing values can be clearly seen in time 4 and 5 for Pt Lowly West. It is clear from this plot that the Urchin habitat has higher counts than the Algal habitat. Within this habitat the sites that were originally closed to commercial fishing, namely, Stony Pt, Santos, Pt Lowly West indicate smaller counts of cuttlefish than the fished sites, Black Pt, 3rdDip, WOSBF.

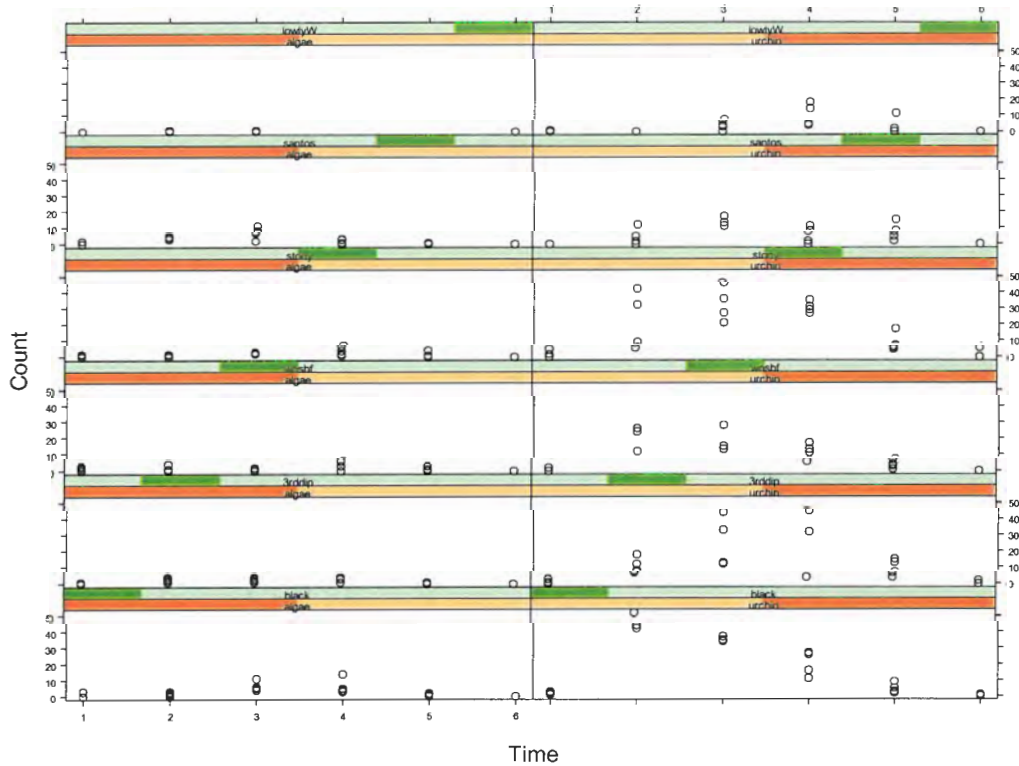


Figure 4: Cuttlefish counts over the spawning season of 1998 across six sites (Black Pt (BP), Stony Pt (SP), 3rdDip, WOSBF, Santos, Pt Lowly West) and two habitat types (Urchin and Algal)

At the beginning and the end of the spawning season many zeroes exist in each of the replicates for each habitat within each site. The number of zeroes increases further for all sites in the algal habitat. Special precautions must be taken to include these zeroes. Furthermore, one can expect that the counts generated at adjacent sampling times would be correlated. A simple example explanation of this correlation can be taken from Figure 1. The observations at time 3 would be expected to increase as the last two intervals show an increasing sequence.

The response, counts of cuttlefish, is not a Normally distributed variate. Although, this variable can be transformed in a number of ways to ensure the aforementioned assumption. To maintain the possible high correlation between the observations at the pseudo-replicate level and the serial correlation that may exist between times it is suggested that the response be transformed using the mathematical square root. If y_i is the i th count of cuttlefish the new response would be

$$y_i^* = \text{sqrt}(y_i + 1)$$

The included unit value is to ensure that the square root does not calculate $\text{sqrt}(0)$, which is undefined. This transformation can be assessed by fitting the full model (see next section) with the transformed response.

Peak Time Sampling

Figure 2 describes the counts of the cuttlefish at peak times of each year from 1998 to 2000. Again all the urchin habitats have higher numbers of cuttlefish at the height of the spawning season. Also the counts are slightly lower for the area originally closed to fishing (Stony Pt. Santos, Pt Lowly West) in both habitats.

It is apparent that there were low numbers of recorded counts in 1998 (the year that commercial fishing occurred) compared to the following years. This suggests that there may be a strong year effect as well as an area effect.

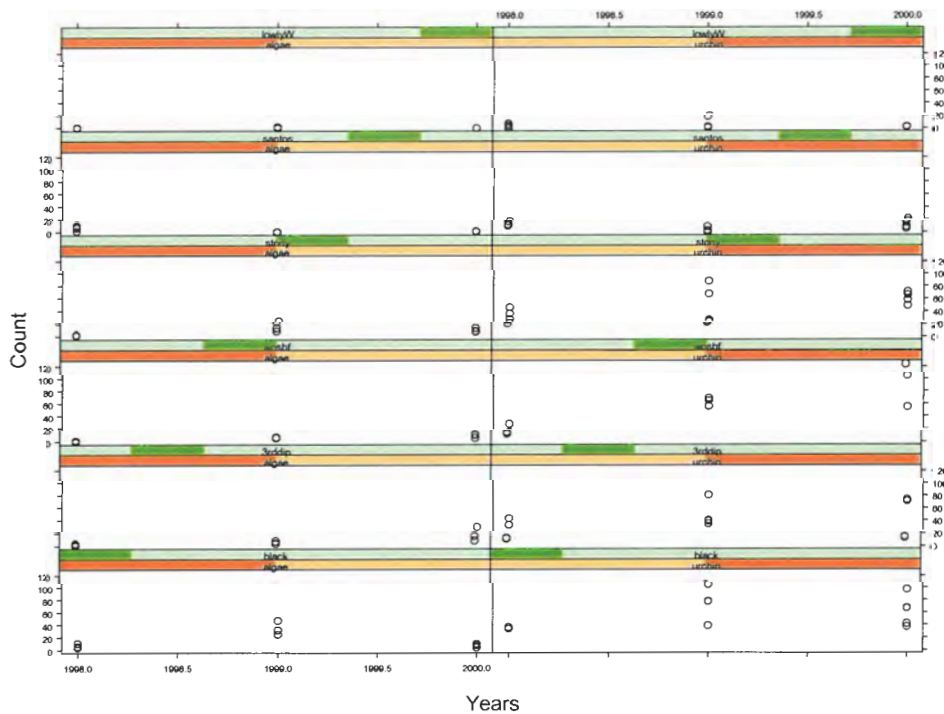


Figure 5: Cuttlefish counts at peak times from 1998-2000 across six sites ((Black Pt (BP), Stony Pt (SP), 3rdDip, WOSBF, Santos, Pt Lowly West) and two habitat types (Urchin and Algal)

The response for this experiment, the number of cuttlefish, is a count and therefore is not a Normally distributed variate. However, this variate may be transformed to Normality using natural logs. If y_i is the i th count of cuttlefish the new response would be

$$y_i^* = \log(y_i + 1)$$

The included unit 1 is to prevent the log function from calculating $\log(0)$. These new responses may be back transformed after performing analysis to ensure a valid result.

Figure 3 describes the Fitted vs Residual Plot from this full model and the histogram for the different habitats included in the model. The Fitted vs Residual plot shows an even scatter around the zero line and the histogram describes a normal distribution for the residuals of the full model.

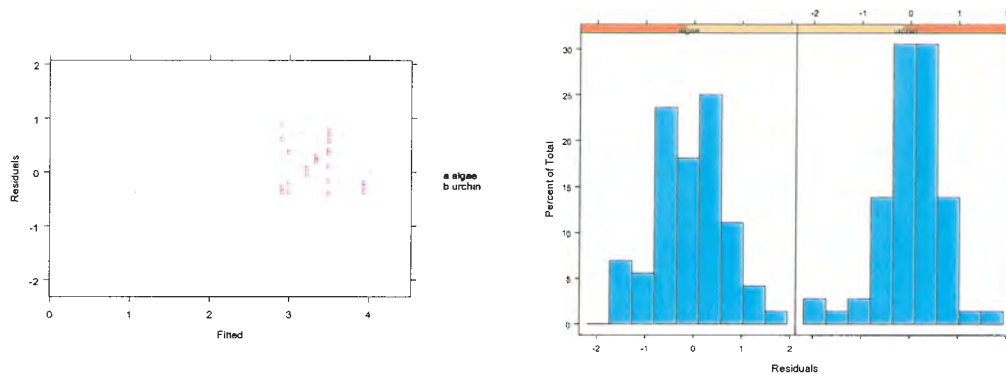


Figure 3: Residuals vs Fitted Plot and histograms of the residuals for the full model described in

METHODS

The techniques detailed in this section will be presented for each independent data set. The techniques from data set to data set will alter due to the temporal conditions of the data and the nature of the zeroes present.

1998 Sampling

The temporal nature of the 1998 data requires the correlation between time intervals to be explained appropriately. This repeated measures component of the model becomes a more difficult model due to the non-linear nature of the counts over time. This non-linearity can be seen clearly in Figure 1.

The extra zeroes that are abundant at the start and end of the season enhance the difficulty of the modelling process again due to the response being bounded at zero. To help this inflexibility the sixth time component of the data set has been discarded due to the fact that it contains nearly all zeroes across all sites and habitats.

The type and accuracy of the analysis depends on using the appropriate structure for the data. Here the following structure is proposed. The Sites are nested within Habitats due to there being different sites inside the Open-Closed and Closed-Closed Area. The two Habitats have been sampled to obtain crossed effects across all Sites but the physical difference, say, between two Algal habitats is prevalent when changing Sites. Therefore the habitats are deemed to be nested inside each site to ensure this difference is accounted for.

The Time component of the model is treated as a covariate. To allow for the inherent non-linear nature of this dependence over time a complex structure is proposed. This structure requires the use of piecewise cubic splines. The use of the cubic splines is to allow for smooth components of the data over time which deviate from linearity. This non-linearity can be clearly seen from Figure 1. These deviations may then be tested for inclusion and inevitably decide the overall fit of the model.

Alongside this non-linear temporal structure the observations at nearby times are expected to be correlated. This correlation can be modelled using an autoregressive time series, which allows the slow degradation of dependence over time. This degradation is apparent in the physical differences expected over time of the cuttlefish counts over the spawning season.

The reps at the bottom level of the 1998 experiment are considered to be correlated with each other. This correlation is due to the physical locality of the adjacent transects at the same bottom level of the structure. Incorporating this correlation will ensure that the "pseudo-reps" will be treated appropriately. The proposed correlation for this experiment requires certain limitations to ensure appropriate estimation. Here, it is proposed that within each habitat, Algal and

Urchin, the four transects are correlated differently. Therefore for higher level structures, i.e sites within each year, the correlation model will be identical and the parameter estimates for the correlations will be pooled to reflect this.

The full model can therefore be expressed as

$$\mathbf{y}_{ijk} = \text{site}_i + \text{site.habitat}_{ij} + \text{time}_{(k)} + \text{site : habitat : time}_{ij(k)} + \text{spl}(\text{time}_{(k)}) + \text{time}_k^* + \text{site.habitat.time}_{ijk}^* + \text{spl}(\text{site.habitat.time}_{ij(k)}) + \boldsymbol{\varepsilon}_{ijk}$$

where, $i = 1,2,3; j = 1,2; k = 1,\dots,5$; and $\boldsymbol{\varepsilon}_{ijk}$ is distributed as mean zero and variance

$$\Omega = \text{Var}(\boldsymbol{\varepsilon}_{ijk}) = \begin{pmatrix} \sigma_j^2 & \rho_j & \rho_j & \rho_j \\ \rho_j & \sigma_j^2 & \rho_j & \rho_j \\ \rho_j & \rho_j & \sigma_j^2 & \rho_j \\ \rho_j & \rho_j & \rho_j & \sigma_j^2 \end{pmatrix}, j = 1,2$$

where $\rho_j, j = 1,2$ is the correlation in the Algal and Urchin habitat respectively. This structure ensures that the correlation will be different for each habitat whilst maintaining a simplistic approach to allow for the similarities between sampled transects.

Each of the terms in the above model needs to be explained further. This model contains a combination of random and fixed effects and each term in the model is a reflection of either of these effects. The terms are given below with the proposed effects structure.

site: will be considered to be fixed effect of interest to help determine the differences between the different sites sampled in the project.

site.habitat: will be considered to be a fixed effect of interest. Here, the difference between habitats within sites can be determined.

time: is used as a covariate to determine any linear effect over time. The cubic smoothing spline requires this term in the fixed component of the model to ascertain any changes from linearity over time.

site.habitat.time: is used as a covariate to help the piecewise cubic spline fit correctly to each habitat within each site.

*time**: is used to describe an overall random effect for time to pick up systematic changes over time that might be present over all sites and habitats.

*site.habitat.time**: is considered to be a random effect associated with systematic changes over time that might be present within each of the habitats inside each site.

spl(time): is considered a random effect associated with an *overall* spline smoothing component. This used to help describe the *overall* non-linear component of the model.

site.habitat:spl(time): is considered a random effect associated with a spline smoothing component for each habitat within each site. This effect ensures that different splines can be fitted at different levels of the structure. This inclusion is necessary due to the obvious different non-linear structures in Figure 1.

All fixed effects are tested using a Wald statistic which is asymptotically distributed as chi-square. These effects are tested sequentially and allow the determination of significant terms such as differences between Sites or differences between habitats within sites.

The blocking variables, random effects and cubic smoothing spline terms with their associated variance/correlation parameters can also be tested for inclusion in the model by assuming a null hypothesis that the associated variance component is zero. Using Restricted Maximum Likelihood (REML, see Patterson and Thompson, 1971) theory, twice the difference between the original likelihood L_1 and the null hypothesis likelihood, L_0 is distributed asymptotically chi-squared with one degree of freedom, namely,

The probability of obtaining a value greater than 3.84, in this case, is 5 %. This shall be

$$-2(L_1 - L_0) \sim \chi_1^2$$

reserved as the cut-off value.

(Note: area has not been included in the model as a fixed or random effect. This is due to the ability to formulate the model to test the three sites inside the closed area against the three sites in the open area (see the next section).)

Peak Time Sampling

This particular data can be handled more simply than the previous data. As the data is for one time during each season the temporal component of the model is not required. There are few non-zero counts in the response due to the peak time being taken in each season. This also allows a simpler model to be utilized.

For this particular set of data the following structure is proposed. The Open-Closed and Closed -Closed Areas are crossed with the Year. Inside this crossing of factors the Sites are nested inside the Open-Closed and Closed-Closed Area. This is because Stony Pt, Santos, Pt Lowly West are not found inside the Open-Closed area and Black Point, 3rdDip and WOSBF do not appear in the Closed-Closed area. Within each of these sites the habitats could be deemed as being crossed but have been allocated as being nested due to the different nature of each site included in the analysis.

At the bottom level of the structure the supposed "reps" are most likely highly correlated due to the measurements coming from adjacent transects and in the same habitat inside each site at each area within each year. This proposed correlation requires certain limitations to ensure appropriate estimation. Here, it is proposed that within each habitat, Algal and Urchin, the four transects are correlated differently. Therefore for higher level structures, i.e. sites within each year, the correlation model will be identical and the parameter estimates for the correlations will be pooled to reflect this. Theoretically the full final model will have the form

$$y_{ijkl} = year_i + area_j + site_k + year.site_{ik} + site.habitat_{kl} + year.site.habitat_{ikl} + \boldsymbol{\varepsilon}_{ijkl} \quad (1)$$

where, $i = 1,2,3; j = 1,2; k = 1, \dots, 6; l = 1,2$ and $\boldsymbol{\varepsilon}_{ijkl}$ is distributed with mean zero and variance

$$Var(\boldsymbol{\varepsilon}_{ijkl}) = \begin{pmatrix} \sigma_l^2 & \rho_l & \rho_l & \rho_l \\ \rho_l & \sigma_l^2 & \rho_l & \rho_l \\ \rho_l & \rho_l & \sigma_l^2 & \rho_l \\ \rho_l & \rho_l & \rho_l & \sigma_l^2 \end{pmatrix}, \quad l = 1,2$$

where $\rho_l, l = 1,2$ is the correlation between sampled transects in the Algal and Urchin habitat respectively. This structure ensures that the correlation will be different for each habitat whilst maintaining a simplistic approach to allow for the similarities between sampled transects.

The model proposed above consists of effects of interest (fixed effects) and effects that are considered to be blocking variables or random effects. The blocking structure allows the variation of the associated variable to be accounted for without estimating its mean. For this particular model the effects are given below along with the associated reasons.

Year: will be considered to be a blocking variable. This is due to the fact that at the top level of the design there is no replication for Year. Without appropriate replication the Year cannot be estimated as a fixed effect accurately.

Area: will be a fixed effect. This variable is of interest in determining the differences between the commercially fished area and the non-commercially fished area.

Site: will be a blocking variable. This blocking variable is a product of the sampling areas required for the experiment and therefore is not of interest for fixed estimation. Each site is inside each year and therefore crossed with year.

Habitat: will be a blocking variable. This is also a product of the sampling area used. The two habitats are nested within each site and crossed with each year.

(Note: Any effects present in the model that are an interaction of fixed effects with random effects are deemed to be random also.)

The fixed effects can be tested easily using a Wald statistic which has an asymptotic chi-squared distribution. The blocking variables with their associated variance/correlation parameters can also be tested for inclusion in the model by assuming a null hypothesis that the associated variance component is zero. Using likelihood theory, twice the difference between the original likelihood L_1 and the null hypothesis likelihood, L_0 is distributed asymptotically chi-squared with one degree of freedom, namely,

$$-2(L_1 - L_0) \sim \chi_1^2$$

The probability of obtaining a value greater than 3.84, in this case, is 5%. This shall be reserved as the cutoff value.

Using these tests the final model can be concluded by iteratively dropping terms from the model and checking the differences between the likelihoods. If the statistic is below 3.84 and is the smallest then the term that was being tested is discarded from the final model.

All models and model testing was achieved in S-PLUS 6.0 (Insightful Inc.). The peak time experiment models were fit using `samm()` which uses ASReml.

RESULTS & CONCLUSIONS

This section will also be presented in stages.

1.1 1998 Sampling

The full model presented in the previous section was fitted using `samm()` and the results can be found in Output 1. The results show that the objective function did not converge under this model and a new simpler model must be sought. Using the next section **Results 1.2** it has been suggested that the correlation between each transect is minimal and can be ignored. This adjustment was made and the resultant fit can also be found in Output 1.

An initial glance at the significance of the fixed effects at the 5% level shows the habitat within site effects are large. This is clearly suggested from Figure 1 where the large difference in the counts over the season is obvious.

The accuracy of the fixed effect relies on the appropriate null model being fit to the data. The correlation parameters and the variance/covariance parameters can be tested for inclusion in the model by using the asymptotic likelihood of the previous section. The correlation between transects has been dropped and therefore only a single variance parameter exists between transects inside each habitat.

The autoregressive correlations that exist across the times for each habitat type can be tested for inclusion in the model. Each autoregressive correlation parameter was dropped from the model and tested using the asymptotic likelihood ratio statistic. The autoregressive parameter associated with the times over the algal habitat is not significant at the 5% level (p-value = 0.380) and is dropped from the model. The autoregressive parameter associated with the times over the urchin habitat shows significance at the 5% level (p-value = 0.0354) and is retained in the final model.

The variance components for the overall time random effect and the time within habitat within site random effects are small and the change in likelihood after reducing the model without them is negligible. They are discarded from the model.

The final variance components to test are the spline variance parameters which determine the overall non-linear curvature and the curvature that required at the habitat within each site. Dropping each one from the model alternately it was found that the non-linearity for each habitat within each site is significantly different. The overall spline variance was not significant at the 5% level (p-value = 0.07367) and can therefore be dropped from the final model.

The Analysis of Variance table can be found at the end of Output 1. It shows there is significantly different linearity between habitats with each site. This is to be expected as there are more cuttlefish found in the Urchin habitat. There is also differences between the habitats within each site themselves. This difference is obvious in Figure 5, which describes the fitted values of the final model.

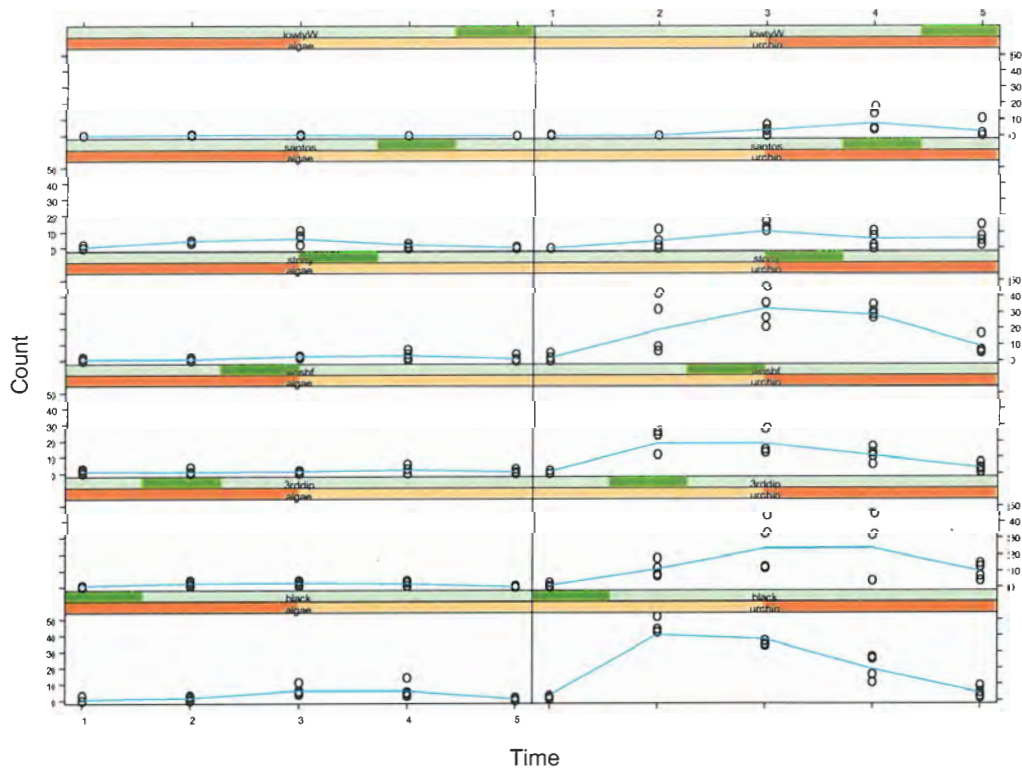


Figure 5: Fitted values of cuttlefish counts over the spawning season of 1998 across six sites (Black Pt (BP), Stony Pt (SP), 3rdDip, WOSBF, Santos, Pt Lowly West) and two habitat types (Urchin and Algal).

1.2 Peak Time Sampling

The full model suggested in the previous section was fit using `samm()` with the appropriate fixed and random effects. The results can be found in Output 2. In the same Output the area fixed effect is tested using the Wald statistic. It can be seen, in the first instance, that there is no significant difference (pvalue = 0.09954) at the 5% level between the commercially fished and non-commercially fished areas. Although at the 10% level it is clear that there is a significant difference.

To ensure accurate area effects, each correlation parameter and variance parameter can be tested for inclusion in the model using the asymptotic likelihood ratio statistics of the previous section. Each habitat was initially given its own 4 x 4 correlation matrix pooled across all the sites. This correlation inside each habitat can be tested for inclusion. Consider the null hypothesis,

$$H_0: \rho_1 = \rho_2 = 0$$

As the difference between the original model and the new null hypothesis model is two parameters then the probability that the asymptotic statistic is distributed as χ^2 with two degrees of freedom. Then the probability that the statistic has a value greater than 5.99 is 5%. The results of this test can be found in Output 2. The value for the statistics 0.19 and therefore the null hypothesis is retained and it is confirmed that at the 5% significance level the correlation parameters are zero. The model can be refit allowing only different variance components with *no* correlation between the transects for each habitat.

The new model can be found in Output 2. To test whether the variance components are the same for the habitats, Algal and Urchin, the new null hypothesis to consider is

$$H_0: \sigma_1^2 = \sigma_2^2$$

This can be tested using the asymptotic likelihood statistic of the previous section. The resultant test can be found in Output 2. The value for the statistic is 4.77 which is greater than 3.84 and therefore we reject the null hypothesis at the 5% level and conclude that the variance of the transects for the Algal habitat are not the same as the variances of the transects for the Urchin habitat. The previous model is retained.

The variance components associated with each random effect within this model can now be tested by dropping each effect in turn to produce a null model. Each of these null models has only one parameter difference from the full model, i.e. the variance component associated with the effect. Here from model (1) in the previous section the aforementioned random effects will be *year*, *site*, *year.site*, *site.habitat*, *year.site.habitat*. The results of dropping each effect in turn can be found in Output 2.

From the first elimination process *year* and *site* offer no contribution to the overall likelihood. This provides evidence for no year variation effect and therefore no change of the counts of cuttlefish can be concluded from this data. Similarly the site variation effect is very small. This suggests the pattern of spawning cuttlefish does not change from site to site across years.

Dropping these two terms from the model the remaining random effects can then be retested exactly as above. The results of this test can be found in Output 2. With a value of 3.47 for the asymptotic likelihood ratio the habitat within site within year effect is not significant at the 5% level and therefore can be dropped from the model.

The final model can be written as

$$y_{ijkl} = area_j + year.site_{ik} + site.habitat_{kl} + \epsilon_{ijkl} \quad (2)$$

where $i = 1,2,3; j = 1,2; k = 1,\dots,6; l = 1,2$ and *year.site* and *site.habitat* are random effects and *area* is the fixed effect of interest. Here, ϵ_{ijkl} has a mean zero and variance

$$Var(\epsilon_{ijkl}) = \begin{pmatrix} \sigma_l^2 & 0 & 0 & 0 \\ 0 & \sigma_l^2 & 0 & 0 \\ 0 & 0 & \sigma_l^2 & 0 \\ 0 & 0 & 0 & \sigma_l^2 \end{pmatrix}, \quad l = 1,2$$

In this model the variance components associated with *year.site* and *site.habitat* are $\sigma_{ys}^2 = 0.360$ and $\sigma_{sh}^2 = 1.182$. These seem small but have a significant effect on the fit of the model. This suggests that the variation of the cuttlefish between sites in each year is very significant. This is expected as the different areas (Closed-Closed, Open-Closed) will markedly increase the variation among the sites in any given year. Similarly the variation effect of habitats within each site across all three years is also very significant. This is also expected as the counts of cuttlefish from the urchin and algal habitats are different providing larger variation. Also, differences of the habitats due to the area are also possible.

Please note that the significance of these effects is not of particular interest. The estimation of these variance components is to allow for the spatial and temporal component of the design to ensure the accurate estimation of the area effects.

The final model in (2) provides slightly different estimates for the fixed effects due to the discarding of variance components from the model. From the end of Output 2 it can be seen at the 5% level (pvalue = 0.10667) the area effects are not significant. However, biologically this may be viewed as a large enough difference to be deemed important.

OUTPUT 1 - 1998 SAMPLING

Initial fit

```
> fm98ExpS <- samm(countq ~ site + timev + site:habitat + site:habitat:timev,
random = ~time + site:habitat:time + spl(timev) + site:habitat:
+ spl(timev), rcov = ~ site:at(habitat):ar1(time):cor(rep), data =
cutt98FinalA, maxiter = 25)
Convergence monitoring: WARNING: LogLikelihood not converged
Error in samm.aireml(data, asr.inter, asr.struc, asr.glm, weights, o...: ifault
!= 0
```

```
> fm98Exp <- samm(countq ~ site + timev + site:habitat + site:habitat:timev,
random = ~time + site:habitat:time + spl(timev) + site:habitat:
+ spl(timev), rcov = ~ site:at(habitat):ar1(time):id(rep), data =
cutt98FinalA, maxiter = 25)
```

```
> summary(fm98Exp)$varcomp
```

| | gamma | component | std.error | z.ratio | constraint |
|-------------------------|----------------|----------------|------------|------------|---------------|
| time | 1.263752e-007 | 1.263752e-007 | NA | NA | Boundary |
| site:habitat:time | 8.284521e-002 | 8.284521e-002 | 0.12173312 | 0.6805478 | Positive |
| spl(timev) | 6.932027e-001 | 6.932027e-001 | 0.77015824 | 0.9000782 | Positive |
| site:habitat:spl(timev) | 1.775545e+000 | 1.775545e+000 | 0.89597870 | 1.9816827 | Positive |
| habitat_urchin!variance | 4.040633e-001 | 4.040633e-001 | 0.06135481 | 6.5856823 | Positive |
| habitat_urchin!time.ar1 | 1.630308e-001 | 1.630308e-001 | 0.10356329 | 1.5742140 | Unconstrained |
| habitat_algal!variance | 5.532080e-001 | 5.532080e-001 | 0.08195698 | 6.7499807 | Positive |
| habitat_algal!time.ar1 | -9.640199e-002 | -9.640199e-002 | 0.11421833 | -0.8440151 | Unconstrained |

```
> anova(fm98Exp)
Wald tests for fixed effects
```

Response: countq

Terms added sequentially; adjusted for those above

| | Df | Sum of Sq | Wald statistic | Pr(Chisq) |
|--------------------|----|-----------|----------------|-------------|
| (Intercept) | 1 | 1678.311 | 1678.311 | 0.000000000 |
| site | 5 | 86.224 | 86.224 | 0.000000000 |
| timev | 1 | 12.123 | 12.123 | 0.000498028 |
| site:habitat | 6 | 244.951 | 244.951 | 0.000000000 |
| site:habitat:timev | 11 | 25.913 | 25.913 | 0.006685926 |
| residual | | 1.000 | | |

Testing correlations

```
fm98ExpA <- samm(countq ~ site + timev + site:habitat + site:habitat:timev,
random = ~ time + site:habitat:time + spl(timev) + site:habitat:spl(timev),
rcov = ~site:at(habitat, 1):ar1(time):id(rep), data = cutt98FinalA, maxiter = 25)
```

```
> summary(fm98ExpA)$varcomp
```

| | gamma | component | std.error | z.ratio | constraint |
|-------------------------|----------------|----------------|------------|------------|---------------|
| time | 1.263752e-007 | 1.263752e-007 | NA | NA | Boundary |
| site:habitat:time | 8.296219e-002 | 8.296219e-002 | 0.12079329 | 0.6868112 | Positive |
| spl(timev) | 6.848857e-001 | 6.848857e-001 | 0.75710142 | 0.9046155 | Positive |
| site:habitat:spl(timev) | 1.742840e+000 | 1.742840e+000 | 0.88084130 | 1.9786078 | Positive |
| habitat_urchin!variance | 4.066368e-001 | 4.066368e-001 | 0.06080750 | 6.6872809 | Positive |
| habitat_algal!variance | 5.534116e-001 | 5.534116e-001 | 0.08200032 | 6.7488952 | Positive |
| habitat_algal!time.ar1 | -9.651770e-002 | -9.651770e-002 | 0.11421877 | -0.8450249 | Unconstrained |

```
> -2*(fm98ExpA$loglik - fm98Exp$loglik)
[1] 2.268738
> 1 - pchisq(2.268738, 1)
[1] 0.1320074
```

```
fm98ExpB <- samm(countq ~ site + timev + site:habitat + site:habitat:timev,
random = ~ time + site:habitat:time+ spl(timev) + site:habitat:spl(timev),
rcov = ~ site:at(habitat, 2):ar1(time):id(rep), data = cutt98FinalA, maxiter
= 25)
```

```
> summary(fm98ExpB)$varcomp
              gamma      component  std.error  z.ratio  constraint
time 1.263752e-007 1.263752e-007      NA      NA      Boundary
site:habitat:time 7.866147e-002 7.866147e-002 0.12186177 0.6454976      Positive
spl(timev) 6.893145e-001 6.893145e-001 0.77067772 0.8944264      Positive
site:habitat:spl(timev) 1.803504e+000 1.803504e+000 0.90754808 1.9872264      Positive
habitat_urchin!variance 4.040616e-001 4.040616e-001 0.06135088 6.5860764      Positive
habitat_urchin!time.ar1 1.630632e-001 1.630632e-001 0.10355965 1.5745820 Unconstrained
habitat_algal!variance 5.540843e-001 5.540843e-001 0.08156076 6.7935157      Positive
```

```
> -2*(fm98ExpB$loglik - fm98Exp$loglik)
[1] 0.7693932
> 1 - pchisq(0.7693932, 1)
[1] 0.3804048
```

```
fm98ExpC <- samm(countq ~ site + timev + site:habitat + site:habitat:timev,
random = ~ time + site:habitat:time+ spl(timev) + site:habitat:spl(timev),
rcov = ~ site:habitat:ar1(time):id(rep), data = cutt98FinalA, maxiter = 25)
```

```
> summary(fm98ExpC)$varcomp
              gamma      component  std.error  z.ratio  constraint
time 0.00000010 4.823604e-008 5.084787e-009 9.4863432      Boundary
site:habitat:time 0.15235069 7.348794e-002 1.189509e-001 0.6178006      Positive
spl(timev) 1.41690158 6.834572e-001 7.566834e-001 0.9032273      Positive
site:habitat:spl(timev) 3.60564462 1.739220e+000 8.765951e-001 1.9840633      Positive
R!variance 1.00000000 4.823604e-001 5.084787e-002 9.4863432      Positive
R!time.ar1 0.01362347 1.362347e-002 7.828208e-002 0.1740305 Unconstrained
```

```
> -2*(fm98ExpC$loglik - fm98ExpB$loglik)
[1] 4.425044
> 1 - pchisq(4.425044, 1)
[1] 0.0354152
```

Testing variance components

```
fm98ExpD <- samm(countq ~ site + timev + site:habitat + site:habitat:timev,
random = ~ spl(timev) + site:habitat:spl(timev),
rcov = ~ site:at(habitat, 2):ar1(time):id(rep), data = cutt98FinalA, maxiter
= 25)
```

```
> summary(fm98ExpD)$varcomp
              gamma component  std.error  z.ratio  constraint
spl(timev) 0.6170240 0.6170240 0.72246048 0.8540592      Positive
site:habitat:spl(timev) 2.3543274 2.3543274 0.80754610 2.9154093      Positive
habitat_urchin!variance 0.4074930 0.4074930 0.06181520 6.5921163      Positive
habitat_urchin!time.ar1 0.1645623 0.1645623 0.10363377 1.5879217 Unconstrained
habitat_algal!variance 0.5569818 0.5569818 0.08166557 6.8202772      Positive
> -2*(fm98ExpD$loglik - fm98ExpB$loglik)
[1] 0.3752619
```

```
fm98ExpE <- samm(countq ~ site + timev + site:habitat + site:habitat:timev,
random = ~ spl(timev),
rcov = ~ site:at(habitat, 2):ar1(time):id(rep), data = cutt98FinalA, maxiter
= 25)
```

```
> summary(fm98ExpE)$varcomp
              gamma component  std.error  z.ratio  constraint
spl(timev) 0.8187718 0.8187718 0.7471808 1.095815      Positive
habitat_urchin!variance 1.0710041 1.0710041 0.1532726 6.987578      Positive
habitat_urchin!time.ar1 -0.1713813 -0.1713813 0.1023132 -1.675065 Unconstrained
habitat_algal!variance 0.7805549 0.7805549 0.1103007 7.076608      Positive
> -2*(fm98ExpE$loglik - fm98ExpD$loglik)
[1] 84.63174
> 1 - pchisq(84.63174, 1)
[1] 0
```

```
fm98ExpF <- samm(countq ~ site + timev + site:habitat + site:habitat:timev,
random = ~ site:habitat:spl(timev),
```

```
rcov = ~ site:at(habitat, 2):ar1(time):id(rep), data = cutt98FinalA, maxiter
= 25)
```

```
> summary(fm98ExpF)$varcomp
      gamma component  std.error  z.ratio  constraint
site:habitat:spl(timev) 3.2532781 3.2532781 0.97904952 3.322894    Positive
habitat_urchin!variance 0.4013245 0.4013245 0.06055562 6.627370    Positive
habitat_urchin!time.ar1 0.1633049 0.1633049 0.10322852 1.581975 Unconstrained
habitat_algal!variance 0.5542247 0.5542247 0.08135031 6.812817    Positive
```

```
> -2*(fm98ExpF$loglik - fm98ExpD$loglik)
[1] 3.199081
> 1 - pchisq(3.199081, 1)
[1] 0.07367966
```

```
> anova(fm98ExpG)
Wald tests for fixed effects
```

Response: countq

Terms added sequentially; adjusted for those above

| | Df | Sum of Sq | Wald statistic | Pr(Chisq) |
|--------------------|----|-----------|----------------|----------------|
| site | 6 | 2736.745 | 2736.745 | 0.000000000000 |
| timev | 1 | 19.113 | 19.113 | 0.00001231754 |
| site:habitat | 6 | 464.618 | 464.618 | 0.000000000000 |
| site:habitat:timev | 11 | 45.881 | 45.881 | 0.00000339379 |
| residual | | 1.000 | | |

```
> anova(fm98ExpG, ssType = 3)
Wald tests for fixed effects
```

Response: countq

Terms adjusted for all others

| | Df | Sum of Sq | Wald statistic | Pr(Chisq) |
|--------------------|----|-----------|----------------|-----------|
| site | 6 | 117.3618 | 117.3618 | 0.0000000 |
| timev | 1 | 1.6183 | 1.6183 | 0.2033307 |
| site:habitat | 6 | 76.9743 | 76.9743 | 0.0000000 |
| site:habitat:timev | 11 | 45.8810 | 45.8810 | 0.0000034 |
| residual | | 1.0000 | | |

OUTPUT 2 - PEAK TIME-SAMPLING

Initial Fit

```
fmNormRand <- samm(countlog ~ area, random = ~ year*(site/habitat),rcov = ~
year:site:at(habitat):cor(rep), family = samm.gaussian(), maxiter = 15, data =
cuttPeakFinal, stepsize = 0.05)
```

Spatial Analysis <> Mixed Models (Version: 1.0 AISamm license: 30 Jun 2002)
SAMM Convergence Monitoring: Fri Mar 08 12:27:16 2002

```
Seq Component
1 year
2 site
3 site:habitat
4 year:site
5 year:site:habitat
6 habitat_urchin!variance
7 habitat_urchin!rep.cor
8 habitat_algal!variance
9 habitat_algal!rep.cor
```


Equations: 83 (4 dense)
 Initial update shrinkage factor: 0.224
 Singularities: 1 (more)

| 5 | LogLik | S2 | DF | 1 | 2 | 3 | 4 |
|-------------------|-------------------|-------|-------|-------|----------|-------|-------|
| 6 | 7 | 8 | 9 | | | | |
| -46.6335 | 1.0000 | 138 | 0.095 | 0.095 | 0.095 | 0.095 | 0.095 |
| 0.095 | 0.761 | 0.100 | 0.543 | 0.100 | 12:27:16 | | |
| -28.0292 | 1.0000 | 138 | 0.052 | 0.088 | 0.159 | 0.154 | |
| 0.045 | 0.384 | 0.257 | 0.474 | 0.102 | 12:27:16 | | |
| -19.5878 | 1.0000 | 138 | 0.019 | 0.094 | 0.264 | 0.223 | |
| 0.004 | 0.305 | 0.338 | 0.416 | 0.148 | 12:27:16 | | |
| -15.6337 | 1.0000 | 138 | 0.000 | 0.104 | 0.432 | 0.284 | |
| 0.004 | 0.257 | 0.314 | 0.386 | 0.167 | 12:27:16 | | |
| -13.7701 | 1.0000 | 138 | 0.000 | 0.010 | 0.764 | 0.334 | |
| 0.004 | 0.228 | 0.248 | 0.420 | 0.246 | 12:27:16 | | |
| -13.3627 | 1.0000 | 138 | 0.000 | 0.000 | 1.158 | 0.358 | |
| 0.004 | 0.194 | 0.138 | 0.421 | 0.258 | 12:27:16 | | |
| -13.3990 | 1.0000 | 138 | 0.000 | 0.000 | 1.181 | 0.342 | |
| 0.004 | 0.180 | 0.076 | 0.432 | 0.276 | 12:27:16 | | |
| -13.3481 | 1.0000 | 138 | 0.000 | 0.000 | 1.164 | 0.339 | |
| 0.004 | 0.196 | 0.130 | 0.423 | 0.263 | 12:27:16 | | |
| -13.3447 | 1.0000 | 138 | 0.000 | 0.000 | 1.164 | 0.339 | |
| 0.004 | 0.201 | 0.148 | 0.418 | 0.255 | 12:27:16 | | |
| -13.3446 | 1.0000 | 138 | 0.000 | 0.000 | 1.165 | 0.339 | |
| 0.004 | 0.202 | 0.150 | 0.419 | 0.255 | 12:27:17 | | |
| FINAL | parameter values: | | 0.000 | 0.000 | 1.165 | 0.339 | |
| 0.004 | 0.202 | 0.151 | 0.418 | 0.255 | | | |
| Constraint codes: | | | B | B | P | P | |
| S | P | U | P | U | | | |

Exit status: 0 - LogLikelihood Converged

Finished on: Fri Mar 08 12:27:17 2002

```
> anova(fmNormRand)
Wald tests for fixed effects
```

Response: countlog

Terms added sequentially; adjusted for those above

| | Df | Sum of Sq | Wald statistic | Pr(Chisq) |
|-------------|----|-----------|----------------|------------|
| (Intercept) | 1 | 11.04536 | 45.76137 | 0.00000000 |
| area | 1 | 0.65477 | 2.71275 | 0.09954925 |
| residual | | 0.24137 | | |

Testing Correlations

```
> fmNormRandA <- samm(fixed = countlog ~ area, random = ~ year + site +
year:site + site:habitat + year:site:habitat, rcov = ~
year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 15, stepsize =
0.05)
> -2*(fmNormRandA$loglik - fmNormRand$loglik)
[1] 0.1978598
```

Testing Variance Components

```
> fmNormRandAAAt <- samm(countlog ~ area, random = ~ year + site + year:site +
site:habitat + year:site:habitat, rcov = ~
+ year:site:habitat:id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+ 0.05)
Convergence monitoring: LogLikelihood Converged
> -2*(fmNormRandAAAt$loglik - fmNormRandA$loglik)
```

```

[1] 4.777515

> fmNormRandAYear <- samm(countlog ~ area, random = ~ site + year:site +
site:habitat + year:site:habitat, rcov = ~
+   year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+   0.005)
Convergence monitoring: LogLikelihood Converged
> fmNormRandASite <- samm(countlog ~ area, random = ~ year + year:site +
site:habitat + year:site:habitat, rcov = ~
+   year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+   0.005)
Convergence monitoring: LogLikelihood Converged
> fmNormRandAYearSite <- samm(countlog ~ area, random = ~ year + site +
site:habitat + year:site:habitat, rcov = ~
+   year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+   0.005)
Convergence monitoring: LogLikelihood Converged
> fmNormRandASiteHab <- samm(countlog ~ area, random = ~ year + site +
year.site + year:site:habitat, rcov = ~
+   year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+   0.005)
Error in samm.modelFrame(form, data = data, na.method.Y = na.method...: Object
"year.site" not found
> fmNormRandASiteHab <- samm(countlog ~ area, random = ~ year + site +
year:site + year:site:habitat, rcov = ~
+   year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+   0.005)
Convergence monitoring: LogLikelihood Converged
> fmNormRandAYearSiteHab <- samm(countlog ~ area, random = ~ year + site +
year:site + site:habitat, rcov = ~
+   year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+   0.005)
Convergence monitoring: LogLikelihood Converged

> -2*(fmNormRandAYear$loglik - fmNormRandA$loglik)
[1] 3.858977e-007
> -2*(fmNormRandASite$loglik - fmNormRandA$loglik)
[1] 0.0002340961
> -2*(fmNormRandAYearSite$loglik - fmNormRandA$loglik)
[1] 8.212113
> -2*(fmNormRandASiteHab$loglik - fmNormRandA$loglik)
[1] 21.85282
> -2*(fmNormRandAYearSiteHab$loglik - fmNormRandA$loglik)
[1] 3.457236

> fmNormRandB <- samm(countlog ~ area, random = ~ year:site + site:habitat +
year:site:habitat, rcov = ~
+   year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+   0.05)
Convergence monitoring: LogLikelihood Converged
> fmNormRandBYearSite <- samm(countlog ~ area, random = ~ site:habitat +
year:site:habitat, rcov = ~
+   year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+   0.05)
Convergence monitoring: LogLikelihood Converged
> fmNormRandBSiteHab <- samm(countlog ~ area, random = ~ year:site +
year:site:habitat, rcov = ~
+   year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =

```

```

+      0.05)
Convergence monitoring: LogLikelihood Converged
> fmNormRandBYearSiteHab <- samm(countlog ~ area, random = ~ year:site +
site:habitat, rcov = ~
+      year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+      0.05)
Convergence monitoring: LogLikelihood Converged
> -2*(fmNormRandBYearSite$loglik - fmNormRandB$loglik)
[1] 8.533221
> -2*(fmNormRandBSiteHab$loglik - fmNormRandB$loglik)
[1] 25.55181
> -2*(fmNormRandBYearSiteHab$loglik - fmNormRandB$loglik)
[1] 3.457245

> fmNormRandC <- samm(countlog ~ area, random = ~ year:site + site:habitat,
rcov = ~
+      year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+      0.05)
Convergence monitoring: LogLikelihood Converged
> fmNormRandCYearSite <- samm(countlog ~ area, random = ~ site:habitat, rcov =
~
+      year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+      0.05)
Convergence monitoring: LogLikelihood Converged
> fmNormRandCSiteHab <- samm(countlog ~ area, random = ~ site:habitat, rcov =
~
+      year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+      0.05)
Convergence monitoring: LogLikelihood Converged
> fmNormRandCSiteHab <- samm(countlog ~ area, random = ~ year:site, rcov = ~
+      year:site:at(habitat):id(rep), data = cuttPeakFinal, family =
samm.gaussian(), maxiter = 25, stepsize =
+      0.05)
Convergence monitoring: LogLikelihood Converged
> -2*(fmNormRandCYearSite$loglik - fmNormRandC$loglik)
[1] 63.39083
> -2*(fmNormRandCSiteHab$loglik - fmNormRandC$loglik)
[1] 140.2611

> anova(fmNormRandC)
Wald tests for fixed effects

```

Response: countlog

Terms added sequentially; adjusted for those above

| | Df | Sum of Sq | Wald statistic | Pr(Chisq) |
|-------------|----|-----------|----------------|-----------|
| (Intercept) | 1 | 44.34813 | 44.34813 | 0.0000000 |
| area | 1 | 2.60278 | 2.60278 | 0.1066764 |
| residual | | 1.00000 | | |