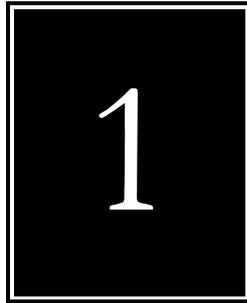


Preface:
The History of Giant Crab
Exploitation and Research



Taxonomy

The current taxonomy of *Pseudocarcinus gigas* is:

Phylum	CRUSTACEA
Class	MALACOSTRACA
Order	DECAPODA
Infraorder	BRACHYURA
Section	HETEROTREMATA
Superfamily	Xanthoidea MacLeay 1838
Family	Eriphiidae ¹ MacLeay, 1838
Subfamily	Oziinae Alcock 1898
G & Sp.	<i>Pseudocarcinus gigas</i> (Lamarck, 1818).

Pseudocarcinus gigas was originally described as *Cancer gigas* by Jean-Baptiste Lamarck in 1818 and later placed into *Pseudocarcinus* by Henri Milne Edwards after he created the genus in 1834.

The family and subfamily status of the genus is unclear as *Pseudocarcinus* has not been included in recent reviews of the Xanthoidea by Guinot (1977, 1978, 1979) and Holthuis (1993) due to inadequate published descriptions. Critically, the male pleopods and sternum have not been described or illustrated and few specimens are held by international museums. Specimens and photographs were sent to xanthoid taxonomists Mr Peter Davie (Queensland Museum) and Dr Danièle Guinot (Museum National d'Histoire Naturelle, Paris) and their opinion was that *Pseudocarcinus* lies in the taxonomic cascade listed above. Further work on the taxonomic relationships of *Pseudocarcinus* is underway using sperm morphology (Prof. Barrie Jamieson, Queensland University and Dr Danièle Guinot) so the status should soon be resolved.

The common name for *Pseudocarcinus gigas* was traditionally “giant crab” although the name “king crab” was widely adopted after 1980. Use of “king crab” seems to have resulted

¹ Eriphiidae is synonymous with Oziidae and Menippidae (Holthuis 1993).

from confusion with the commercially important lithodid crabs which are generically termed “king crabs” (Dawson, 1989). In 1995, the Federal Department of Primary Industries and Energy published a report on marketing names for Australian seafood and it was concluded that “king crab” was misleading so “giant crab” was adopted as the official marketing name (MNFSA, 1995).

Distribution

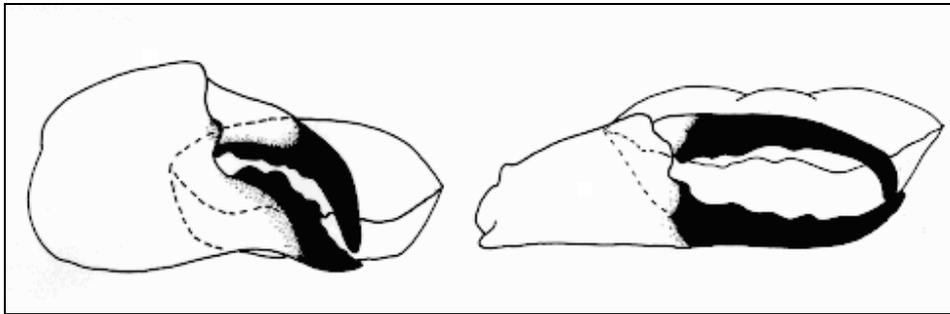
In temperate regions of the world, the coastal waters of the continental shelf tend to be inhabited by at least one very large species of crab with large crab species seldom found in tropical waters. This trend may be due to a female defence polygynous mating system in open habitat, which favours larger, stronger males (Christy, 1987; Orensanz et al., 1995). The trend is worth noting as it may be of value in understanding the origins and niche of *P. gigas*. This position of large crab is filled by different taxa in various temperate regions and examples of genera are: *Macrocheira*, *Chionoecetes* (Majidae) and *Erimacrus* (Atecyclidae) in the north-west Pacific; *Chionoecetes* (Majidae) and *Paralithodes* (Lithodidae; Anomura) in the north-eastern Pacific; *Chionoecetes* (Majidae) in the north-west Atlantic; *Cancer* (Cancridae) and *Maja* (Majidae) in the north-east Atlantic; *Geryon* (Geryonidae) around southern Africa; and *Lithodes*, *Paralomis* (Lithodidae, Anomura), and *Geryon* (Geryonidae) around southern America.

While *Pseudocarcinus gigas* is the most abundant large crab in Australian temperate waters, other relatively large species are also found: *Hypothalassia armata* (Xanthoidea), *Chaceon bicolor* (Geryonidae), and deepwater lithodid species. *Pseudocarcinus* is an endemic, monospecific Australian genus belonging to the superfamily Xanthoidea — an extremely diverse taxon with approximately 166 species and 47 genera in Australia alone (Griffin and Yaldwyn, 1968). Xanthoids are found world-wide although they appear to be slightly more diverse through the Indo-West Pacific. Despite their wide distribution and diversification, relatively few xanthoids have attained large size with notable exceptions being three commercial genera *Pseudocarcinus*, *Menippe* (Americas), and *Hypothalassia* (north-west Pacific and south-west Australia).

It is interesting that during the mid-Tertiary, the dominant large crab of the southern coastal shelf of nearby Tasmantis — the land mass bearing New Zealand and Lord Howe

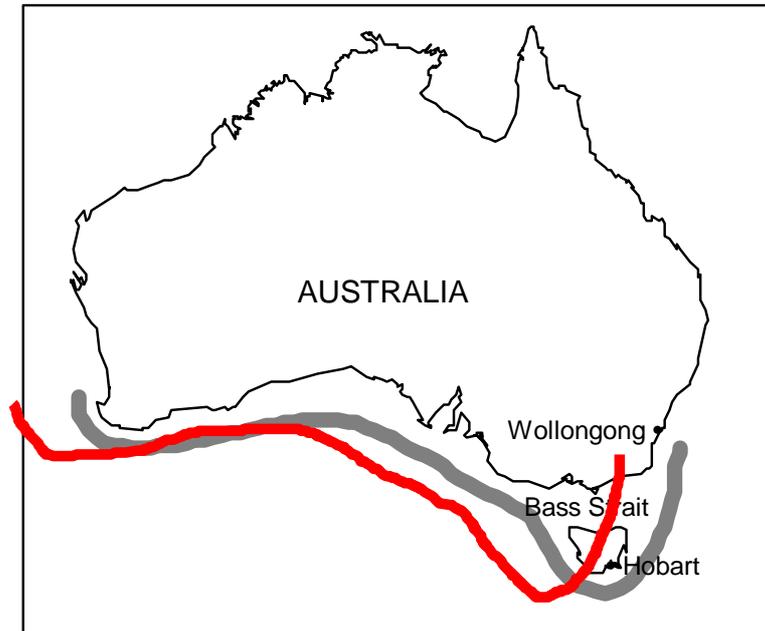
Island — was also a xanthoid. This species, *Tumidocarcinus giganteus*, is common in New Zealand middle and upper Miocene sediments laid down in deeper coastal waters (Fleming, 1962). As with *P. gigas*, the males had exceptionally large right chelae although overall body size appears to be somewhat smaller (Fleming, 1962; Fig. 1). This species may have been displaced by *Cancer novaezelandiae* (McLay, 1988), the only cancrid crab to reach Tasmantis or Meganesia (Australia and New Guinea), from South America via Antarctica in the mid-Tertiary (Nations, 1975).

Figure 1. Frontal views of the extinct xanthoid crab, *Tumidocarcinus giganteus*, from New Zealand (left: after Fleming, 1962) and *Pseudocarcinus gigas* showing development of large right chelae.



The present distribution of *P. gigas* stretches from south-west Western Australia, across southern Australia, to mid-New South Wales, with the most northerly record on the east coast from Five Islands off the coast of Wollongong NSW (McNeill, 1920; Fig. 2). The southern extent of their distribution appears to be the continental shelf around Tasmania and specimens have been obtained by rock lobster fishers from around this entire region. Although specimens have been collected off southern Tasmania, crabs are not common and commercial fishers seldom work below 42°30' S. Bass Strait is popularly considered the major region inhabited by *P. gigas* (e.g. Griffin, 1970), possibly from repeated citation of early collections by Haswell (1882) and Rathbun (1926) in this area. However, most *P. gigas* are found at depths between 120 and 370 m (Levings et al., 1996) which effectively excludes Bass Strait as it is generally shallower than 100 m.

Figure 2. Distribution of *Pseudocarcinus gigas* (shaded area) and important Australian cities.



History of harvest with emphasis on Tasmania

Aboriginal history

Although giant crabs are normally found in deep water out of reach of divers, large individuals are occasionally collected in shallow areas of less than 10 m (Hale, 1927).

Francois Péron visited Maria Island on the east coast of Tasmania in 1802 as the zoologist for the Baudin expedition and his diary reports that giant crabs formed part of the diet of local Aborigines.

Péron was one of the more enlightened explorers of his time, he had a great personal interest in human diversity and was one of the first people to coin the term “anthropology” (Plomley et al., 1990; Flannery, 1994). His diary record of the expedition’s visit to Bruni (now Bruny) Island shows great humanity (Wallace, 1984).

As a consequence of his interest, Péron's records of aboriginal lifestyle in Tasmania are particularly detailed and are in his words "minutely exact" or accurate; they provide one of the best records now available. They are also full of boundless enthusiasm. Péron describes his discovery of a giant crab cheliped on Maria Island and his reactions to the capture of these crabs by Aborigines thus:

"Upon the beach at the head of the eastern bay, I, myself came upon the monstrous claw of a crab; the individual to which this redoubtable weapon had belonged must not have weighed less than 30 or 40 pounds. Moreover, these large species supply the natives of these regions with part of their diet. It is the women who dive to great depths for them, and I confess that I can scarcely imagine how they manage to pull from their rocky dwellings creatures so big and frightfully armed" (Plomley et al., 1990).

The "monstrous claw" collected by Péron was transported back to France and it is mentioned in the original description by Lamarck (1818). As Péron notes, it is difficult to imagine how the women were able to collect giant crabs. Maria Island is close to the edge of the continental shelf so giant crabs will be found closer to land than in most other areas of Tasmania and occasional animals may have wandered in close to land. These may have been washed ashore as with the specimen collected by Péron or they may have been actually collected by diving as Péron asserts. Oysters (*Ostrea angasi*), abalone (*Haliotis rubra*), and southern rock lobsters (*Jasus edwardsii*) were collected by diving so it is conceivable that giant crabs may have been encountered.

Colonisation of Tasmania by Europeans occurred in 1802 and resulted in the near extermination of Aborigines so that by 1847 none were left in Tasmania save small populations on the Bass Strait Islands (Flannery, 1994). As a result, subsequent fishing for giant crabs was mainly by Europeans.

Post-Colonisation History

Harvest of rock lobsters appears to have commenced soon after colonisation and was clearly productive in inshore areas. In 1802, the Baudin expedition assigned a few crew members to catching rock lobsters with lines, a fairly ineffective method, yet they were able to catch enough within a few hours to feed the entire crew (Plomley et al., 1990). By 1884, William Saviile-Kent, the colony of Tasmania's superintendent and inspector of fisheries,

had already noticed effects of fishing on the east coast with declines in size and abundance of rock lobsters (Saville-Kent, 1884).

Giant crabs were occasionally collected as bycatch of this rock lobster fishery although a royal commission into the state of the fisheries in Tasmania concluded that though they were a splendid animal, they were only brought to market occasionally and were not of much commercial importance (Royal Commission Report, 1882). At around the same time in Victoria, McCoy (1889) reported that giant crabs, especially females, were occasionally brought to market and were especially common along the Victorian coast near Portland. This is the region where most of the Victorian catch is harvested today. William Saville-Kent appears to have been appointed following presentation of the Tasmanian royal commission report in 1882 and was keen to see the harvest of crustaceans diversified to provide greater variety of seafood to the Tasmanian public. He seemed to be exasperated by the state of Tasmanian technology and wrote:

“The use of crab pots, as utilised in almost every other country on the face of the globe, might be advantageously recommended to the fishermen of Tasmania” (Saville-Kent, 1884).

Saville-Kent was one of the visionaries of Australian marine science in many respects, notably in aquaculture where he: published methodology for rearing of the European lobster (*Homarus gamarus*; Saville-Kent, 1883); developed the Australian pearl industry in northern Western Australia; proposed oyster farming in Tasmanian Bays, Pipeclay Lagoon and Pittwater (which occurred almost 100 years later); and proposed the construction of a state hatchery for aquaculture trials with the giant Tasmanian crayfish (*Astacopsis gouldii*) and the striped trumpeter (*Latris lineata*) (which occurred around 100 years later). As with most of these other projects, Saville-Kent’s ideas on crab fishing did not develop further until the 1970’s when Tasmanian and Victorian crab fisheries were investigated in two projects: “Development of Small Scale Invertebrate Fisheries in Tasmanian Waters” (by the Tasmanian Fisheries Development Authority; Sumner and Dix, 1980); and “Experimental Trapping of the Giant Crab *Pseudocarcinus gigas*” (by the Fisheries and Wildlife Division, Victoria; Winstanley, 1979).

The Tasmanian project was headed by Colin Sumner and Trevor Dix and addressed fisheries for a variety of invertebrates including three crab species, the sand crab (*Ovalipes australiensis*; Portunidae), the spider crab (*Leptomithrax gaimardii*; Majidae), and giant crabs. The survey concluded that there was potential for development of a fishery for giant crabs,

primarily as bycatch from the rock lobster (*Jasus edwardsii*) fishery, rather than as a targeted species. The development of a fishery was considered to rest on marketing as prices were generally too low to warrant fishers bothering with crabs (20c/kg, 1977/78). Fishing for giant crabs involves greater expense than for rock lobsters as giant crabs inhabit deeper, offshore areas; vessels need to be large to withstand high seas and gear is more expensive with larger pot haulers and more rope required. Crabs were processed by hand picking the meat and marketing trials were also made with whole cooked crabs. There appeared to be promise for the development of a fishery and further marketing trials were recommended (Sumner and Dix, 1980).

Further market research was not undertaken and market prices did not improve for several years so most giant crab bycatch remained under utilised. Although most bycatch was taken by rock lobster fishers, trawlers also captured some giant crabs on muddy substrates and these were generally discarded. Crabs collected by rock lobster fishers were usually smashed so that they could be removed more easily from the wicker lobster pots and also because they were considered to interfere with the entry of rock lobsters (Sumner and Dix, 1980).

Research conducted to improve live transport of rock lobsters in the late 1980's allowed processors to gain higher prices for live rock lobster exports to Asia. The improved methods also enhanced survival of giant crabs so that they could be sold for far higher prices than was previously possible (VDCNR, 1995a). This opened the way for the development of a giant crab fishery as processors began to offer prices that were high enough to allow large vessels to profitably fish the deeper waters at the edge of the continental shelf (Yasuhara, 1995; Fig. 3). Tasmanian catches grew from 133 kg in 1990 to 243 tonnes in 1995 and Victorian catches also increased dramatically (Fig. 4; VDCNR, 1995b; TDPIF, 1995). As the market has become increasingly aware of giant crabs, prices have steadily climbed so that beach price was over \$50/kg for small crabs in 1997. This high price is based on the colour of crabs, not their unique size, as small crabs of less than 3 kg receive around double that of crabs greater than 5 kg (on a per kg basis).

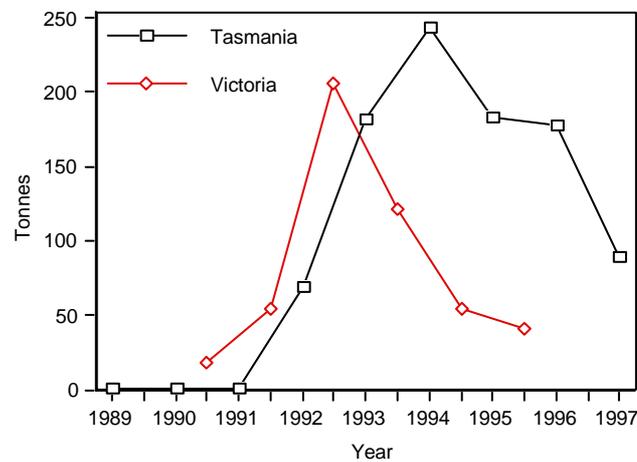
The giant crab fishery was initially subject to only two levels of input restriction, seasonal closures and pot limit — artefacts of the rock lobster fishery as similar gear was used for both species. The fishery grew at such a rapid rate that a minimum size restriction of 150 mm carapace length was introduced in 1994 across southern Australia which resulted in a decline in catch in Victoria (Fig. 4). This size limit was introduced as an interim measure

until valid biological information could be collected and it is still in place. A more detailed discussion of changes in fishery management is documented elsewhere (Gardner, 1998).

Figure 3. Commercial fishing operation targeting giant crabs with purpose-built traps.



Figure 4. Historical patterns of catch of giant crabs in Tasmania and Victoria since 1989 (data from the Victorian Department of Conservation and Natural Resources and the Tasmanian Department of Primary Industries and Fisheries).



History of research

The oldest written record of giant crabs exists in Francois Péron's accounts of his journey around Tasmania (1802), as discussed earlier. Péron was interested in the relationship

between organisms and the environment and his ideas contributed to the formation of the science of ecology some 50 years later. He developed the idea of habitat preference in relation to giant crabs, and other crustaceans, by first discussing the terrain and then the interaction with crabs and lobsters².

“... everything here [on Maria Island] bears the marks of the worlds upheavals, everything here attests to its great antiquity, everything recalls the painful struggle that it had to carry on against the fury of the waves, everything speaks of their ancient dominion over the land... the traces of their gradual recession are to be seen everywhere and in the shape and distribution of the rocks as well as their nature. There, ramparts of granite seem to present an insurmountable barrier to the ocean. Steep and sheer they rise to a height of two or three hundred feet. In their sides there are sometimes more or less large caves, in which the waters, as they surge tumultuously in produce dull boomings like the sound of distant thunder.

..it remains for me to say a few words about the genus Cancer, using the name in all its Linnæan generality. Among the rocks that I have described and in the furthest depths of those caves which I have spoken, it is easy to conceive that the largest species of this genus [, the giant crab,] must not only multiply freely, but also reach a gigantic size.... Be that as it may, one further finds in connection with this genus of animals, a new and striking proof of the influence of nature of the seabed upon the existence of such and such a species in preference to all others. Lobsters, which seek out holes in rocks and their debris, exist in prodigious numbers around Maria Island, they were generally rare in D’Entrecasteaux Channel....On the other hand, spider crabs, which delight in filth and mud, abounded to excess on every point in the Channel and yet apparently did not exist around Maria Island. The different nature of the terrain must in fact repel them...”(Plomley et al., 1990).

Following on from Péron’s report, several references to giant crabs have been made, generally of a taxonomic nature, although a small amount of biological information was also collected. The first taxonomic account was by Jean-Baptiste Lamarck, who is famous for his early contribution to evolution, published in “Zoological Philosophy” (1809). Although he invented the term “biology”, Lamarck’s original career plans had been with the army until a neck injury forced him to seek other directions (Elliot, 1914). At age 50, he had spent 25 years studying botany when three chairs were created at the Museum

² As an aside, it is also interesting to note his views on the earth’s age as the day of creation had been fixed in Péron’s era at 23 October 4004 BC by Archbishop Ussher. This view was beginning to be questioned in the early nineteenth century and was finally shattered by Charles Lyell’s “Principles of Geology, 1830-33”(Carey, 1995).

National d'Histoire Naturelle in Paris. The botany chair had been filled so Lamarck was appointed professor of zoology, insects, worms and microscopic animals. He practically abandoned botany and launched into the epic task of classifying the world's invertebrate animals; the results of his research were then published in *Histoire Naturelle des Animaux sans vertèbres*, a seven volume work published from 1815-1822. The giant crab was included in the 1818 volume and it is the last crab that Lamarck described before moving onto another invertebrate group. His description is very brief and sounds almost tired:

“Giant Crab (Cancer gigas)

Inhabits the waters of New Holland, in Port Jackson. Péron and Lesueur [collectors]. The shell of an entire individual is 10 inches width; however according to a found anterior leg which is of human arm size, it can be of a huge size. The front of the shell bears four small teeth. Its posterior sides bear small sparse tubercles. The end articulation of the legs are slightly spiky. Etc.

It is in the museum collection, which owns plenty of other species still undescribed”(Lamarck, 1818).

The holotype is male of around 27-28 cm carapace width and is still held at the French Museum National d'Histoire Naturelle although it is dry and some legs are detached (MNHN-B 13171; pers. comm., Danièle Guinot).

Lamarck lists the collecting location of this holotype as Port Jackson, generally known as Sydney Harbour although the apparently old label on the holotype indicates “Tasmanic” as the collecting location. The location of Sydney is unlikely given the current knowledge of the distribution and appears to be an error, possibly introduced by Péron or Lesueur. The large anterior leg that Lamarck mentions is almost certainly that found by Péron on Maria Island, and the whole specimen may have been one collected by Aborigines from the same area. The large leg can no longer be located in the collection of the Museum National d'Histoire Naturelle.

Thomas Whitelegge (1889) continued the probable error in the collecting location of the holotype by including the giant crab in a species list for Port Jackson and he lists the collecting location as Lane Cove River, a sheltered brackish estuary. Whitelegge notes that his task was difficult given the paucity of books in the colony and in many cases his quotations are second hand. This difficulty seems to have affected his inclusion of the giant crab, he cited Lamarck's description incorrectly and included Lane Cove River as a collecting site when it was not previously listed.

Taxonomic accounts of the giant crab included those by Milne Edwards (1834), Haswell (1882), McCoy (1889), Rathbun (1926), and Hale (1927-29). The description by McCoy is by far the most accurate and detailed and includes the earliest known illustrations of the species including the dissected mouth-parts and abdomens of both sexes (Fig. 5). McCoy completed his description as part of a massive two volume series on the natural history of Victoria, "A Prodromus of the Natural History of Victoria". Only a few copies of this publication have survived so his description is reproduced in Appendix 1.

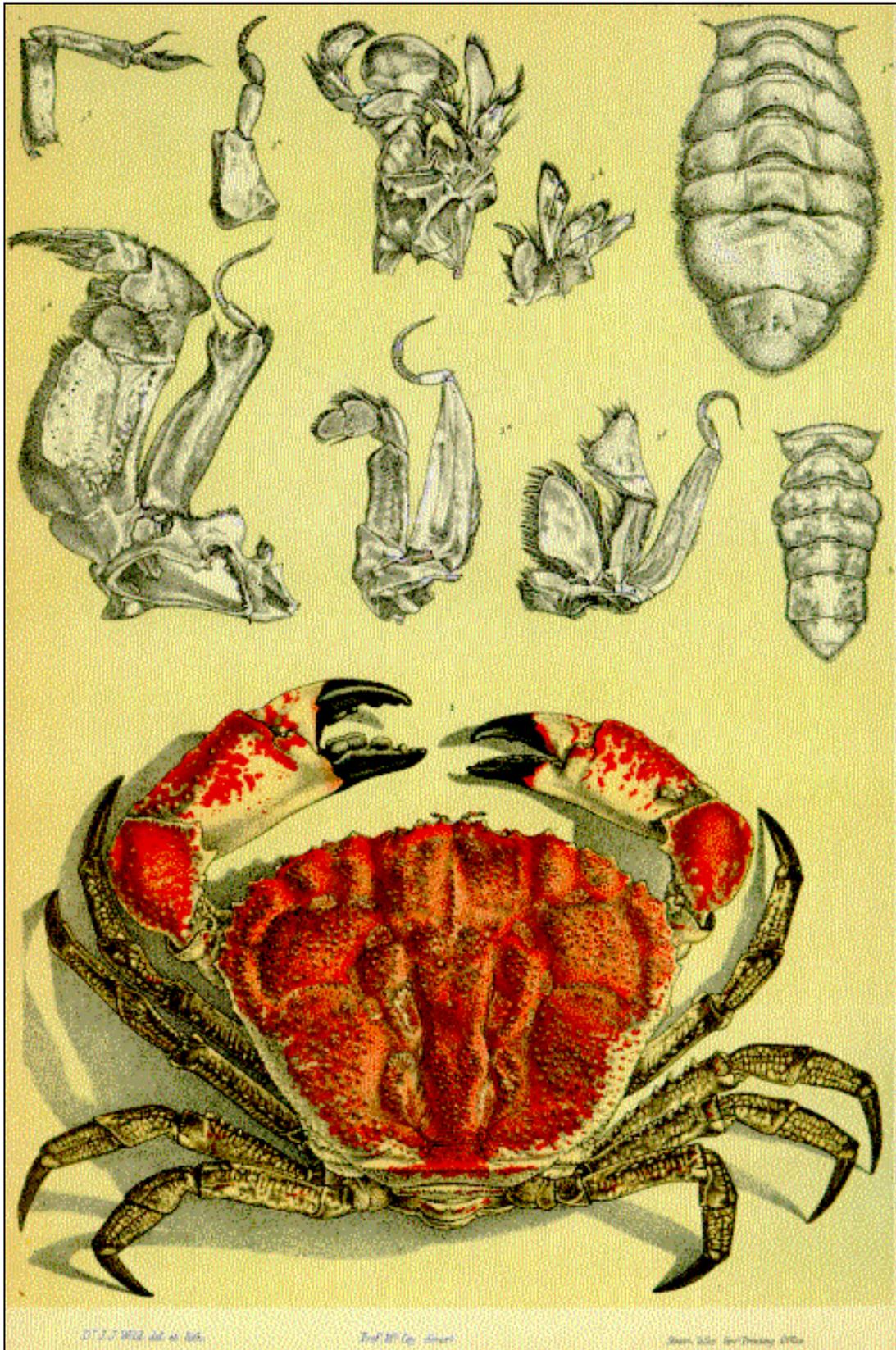
Figure 5 (proceeding pages). Illustrations of female (first) and male (second) P. gigas by McCoy (1889) including dissected mouthparts. The plates were produced by lithography which reverses the image – the original crabs depicted both bore the larger molariform cheliped on the right hand side which is typical.

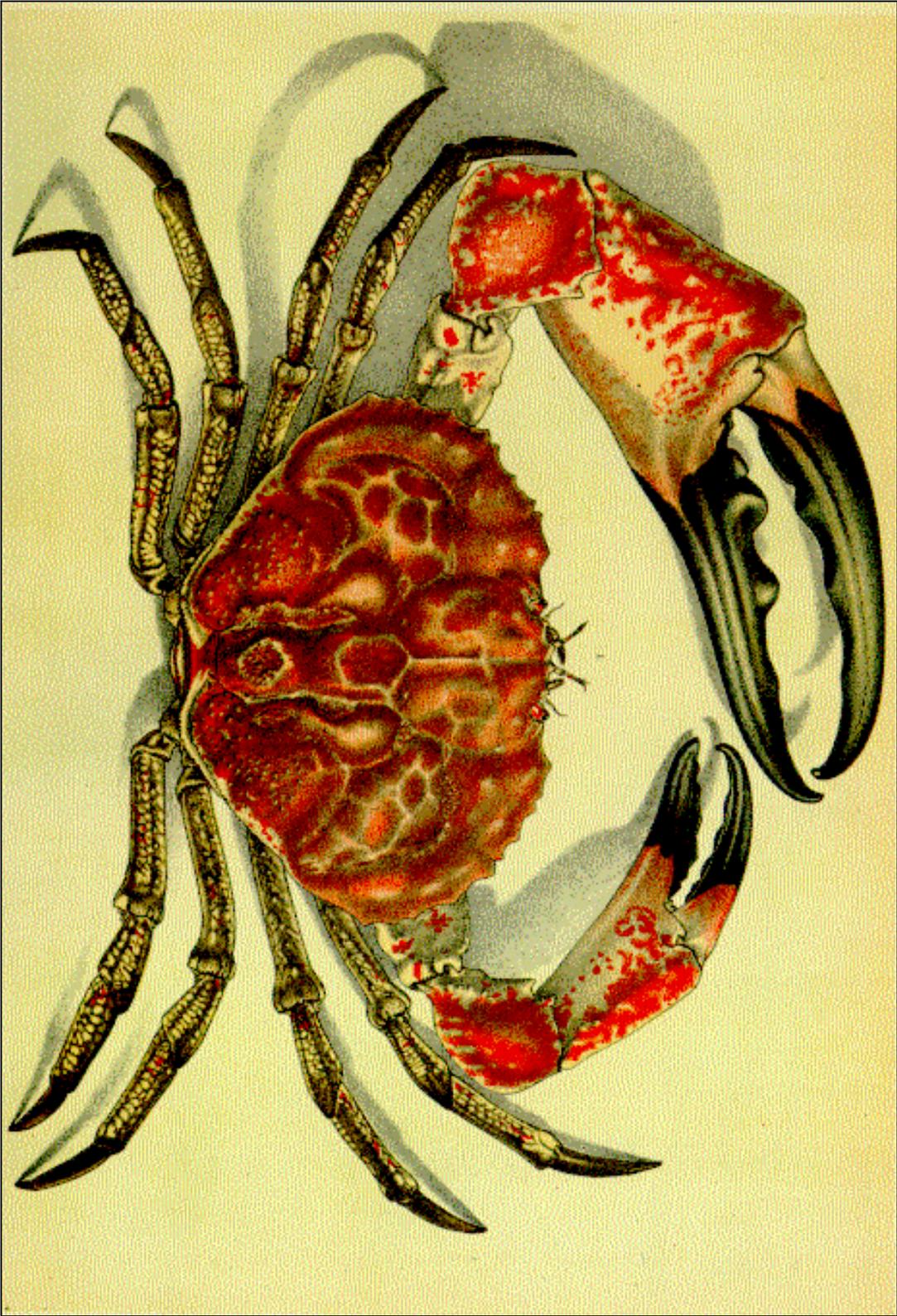
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EXPLANATION OF FIGURES.

PLATE 179.-Fig. 1, female, about one third natural size. Fig 1a, abdomen of female, one third the natural size. Fig.1b, antennules, or inner antennae, movable portion without great fixed base, twice the natural size. Fig 1c, antennae, or outer antennae, without the small basal joint, twice the natural size. Fig. 1g, mandible and first and second maxillipedes, natural size. Fig. 1d, third or external maxillipede, natural size. Fig. 2, abdomen of male, one-third natural size.

PLATE 180 -Fig. 1, male, about one-third natural size. (For abdomen, see pl. 179, f. 2).





Most of the descriptive texts also mention some biological information about giant crabs. Haswell (1882) states that the carapace is sometimes 2 feet in breadth; however, using regressions derived for male crabs in the submitted study, this would equate to the unlikely weight of around 60 kg. McNeill (1920) also discusses the size of giant crabs and he gives more accurate measures from a specimen at the Tasmanian Museum and Art Gallery (still on display) of 330 mm carapace width and hand length of 438 mm. Several authors have noted the beautiful colouring which seems to vary for each animal in an individual pattern (McCoy, 1889; McNeill, 1920; Rathbun, 1926; Hale, 1927). McCoy (1889) and Rathbun (1926) observed increased dimorphism of the chelae with increased body size and that most animals were right handed. Rathbun's (1926) work was based on collections made by the federal trawler, "The Endeavour", from 1909-1914 and McNeill (1920) presented information recorded by a member of the staff aboard the vessel for these expeditions, Mr A.R. McCulloch. He noted that several juvenile specimens, as little as 1 inch carapace width, were found in sponge cavities on different occasions. This remains the only reliable information published on the habitat occupied by juvenile giant crabs.

Very little research of any nature was conducted on giant crabs until the fisheries research by Sumner and Dix in the late 1970's. Trevor Dix (1980) also suggested that giant crabs might be cultured although there was insufficient knowledge to assess their potential.

It has not been until the 1990's, after the development of the fishery, that the biology of the giant crab has begun to be studied in any detail; among the information collected is that reported in this thesis.

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General Introduction:
Larval Biology of the Giant Crab
Pseudocarcinus gigas



Research on the larval biology of the giant crab *Pseudocarcinus gigas* (Lamarck) was directed towards two aims: to provide biological information to assist research on recruitment processes; and to conduct a preliminary assessment of the potential for hatchery production of *P. gigas*.

The rapid increase in the value of giant crabs from 1992 prompted high levels of fishing exploitation in some regions leading to concerns that crabs may be depleted from some areas, particularly in north-western Tasmania and western Victoria (see Chapter 16). The impact of depletion of crabs through fishing is especially severe where dispersal is limited, as localised recruitment failure may result. Although crabs may walk large distances as adults, dispersal is usually during planktonic larval stages (excepting among rafting species) making information on the larvae vital for assessing the potential for dispersal (Hitchcock, 1941; Kingsford and Choat, 1985; Havenhand, 1995).

At the commencement of this project, commercial fishers reported that *P. gigas* larvae had direct development which is unusual, but does occur in other xanthoid crabs in the Australasian region (e.g. *Pilumnus novaezelandiae* and *P. vestitus*; McLay, 1988). This would imply that there was high potential for recruitment failure following localised depletion. However, shortly afterwards, an ovigerous female released free swimming zoeas³ in a tank which demonstrated that development was not direct (A. Levings, Deakin University, Pers. Comm.). Beyond this, nothing was known of the larvae and more information was required on the duration of the planktonic stage. Detailed analysis of dispersal of larvae also requires information on vertical migration behaviour.

The larvae of *P. gigas* had not been described previously and this was undertaken to permit identification from plankton samples. Description of the larvae was also useful taxonomically as *P. gigas* has not been included in recent reviews of xanthoid crabs (see preface, Chapter 1). Plankton archives in Tasmanian marine research facilities were then searched for material collected in oceanic areas during late spring and summer. This material was sorted for *P. gigas* larvae to assess vertical distribution in natural conditions, and to find evidence of vertical migration stimuli such as time of day (i.e. light) and temperature (Chapter 4). Unfortunately, few larvae were obtained from field samples.

³ Zoea and megalopa are pluralised as zoeas and megalopas throughout this thesis as recommended by Martin (1984) and P. Clark (Natural History Museum, London, Pers. Comm., 1998).

The investigation of larval distribution in the field was complemented by laboratory-based experimental projects. Vertical migration of crustacean larvae is influenced by a range of stimuli including pressure, polarisation of light, gravity, absolute light intensity, salinity, change in light intensity, predator fields, prey fields, and temperature (Knight-Jones and Morgan, 1966; Umminger, 1969; Latz and Forward, 1977; Forward et al., 1984; Gliwicz and Pijanowska, 1988; and Forward, 1990). In a seminal paper, Sulkin (1984) considered that vertical migration of crab larvae results from a combination of orientating cues, principally gravity, combined with changes in upward swimming speed in response to various environmental cues, principally light. He also considered that change in pressure was important in regulating swimming speed, particularly in the absence of light (Sulkin, 1973). Consequently, this laboratory study focused on the response of *P. gigas* larvae to gravity, changes in light, and changes in pressure. Most research on the vertical migration behaviour of crab larvae has been with inshore, coastal species. *Pseudocarcinus gigas* are fished around the continental shelf so there is potential for the larvae to migrate through depths of over 300 m. It was considered that change in temperature may be an important migration cue in this environment so additional trials were conducted to assess the effect of temperature and thermoclines.

Research on the potential for hatchery production was intended to provide preliminary information as it was recognised that a considerable research effort is required to achieve commercial production of a new species. Nonetheless, commercial production of giant crabs, or any other species, is highly dependent on hatchery techniques so this was a critical area for initial research. Giant crabs are highly valued with beach prices in 1997 fluctuating seasonally between \$30/kg and \$50/kg for small crabs under 3 kg. Markets would prefer crabs of around 100 mm carapace length which is smaller than the current minimum legal size of 150 mm carapace length, introduced in 1994. This market demand can only be filled by aquaculture and processors have indicated that prices would be higher than for legal sized crabs.

At the commencement of this project, the only growth information available on giant crabs was from the moult of a juvenile giant crab captured in October 1993. This animal moulted from 70 mm to 94 mm carapace length which was an impressive 34% increase in length and indicated some potential for rapid growth (Frusher, 1994). Crab culture worldwide is small and has received relatively little research effort, with the exception of

enhancement operations in Japan, so research on the culture of *P. gigas* larvae is of general interest for crab culture (Cowen, 1982).

Much of the research on behaviour of *P. gigas* larvae overlapped with the second aim of assessing the potential for hatchery production of *P. gigas*. Unlike finfish, crab larvae are negatively buoyant and must swim to avoid sinking. Culture conditions that initiate upward swimming will exhaust energy reserves, while cues that initiate sinking may cause larvae to accumulate at the base of culture tanks, thus increasing contact with detritus. Research on optimising larval rearing investigated optimal lighting and temperature as these parameters affect swimming activity, intermoult duration, metabolism, rate of cannibalism, larval size, utilisation of energy reserves, feeding rate, and metamorphosis (Eagles et al., 1986; Waddy and Aiken, 1991; Hecht and Pienaar, 1993; Minagawa, 1994).

The final aspect of research on assessing hatchery production of *P. gigas* was to control disease. In preliminary trials, larval mycosis caused high mortality so additional research was undertaken to refine the use of prophylactic treatments for this species.

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*Larval Development of the Giant Crab
Pseudocarcinus gigas (Lamarck,
1818)(Decapoda: Eriphiidae) Reared in
the Laboratory*

3

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Abstract

The larvae of *Pseudocarcinus gigas*, obtained from females collected from eastern Tasmania, were reared from hatching to metamorphosis. The larval series has five zoeal and one megalopal stages. This chapter presents a description of these stages and a comparison with other members of the sub-family Oziinae.

Introduction

Pseudocarcinus is a monospecific genus and has not been included in recent reviews of Xanthoidea (e.g. Guinot, 1978; Serene, 1984) although it appears to belong to the subfamily Oziinae within the family Eriphiidae⁴ (P. Davie, Queensland Museum, personal communication). Larval information is available for several other species within the Oziinae including *Ozius rugulosus* (Kakati and Nayak, 1977); *O. truncatus* (Wear, 1968; Wear and Fielder, 1985); *Baptozizus vinosus* (Saba et al., 1978a); *Menippe adina* (Martin, 1988; Martin et al., 1988); *M. nodifrons* (Scotto, 1979); *M. rumphii* (Kakati, 1977); *M. mercenaria* (Porter, 1960); and *Epixanthus dentatus* (Saba et al., 1978b).

This chapter expands upon a preliminary description of the first stage zoea of *P. gigas* (Quintana et al., 1996; Appendix 2) to fully describe the zoeal stages and megalopa of *P. gigas*. The implications for taxonomic affinities are discussed.

Materials and methods

Ovigerous crabs were collected from depths in the range of 300 – 380 m off the east coast of Tasmania (41°17'S; 148°40'E) in June 1995. Females ranged in size from 2.2 – 3.5 kg and

⁴ Eriphiidae is synonymous with Oziidae and Menippidae (Holthuis 1993).

were held communally in 4 m³ tanks with flow through, unfiltered, seawater. Larvae were collected at dusk on the same day from two tanks to ensure that larvae were not from a single parent; further mixing probably occurred as several females were releasing larvae in each tank.

Newly hatched larvae were rinsed in 0.2 µm filtered seawater (32‰ salinity) then transferred to 1.8 l, black, rectangular culture vessels. One hundred larvae were placed in each vessel and maintained in a temperature control room at 15.5°C. Cultures were not aerated (Appendix 3). Zoeal stages were fed a mix of Protein Selco™ (INVE aquaculture, Oeverstraat 7, Baasrode, Belgium) enriched rotifers (*Brachionus plicatilis*) and artemia⁵ nauplii for the first two instars and enriched artemia only thereafter. Ongrown artemia (7 d) were supplied to megalopa (Appendix 4). Larvae were transferred by pipette into cleaned sterile containers (2 l) with fresh, 0.2 µm filtered seawater daily (32-34‰ salinity). Larvae and exuvia from each stage were preserved for description in buffered 5% formalin. Voucher specimens have been deposited with the Tasmanian State Museum, the Laboratoire de Zoologie-Arthropodes, Muséum National d'Histoire naturelle and the Natural History Museum, London (registration number 1996.1195).

Measurements and descriptions were usually made from ten larvae for each stage. Measurements taken were: for the zoeal stages, (TT) the distance between tips of dorsal and rostral spines, (CW) between the tips of the lateral spines, and (CL) from the base of the rostral spine to the posterior margin of the carapace; for the megalopa, (CW) the maximum distance across the carapace, and (CL) the maximum distance along the carapace. Dissected appendages were first stained in Lees methylene blue before preparation as wet mounts. Drawings were made using a Wild M-5™ stereo microscope and an Nikon Optiphot-2™ compound microscope, both equipped with a camera lucida. Drawings of the first zoeal stage were then retraced and edited by computer using Adobe Illustrator™. Measurements were made by image analysis using NIH Image™ software. Setal counts are proximal to distal. Setae were classed as simple (S), sparsely plumose (SP), plumose (P), highly plumose (HP), or plumodenticulate (PD) (Greenwood and Fielder, 1984).

⁵ Artemia is not italicized throughout this thesis as it has developed into a common name for a well known organism, as with eucalyptus, melaleuca or gorilla.

Several other larval rearing trials were conducted to investigate the effects of temperature, light intensity, photoperiod, and prophylactic disease treatment. In some of these trials, larvae developed to an intermediate stage after zoea 5, particularly following prolonged exposure to high doses of oxytetracycline (Gardner and Northam, 1997; Chapter 8). None of these larvae developed further. This stage was considered abnormal so it is not included in the present description.

Results

Under rearing conditions used in this trial, mean time taken to reach megalopa was 54.0 d (SD=3.2; n=180) and mean time to complete larval development (crab 1) was 91.8 d (SD=3.93; n=26). The rate of development of *Pseudocarcinus gigas* larvae is discussed in more detail elsewhere (Gardner and Northam, 1997; Chapter 8).

Prezoea (Figure 1A)

Prezoea larvae were obtained from egg masses of females where hatching was in progress and were never collected with free swimming larvae taken from tanks immediately after hatching. As with prezoea larvae of other decapods (Quintana and Konishi, 1986), the prezoeal cuticle enveloped setae so that details of structure could not be seen. Carapace spines were depressed although the abdominal lateroventral spines were clearly apparent.

Zoea I

Carapace (Figs. 1B, 3A; Table 1): eyes immobile; all carapace spines well developed and prominent (frontal spine = 0.63 mm; dorsal spine = 1.36 mm; lateral spines = 0.43 mm); dorsal spine slightly curved backwards and pointed; lateral spines conspicuous and with slight ventral curvature; frontal spine smooth, distally pointed, and with slight anterior flexure; anterodorsal setae absent; 1 pair of posterodorsal setae; the ventral margins of the carapace without setae.

Antennule (Fig. 3C): uniramous, endopod absent; exopod unsegmented with 3 aesthetascs and 2 simple (S) setae.

Antenna (Fig. 3D): protopodal process less than half the length of the frontal spine and bearing around 25 spinules in rows along distal half; endopod absent; exopod rudimentary, unsegmented with 3 unequal setae (S).

Mandible (Fig. 3E): solid with molar and incisor processes well developed (clearly observed in well preserved exuviae); incisor process with small marginal teeth; endopod palp absent.

Maxillule (Fig. 3F): coxa without setae; coxal endite with 7 plumodenticulate (PD) setae and 1 seta (S); basal endite with 5 setae (PD); endopod bisegmented, proximal segment with 1 sparsely plumose (SP) seta, distal segment with 2+2+2 setae (SP); exopod seta absent.

Maxilla (Fig. 3G): coxal endite bilobed with 6+4 setae (PD); basal endite bilobed with 6+5 setae (PD); endopod bilobed with 3+5 setae (PD); exopod (scaphognathite) margin with 4 highly plumose (HP) setae and 1 long, plumose distal stout process.

First maxilliped (Figs. 3H, 3H’): coxa without setae; basis with 2+3+3+3 setae (S); endopod 5-segmented with 2, 2,1,2,5-6 setae (PD except 1-2 simple setae on terminal); exopod 2-segmented, distal segment with 4 terminal setae (HP).

Second maxilliped (Figs. 3I, 3I’): coxa without setae; basis with 4 setae (S); endopod 3-segmented with 1,1,4 setae (PD except 2 simple setae on terminal); exopod 2-segmented, distal segment with 4 terminal setae (HP).

Third maxilliped: absent.

Pereiopods (Fig. 3J): uniramous and rudimentary.

Abdomen (Figs. 3B, 3B’): five somites; somite 1 with mid-posterodorsal spine and no dorsomedial setae; somites 2 and 3 with anterolateral knobs, in addition, the later with sharp lateroventral spines as in somites 4 and 5; somites 2-5 with a posterodorsal prominence bearing 2 minute dorsal setae; somite 2 with a minute mid posteroventral seta; pleopod buds absent.

Telson (Figs. 3B, 3B’): broad, bifurcated, curved backwards; each furca distally pointed, with a well developed, smooth lateral spine with a small spine on the inner margin; inner

margin of furcae with 3+3 biplumose setae lateral to acute median notch; small dorsomedial spine present on each telson furca, arising well posterior to setae bases.

Zoea II

Carapace (Figs. 1C, 2A; Table 1): eyes now mobile; carapace larger, with two pairs of anterodorsal setae and each ventral margin with 3 setae (1 plumose (P) anterior seta and 2 sparsely plumose (SP) posterior setae), posterodorsal setation unchanged; dorsal spine more robust.

Antennule (Fig. 4A): exopod with 4 aesthetascs, otherwise unchanged.

Antenna (Fig. 4F): unchanged.

Mandible (Fig. 5A): unchanged.

Maxillule (Fig. 6A): coxa now with 1 seta (HP); coxal endite with 9-10 setae (PD); basal endite with 7-8 setae (PD); otherwise unchanged.

Maxilla (Fig. 7A): coxal endite with 7+4 setae (PD); basal endite with 7+6 setae (PD); endopod unchanged; exopod (scaphognathite) margin with 17-18 setae (HP) and rounded distally.

First maxilliped (8A): setae on basis unchanged in number but now plumodenticulate; terminal segment of endopod with 5 setae (PD); exopod distal segment with 6 terminal setae (HP); otherwise unchanged.

Second maxilliped (8F): setae on basis unchanged in number but now plumodenticulate; exopod distal segment with 6 terminal setae (HP); otherwise unchanged.

Third maxilliped: absent.

Pereiopods (Fig. 9A): buds more developed and cheliped now chelate.

Abdomen (Figs. 10A, 10B): first abdominal somite with 2 dorsomedial setae; lateroventral spines of abdominal somites 3-5 of similar length, overlapping next somite by approximately three-quarter length; inner margin of furcae with additional biplumose setae lateral to acute median notch giving formula of 4+4; otherwise unchanged.

Zoea III

Carapace (Figs. 1D, 2B; Table 1): larger than in previous stages with ventral margin developed into two notches; 6 pairs of anterodorsal setae; each ventral margin with 9 setae (1 plumose anterior seta and 8 sparsely plumose posterior setae); otherwise unchanged.

Antennule (Fig. 4B): endopod with additional aesthetasc; otherwise unchanged.

Antenna (Fig. 4G): endopod bud present; otherwise unchanged.

Mandible (Fig. 5B): unchanged.

Maxillule (Fig. 6B): coxa with 2-3 setae (HP); coxal endite with 11-12 setae (PD); basal endite with 11-13 setae (PD); endopod unchanged.

Maxilla (Fig. 7B): coxal endite with 8-10+5 setae (PD); basal endite with 8+7 setae (PD); endopod unchanged; exopod (scaphognathite) margin with 28-31 setae (HP).

First maxilliped (Fig. 8B): basis unchanged; terminal segment of endopod with 6 setae (PD); exopod distal segment with 10 setae (HP); otherwise unchanged.

Second maxilliped (Fig. 8G): number of setae on basis unchanged but now plumodenticulate; endopod unchanged; exopod distal segment with 10 setae (HP).

Third maxilliped (Fig. 9B): present, rudimentary and biramous with endopod slightly longer than exopod.

Pereiopods (Fig. 9B): developing with differentiation of segments, segments without setae.

Abdomen (Fig. 10C): now 6-segmented; posterodorsal surface of somite 1 with 6 setae (S); other somites as previously but with ventral swelling in presumptive pleopod region; inner margin of furcae with additional biplumose setae lateral to acute median notch giving formula of 5+5; otherwise unchanged.

Zoea IV

Carapace (Figs. 1E, 2C; Table 1): ridge along the posterior margin is well defined; carapace now with 8 pairs of anterodorsal setae and each ventral margin with 15 setae (1 plumose anterior seta and 14 sparsely plumose posterior setae).

Antennule (Fig. 4C): endopod bud absent; exopod now with 6 aesthetascs and 1 setae (S) terminally and with 1 aesthetasc and 1 setae (S) sub-terminally.

Antennae (Fig. 4H): endopod bud on antenna approximately one-half the length of exopod; otherwise unchanged.

Mandible (Fig. 5C): now with mandibular palp bud.

Maxillule (Fig. 6C): coxa unchanged; coxal endite with 12-15 setae (PD); basal endite with 17-20 setae (PD); endopod unchanged.

Maxilla (Fig. 7C): coxal endite with 10-11+6 setae (PD); basal endite with 9-10+8-10 setae (PD); endopod unchanged; exopod (scaphognathite) margin with 39-43 setae (HP).

First maxilliped (Fig. 8C): basis now with 1+3+3+4 setae (PD); endopod unchanged; exopod distal segment with 14 setae (HP).

Second maxilliped (Fig. 8H): basis and endopod unchanged; exopod distal segment with 14 setae (HP).

Third maxilliped (Fig. 9C): further developed; now with epipod.

Pereiopods (Fig. 9C): further enlarged and differentiated into segments; no setae present.

Abdomen (Fig. 10D): mid-posterodorsal spine on abdominal somite 1 reduced; pleopod buds on abdominal somites 2-6 uniramous with endopod absent; second lateral spine and dorsomedial spine on telson reduced; inner margin of furcae with additional biplumose setae lateral to acute median notch giving formula of 6+6; otherwise unchanged.

Zoea V

Carapace (Fig. 1F, 2D; Table 1): now with 14 pairs of anterodorsal setae and each ventral margin with 24 setae (1 plumose anterior seta and 23 sparsely plumose posterior setae); otherwise unchanged.

Antennule (Fig. 4D): endopod bud present; exopod now with 11-13 aesthetascs and 2 setae (S) terminally, and 2 subterminal aesthetascs.

Antenna (Fig. 4I): endopod bud partially segmented and approximately as long as exopod; otherwise unchanged.

Mandible (Fig. 5D): endopod bud (palp) more developed but unsegmented and unarmed.

Maxillule (Fig. 6D): coxa unchanged; coxal endite with 21 setae (PD); basal endite with 23-25 setae (PD); endopod unchanged.

Maxilla (Fig. 7D): coxal endite with 13-14+8-9 setae (PD); basal endite with 13-15+11 setae (PD); endopod unchanged; exopod (scaphognathite) margin with 51-56 setae (HP).

First maxilliped (Fig. 8D): exopod distal segment with 15 long terminal setae (HP); otherwise unchanged.

Second maxilliped (Fig. 8I): number of setae on terminal segment of endopod unchanged but all now plumodenticulate; exopod distal segment with 17 long terminal setae (HP); otherwise unchanged.

Third maxilliped (Fig. 9D): further developed with partial segmentation; otherwise unchanged.

Pereiopods (Fig. 9D): further enlarged with partial segmentation.

Abdomen (Fig. 10E): somite 1 now with mid-posterodorsal spine further reduced and 10 posterodorsal setae; pleopod buds on abdominal somites 2-6 further developed and elongate, now biramous with endopods present except on pleopod 5 (somite 6); the dorsomedial spine on the telson is further reduced or absent; otherwise unchanged.

Megalopa

Carapace (Fig. 11A): dimensions, CW- 2.92 ± 0.18 mm, CL- 3.57 ± 0.22 mm; carapace roughly quadrangular, broadest immediately below orbits and narrowing posteriorly; surface has a covering of setules, denser towards margins as illustrated; intra-orbital plate simple, over half width of carapace, lateral margins almost parallel, slightly bilobed with medial depression, and directed obliquely downward.

Antennule (Fig. 4E): peduncle 3-segmented with 3, 5, 0 setae (S) respectively; endopod 2-segmented with 2, 6 setae (S) respectively; exopod 4-segmented with 0, 5+6, 5+4, 4+3 subterminal aesthetascs respectively, segment 4 with 3 setae (S).

Antenna (Fig. 4J): peduncle 3-segmented with 7 (2 S, 5 PD), 6 (PD), 6 (S) setae respectively; flagellum 8-segmented with 0, 2, 4, 1-2, 5, 2, 4, 5 setae (S) respectively.

Mandible (Fig. 11B): endopod palp 3-segmented although divisions between segments 1 and 2 sometimes unclear, terminal segment with 19-23 marginal setae (PD).

Maxillule (Fig. 6E): coxa with 6 setae (3 P, 3PD); coxal endite with 33-39 setae (PD); basal endite with 35-37 setae (PD); endopod now unsegmented with 2+2+2+2 setae (PD).

Maxilla (Fig. 7E): coxal endite bilobed with 23-26+11-13 setae (PD); basal endite bilobed with 17-21+16-18 setae (PD); reduced endopod which is no longer bilobate, with 8-14 setae (7-11 HP, 1-3 PD); exopod (scaphognathite) margin with 80-88 setae (HP) and 3 lateral setae (P) on each side.

First maxilliped (Fig. 8E): well developed triangular epipod with 20-26 long setae (S); coxal endite with 22-27 setae (PD); basal endite with 53-61 setae (PD); endopod unsegmented with 5-7 spines, 3-6 subterminal setae (PD) and 2-5 terminal setae (PD); exopod 2-segmented with 5-8 (P) and 6-8 (HP) setae respectively.

Second maxilliped (Fig. 8J): epipod bilobate with 21-22 long setae (HP); coxa with 2 setae (PD); basis with 6 setae (PD); endopod 5-segmented with 4, 5-8, 6, 12-15, 10-12 setae (PD) respectively; exopod 2-segmented, first segment with 4-7 spines and 1 setae (S), terminal segment with 8 setae (HP).

Third maxilliped (Fig. 11C): epipod with 39-53 long setae (P) and arthrobranch gill; coxa and basis not differentiated with 16-28 setae (8-16 S, 8-12 PD); endopod 5-segmented with

57-63, >40, >30, >30, 17-21 setae (PD) respectively; exopod 2-segmented with 6-8 (S), 7-8 (1S, 6-7 HP) setae respectively.

Pereiopods (Figs. 11D-F): cheliped with 2 curved spines on ischium, one more prominent; pereiopods 2-5 thin and setose; dactylus of pereiopods 2-4 with a strong, inwardly flexed terminal spine and with 9-10, 8-9, and 7-8 spines on inner margin respectively; strong spine on distal inner margin of pereiopods 2-4, strongest in pereiopod 2; pereiopod 5 with no spines along inner margin of dactylus, 4-6 small terminal spines, and 3-4 subterminal long setae.

Sternal plates: plates anterior of 2nd pereiopods (plates 1-3) fused with row of 8 setae along anterior third; behind this row of setae is a minute medial spine flanked by 8-10 pairs of setae. Remaining sternal plates unarmed.

Abdomen (Figs. 10F-I): 6 somites present plus telson with dorsal setation as figured; exopods of pleopods 1-4 with respectively 32-34, 33-35, 29-31, and 26-30 natatory setae; endopods with 6-7, 5, 5, 4-5 coupling hooks on the inner margin; uropods without endopod, with 19-20 natatory setae on distal and 1 seta on proximal segments; telson rounded, posterior margin with 5 setae.

Table 1. Dimensions of zoeas of *Pseudocarcinus gigas* (in mm; mean of 10 individuals per zoeal stage, standard deviation in brackets).

Feature	Zoeal Stage				
	1	2	3	4	5
Dorsal to rostral spine (TT)	2.59 (0.10)	2.99 (0.23)	3.61(0.21)	4.44 (0.21)	5.56 (0.33)
Lateral spine range (CW)	1.65 (0.13)	1.72 (0.06)	2.11 (0.09)	2.50 (0.15)	3.32 (0.16)
Base of rostral spine to posterior margin of carapace (CL)	0.93 (0.07)	1.29 (0.07)	1.57 (0.06)	2.05 (0.11)	2.54 (0.12)
Ratio TT/CW	1.6	1.7	1.7	1.8	1.7
Ratio CW/CL	1.8	1.3	1.3	1.2	1.3

Figure 1. *Pseudocarcinus gigas*: A, prezoa, lateral view; B, first zoea; C, second zoea; D, third zoea; E, fourth zoea; F, fifth zoea.

Figure 2. *Pseudocarcinus gigas*: Carapace ventral margin: A, second zoea; B, third zoea; C, fourth zoea; D, fifth zoea.

Figure 3. *Pseudocarcinus gigas*: First zoeal stage: A, frontal view; B and B', abdomen, dorsal view and lateral view; C, antennule; D, antenna; E, mandible; F, maxillule; G, maxilla; H and H', first maxilliped and endopod detail; I and I', second maxilliped and endopod detail; J, pereopod buds. Scale bars are 0.5 mm for A and B; and 0.1 mm for C to J.

Figure 4. *Pseudocarcinus gigas*: Antennule: A, second zoea; B, third zoea; C, fourth zoea; D, fifth zoea; E, megalopa. Antenna: F, second zoea; G, third zoea; H, fourth zoea; I, fifth zoea; J, megalopa.

Figure 5. *Pseudocarcinus gigas*: Mandible: A, B, C, D, second to fifth zoea. Posterior view (left), frontal view (right).

Figure 6. *Pseudocarcinus gigas*: Maxillule: A, B, C, D, second to fifth zoea; E, megalopa.

Figure 7. *Pseudocarcinus gigas*: Maxilla: A, B, C, D, second to fifth zoea; E, megalopa.

Figure 8. *Pseudocarcinus gigas*: First maxilliped: A, B, C, D, second to fifth zoea; E, megalopa. Second maxilliped: F, G, H, I, second to fifth zoea; J, megalopa.

Figure 9. *Pseudocarcinus gigas*: Rudimentary third maxilliped and pereopods: A, B, C, D, second to fifth zoea. The third maxilliped is absent at the second zoeal stage.

Figure 10. *Pseudocarcinus gigas*: Abdomen: A, lateral view, second zoea; B, C, D, E, dorsal view, second to fifth zoea; F, megalopa; G, pleopod 1; H, endopod of pleopod one enlarged; I, telson and uropods.

Figure 11. *Pseudocarcinus gigas*: Megalopa: A, dorsal view; B, mandible; C, third maxilliped; D, cheliped; E, fourth pereopod; F, fifth pereopod.

Discussion

Phylogenetic significance of larval characters

Several authors have attempted to explain phylogenetic relationships of xanthoid crabs based on larval characters. Wear (1970) considered that the most important single zoeal character for distinguishing major groups is the length of the antennal exopod in relation to that of the protopodal process. Rice (1980) divided xanthoid zoea larvae into four groups using several characters with emphasis on the antennal exopod. This scheme was expanded further by Martin (1984) to cover two additional groups although the original groups proposed by Rice (1980) remained unaltered. *Pseudocarcinus gigas* falls into group III of these classifications as the zoea larvae have a robust antennal exopod with three unequal terminal setae. In addition, the other characters used by Rice (1980) and Martin (1984) to define group III are also present although there are five zoeal stages, a character more typical of group IV.

The zoeal groups proposed by Rice (1980) and Martin (1984) correspond only loosely with the classification of Guinot (1978) which was based on adult characters. Zoeal group III includes genera from Eriphiidae, Trapeziidae, and Platyxanthidae while the genera *Menippe* and *Sphaerozys* are in zoeal group IV although these also lie within Guinot's Eriphiidae (termed Menippidae by Guinot, 1978; see Holthuis, 1993). Although the zoeal groupings are broad, they do not challenge the placement of *P. gigas* within the Eriphiidae.

Some of the larval characters of *P. gigas* indicate affiliation with the genus *Ozys* which is also present in southern Australia. The first abdominal somites of *P. gigas* zoea larvae bear a single dorsal spine which is an unusual larval character and has been suggested as a generic feature of *Ozys* based on *O. truncatus* and *O. rugulosus rugulosus* (Wear, 1968; Kakati and Nayak, 1977; Wear and Fielder, 1985). The cheliped ischium of the *P. gigas* megalopa bears a prominent recurved spine which is also present on the two described *Ozys* species, but not for any other member of the Ozinae with described larvae (listed in Introduction).

Martin (1988) presented a detailed analysis of phylogenetic relationships of xanthoid crabs based on 16 megalopal characters; when megalopas of *Ozys* spp. and *P. gigas* are compared with this more extensive list of criteria, any relationship appears to be less clear (see

Appendix 5). Using Martin's (1988) criteria for megalopas, *P. gigas* appears to be more closely affiliated with the genus *Menippe* which also belongs to Oziinae yet falls into group IV of Martin's (1984) grouping of xanthoid zoea larvae. This apparent affiliation of megalopas is largely due to higher setal counts on appendages. Setal counts on mouthpart segments tend to increase with each zoeal stage so it is not surprising that *P. gigas* and *Menippe* spp., each with five zoeal stages, tend to have more setae at megalopa than other members of Oziinae which only have four zoeal stages.

Number of zoeal stages

Most xanthoid crabs have four zoeal stages although this number is variable, even within genera (Wear, 1970); for example, *Pilumnus lumpinus* has only a single non-planktonic larval stage (Wear and Fielder, 1985) while most other *Pilumnus* species have 4 zoeal stages. Martin (1984) discussed trends in the number of larval instars and noted that abbreviated development has been attributed to development within restricted estuarine habitats (Rice, 1980), although he considered this theory untenable given that numerous exceptions exist. Advanced development (greater than four zoeal stages in xanthoids) was discussed by Scotto (1979) who attributed the prolonged development of *Menippe* species to a retained primitive feature, being similar to cancrids. The explanation of Scotto (1979) implies that retention of 5 larval stages is a primitive trait and that *Menippe* is a primitive genus. However, other authors have considered the genus derived (Rice, 1980). The example already listed of variation in the number of larval stages in *Pilumnus* implies that number of zoeal stages is relatively plastic and may be a poor indicator of phylogeny.

Dispersal will be influenced by the number of larval stages although Havenhand (1995) noted that dispersal of adults by rafting or drifting may be more widespread, and more important, than is generally appreciated. Clearly, chances of dispersal of adults by drifting is greatest in small species. The advanced larval duration of *Menippe* and *Pseudocarcinus* may simply be a response to the limited potential for dispersal of the adults as these are among the physically largest genera of the Xanthoidea.

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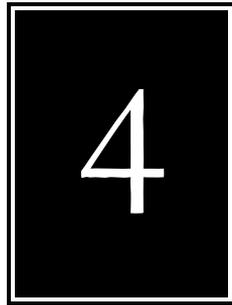
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*A Small Sample of Giant Crab
Pseudocarcinus gigas Larvae
Collected from Southern Tasmania*



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Gardner, C. First record of larvae of the giant crab *Pseudocarcinus gigas* in the plankton. Papers and Proceedings of the Royal Society of Tasmania, 132: 47-48.

An important aspect of the biology of giant crabs *Pseudocarcinus gigas* for management is larval development and its influence on dispersal. Although several laboratory studies conducted on the larvae of the giant crab are described in this thesis, no larvae or recently settled juveniles have been collected from the wild previously. This chapter documents the collection of three stage II *Pseudocarcinus gigas* zoeas from oceanic waters in the vicinity of Pedra Branca off southern Tasmania (within the region: longitude 147°09'32"-147°28'30": latitude 44°11'23"-44°12'30"), an area at the southern limit of the range of *P. gigas*.

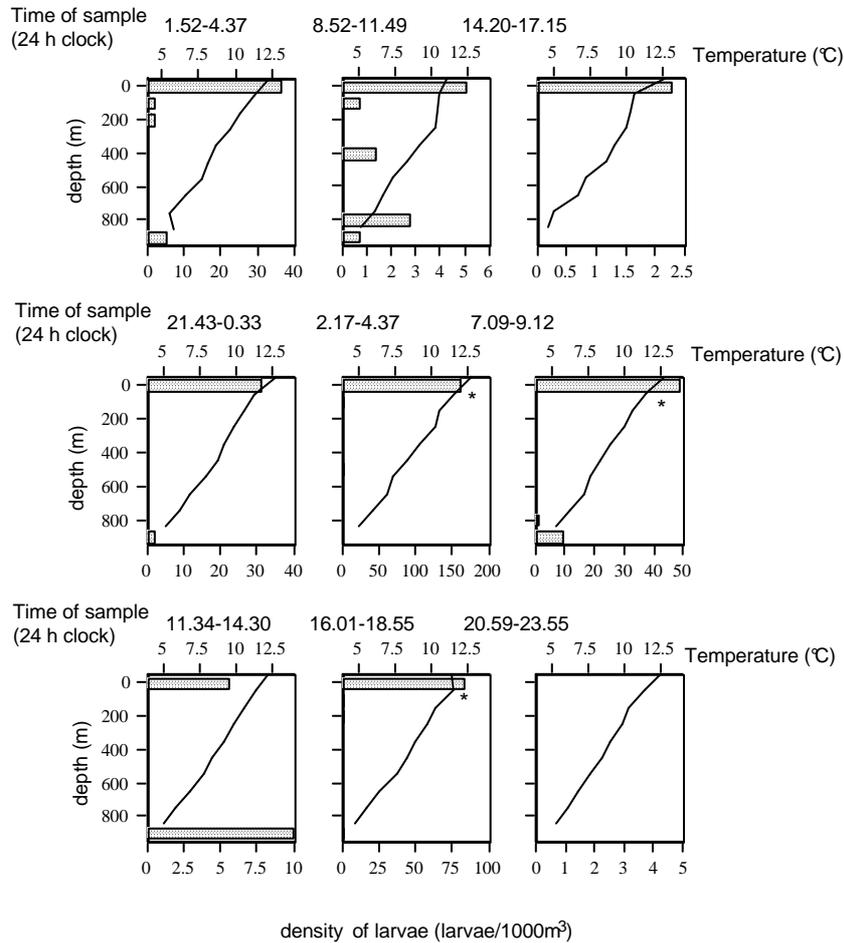
The diel vertical distribution of brachyuran larvae was determined from plankton tows at 10 sampling depths, from 10 to 900 m, collected at 9 periods over 48 hours (Fig. 1). Different depths were sampled in a continuous tow using an EZ plankton net (1 m² mouth) deployed from the *Southern Surveyor*, CSIRO Fisheries Research Vessel (modified Tucker trawl, see Harding et al., 1987). Sampling was conducted in November 1992 near the edge of the continental shelf. Bottom depth ranged from 965 to 1584 m and sampling depth of plankton tows was at 100 m intervals, from 10 to 900 m. The volume of water filtered at each sampling depth ranged between 1650 to 550 m³ and averaged 1140 m³. Sampling was conducted almost continually over 14 and 15 November 1992.

Only higher brachyuran larvae were sorted from plankton samples so counts for total Brachyura probably exclude larvae of crabs from the families Homolidae and Dromiidae, both of which are present in the region. A total of 342 brachyuran larvae were collected and of these, only 3 were identified as *Pseudocarcinus gigas*. Identification was based on form of the telson, presence of a dorsal spine on the first abdominal somite, and on the setation patterns of the maxillule and maxillae (Gardner and Quintana, 1998; Chapter 3). All three *P. gigas* larvae were at the second of the five zoeal stages and were found in the upper 100 m; this depth was also where most of the brachyuran zoeas were collected.

Total brachyuran larvae were distributed predominantly in the surface waters, above 100 m, and this distribution did not appear to be affected by time of sampling. Only one sample appeared to have a different pattern of larval distribution with most brachyuran larvae at >800 m (midday sample: 11.34-14.30; Fig. 1). The presence of zoeas at this depth is rare (Rice, 1979) although very few larvae were captured in this tow (n=13) so the unusual depth distribution may be spurious. The temperature gradient was relatively constant with depth and there were no indications of thermoclines.

The presence of very few *P. gigas* larvae in samples is probably due to the southern latitude of the sampling program. The date of sampling appears to be appropriate as *P. gigas* larvae should have been released prior to the sampling trip. Hatching usually occurs in late October/early November and the presence of stage 2 zoeas also suggests that the plankton sampling was after the peak period of hatch. Likewise, the presence of stage 2 larvae suggests that settlement would not have occurred by this date. Further, the larval duration of laboratory reared larvae is around 50 days which suggests that settlement would not occur before late December (Gardner and Northam, 1997; Chapter 8). Sampling was conducted near the edge of the continental shelf, an appropriate region as it suspected to be where larval release occurs (Levings et al., 1996). Consequently, a possible explanation for the low capture rate of *P. gigas* larvae is the southerly latitude. Although commercial fishers occasionally capture adult *P. gigas* in this region as bycatch, high densities and targeted fishing generally only occurs further north above 42°30'.

Figure 1. Effect of time of day on brachyuran zoeal density (bars) in relation to water depth and temperature (lines) at Pedra Branca on 14th and 15th November 1992. A total of only 3 *Pseudocarcinus gigas* larvae were collected; these samples are marked with an asterisk (*).



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*Behavioural basis of depth regulation in
the first zoeal stage*

5

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Abstract

The responses of the first zoeal stage of *Pseudocarcinus gigas* (Lamarck) to stimuli affecting vertical migration were determined. Larvae are negatively buoyant and sink passively at 0.61 cm/s at 35 ppt salinity. Negative geotactic response was weak in larvae immediately after hatching, however, larvae exhibited strong negative geotaxis in tests at 15 and 20 hours; this pattern was less evident in subsequent samples up to day 13. It is suggested that a strong negative geotactic response is important initially to induce upward migration of larvae from the site of release, at approximately 160-250 m depth, to the surface. Light-adapted larvae were sensitive to change in light intensity from overhead lighting with both decreases and increases in intensity inducing downward migration; this negative phototactic response was only induced at low light intensities (below 230 lux). It is suggested that this may be a shadow response which has not been previously reported for increases in intensity. Distributions of larvae in angled testing columns suggest that the larvae are actively swimming away from the light source when negative phototaxis occurs. Where positive phototaxis occurred, larvae appeared to orientate with gravity while light provided the stimulus for increased locomotory activity. Larvae did not respond to small changes in pressure (0.24-2.70 cm water/s, at surface) of a similar magnitude to that which they would experience by vertical swimming. They were able to detect currents and most larvae could maintain position in currents of 1.12 cm/s.

Introduction

Development of the larvae of giant crabs consists of a prezoaea, 5 zoeal stages and a megalopa (Gardner and Quintana, 1998; Chapter 3). Vertical migration of these larvae, and their response to currents, is likely to influence dispersal, survival and growth. Mechanisms controlling migration in planktonic crustaceans are highly complex and can be influenced by a range of stimuli including pressure, polarisation of light, gravity, absolute light intensity, salinity, change in light intensity, predator fields, prey fields, and temperature (Knight-Jones and Morgan, 1966; Umminger, 1969; Latz and Forward, 1977; Forward et al., 1984; Gliwicz and Pijanowska, 1988; and Forward, 1990). While many stimuli may be

perceived by brachyuran larvae, and contribute to vertical migration, it appears that migration is predominantly controlled by phototaxis in the presence of light (Thorson, 1964), and geotaxis (gravity) and barokinesis (pressure) in the absence of light (Sulkin, 1973).

The response to external stimuli varies between species so that widely different migration strategies exist in relation to depth and diel cycle. Plankton, including decapod larvae, do not show a clear general diel pattern in oceanic water off Tasmania; some species exhibit nocturnal upward migration while others are found on the surface during daylight hours (Pers. comm., Barry Bruce, Div. Mar. Sci., CSIRO, Hobart, Australia, Sept. 1995).

Consequently, no assumptions can be made on the general pattern of larval movement in *P. gigas*. Further, it is not possible to predict larval movements of the giant crab based on other studies as deep-water crabs, or those with oceanic larval stages, have received scant attention in behavioural research. Most other studies on brachyuran larval behaviour have focused on estuarine species with only one other deep water species, *Geryon quinquedens*, having been studied (Kelly et al., 1982).

The aim of this research was to provide behavioural information to assist with understanding movement of giant crab larvae and to contribute to research on deep water crabs in general. An additional aim was to identify larval environmental preferences to assist with small scale production of juveniles. The responses of stage 1 larvae to several stimuli influencing vertical migration were assessed; gravity, spectral sensitivity, absolute light intensity, change in light intensity, orientation of light and change in pressure. Larvae were also tested for their response to lateral current movement.

Materials and methods

Source of larvae

Thirty ovigerous females were collected from depths in the range of 300 – 380 m off the east coast of Tasmania (41°15'S;148°40'E) in May 1994 by a commercial fisher. These females ranged in size from 2.2 – 3.5 kg and were held in two 4 m³ tanks with flow

through, unfiltered, water supply. Crabs were fed twice weekly with abalone (*Haliotis* spp.) or mackerel (*Trachurus* sp.) and food remains were removed after 48 hours.

Hatching of larvae commenced in November 1994 and continued for three weeks. Stage one zoeas were collected for behavioural experiments by first flushing the system of any zoeas present in the tanks, and then reducing flow so that only newly hatched larvae could be drawn from the tanks. Zoeas were mixed by drawing samples from each of the two holding tanks, so that no female contributed more than 50% of the larvae in any trial. Further mixing of larvae was achieved within tanks, as daily monitoring of the egg masses indicated that on any day where larvae were collected, hatching occurred in the egg masses of at least three females.

Sinking and swimming rates

Sinking rates were determined for 30 stage one zoeas collected from each of the two holding tanks and narcotised until immobile in a solution of 0.05% 2-phenoxyethanol in sea water. The larvae were then allowed to sink through a sea water filled plexiglas column and their rate of descent measured for 25 cm after an initial descent of 25 cm.

Swimming rate was measured for 30 larvae which were introduced to a clear, horizontal plexiglas column with illumination from one end (500 lux). The time required for larvae to swim 15 cm without stopping or turning was measured.

General experimental procedures for geotaxis, phototaxis and barokinesis experiments

Zoeas were discarded after use in an experiment and were replaced if held for more than four hours after collection before use in a behavioural trial (with the exception of geotaxis experiments where zoeas up to 13 days old were used).

All experiments were replicated four times with each replicate staggered between other treatment levels. For instance, the behavioural responses of zoeas to each level of light intensity was tested with a single trial at each level and then the entire set of trials was repeated to a total of four times. Trials were conducted at 13°C in a temperature

controlled room. All water used was 0.2 μm filtered seawater of consistently 35 ppt salinity.

The movement of larvae was measured by placing approximately 30 larvae into a clear plexiglas column divided into eleven 5 cm segments (30 mm internal diameter x 550 mm length) and recording their position after subjecting to a stimulus. Larvae were introduced into the middle of the column with a transparent 60 ml syringe. The light intensity that larvae are adapted to has been shown to influence their behaviour in response to light stimulus (Forward, 1974). To prevent confounding of experiments from previous light exposure, larvae were acclimatised to the lighting for 10 min within the transparent syringe. Preliminary trials established suitable duration of trials to be 2 min. Longer periods than this resulted in all the larvae gathering at either end of the testing chamber. Also, no change in the nature of the larval response occurred in trials of 15 min compared with 2 min trials. Barokinesis trials were reduced to 1.5 min due to constraints on the apparatus used to alter pressure.

Injection of larvae into the testing column caused currents which tended to move the larvae vertically upwards. To compensate for this effect, the initial position of the larvae was determined by repeatedly introducing larvae into the testing column and recording their position immediately (433 zoeas in the angled column, 394 in the vertical column). The mean column position of these larvae was used as the point of origin in all trials.

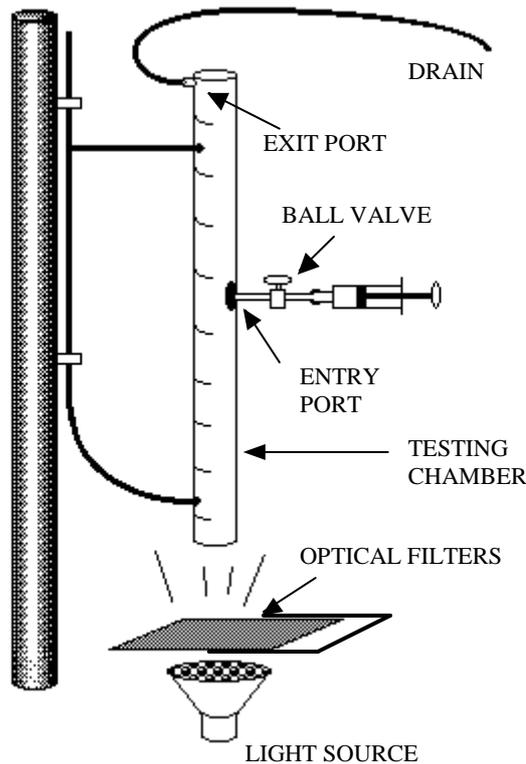
Significant difference between treatments was determined using the method outlined by Sulkin et al. (1980). "Mean position value" was calculated by assigning weights from 1-11 for each of the sections along the testing chamber, multiplying the weights by the number of larvae in each section and dividing the product by the total number of larvae. The mean position value was calculated for each replicate and these values were then used to compare treatments with the non-parametric Mann-Whitney U test (Zar, 1974). Differences in means were considered significant at $P < 0.05$.

Geotaxis

Significant upwards movement of the larvae in the absence of light or pressure changes was attributed to negative geotaxis. The apparatus used to study geotactic response is illustrated in Fig. 1. Geotactic response of larvae was tested at: immediately post-hatch, 15 h, 20 h, 2 d, 6 d, 9 d, and 13 d. Larvae were maintained in a 1000 l tank on a recirculating

water system with UV sterilisation and biofiltration. Larvae were fed 2nd instar artemia nauplii enriched with Protein Selco™ and they moulted to second stage zoeas after 7 days. At the culmination of the geotaxis trials (13 d), larvae were still at second stage zoea.

Figure 1. Detail of testing chamber and experimental apparatus used to measure larval response in darkness and also to light of 617 nm and 478 nm. Wavelength and light intensity was altered with optical filters held in a rack between the light source (quartz halogen flood light) and the testing chamber. Larvae were introduced through the entry port and water and air bubbles displaced through the exit port.



Phototaxis

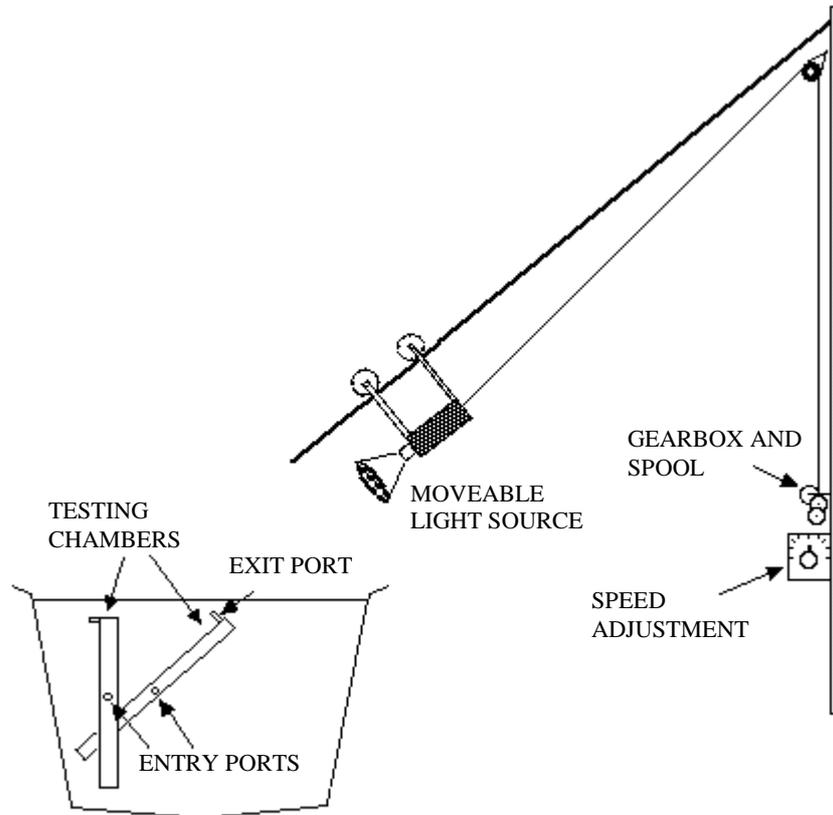
Several aspects of phototactic response were investigated: spectral sensitivity, response to constant light intensity, response to change in light intensity, and the effect of incident angle of light source. All lighting was from quartz halogen globes which was diffused through neutral density optical filters. Generally, intensity of lighting was altered between treatments by changing the wattage of the globe or the neutral density filter although, for change in light intensity trials, the intensity was altered by moving the light source away or towards the testing chamber. Light intensity was measured in lux with a Gossen Profisix™ plan-diffuser light meter.

The apparatus used to study spectral sensitivity is illustrated in Fig. 1. Lighting was from beneath and wavelength was altered with red (Kodak #25, dominant wavelength = 617 nm) and blue (Kodak #47A, dominant wavelength = 478 nm) gelatine filters.

Response to constant light intensity, response to change in light intensity, and the effect of incident angle of light source were determined with the apparatus illustrated in Fig 2. Natural underwater distribution of light was approximated by submerging the testing chamber in a 400 l tank, using angled light and diffusing the light source with neutral density filters. The walls of the outer tank were blackened walls and the tank was filled with 0.2 μm filtered seawater. All trials with constant light intensity were conducted with the light source in the lower position. Test intensities ranged from 3 lux to 40000 lux, recorded from the top of the testing chamber.

To test the effect of change in light intensity, the light source was moved towards or away from the testing chamber by a variable speed 12 V electric motor (Fig. 2). The change in intensity commenced as the larvae were introduced to the column, and continued for the duration of each trial. The effect of change in light intensity was examined for both increasing and decreasing intensities for intensities between 6 and 2000 lux. The range of intensities experienced by larvae for each treatment is given in Fig. 8. Rates of change in light intensity, under natural conditions at sunset, were determined by measuring decline in light intensity on two days in September. Readings were taken every two minutes and rates of change averaged for the two days. Simulated declines in intensity were considerably faster than that which occurs at sunset (Table 1).

Figure 2. Experimental apparatus used to measure larval response to fixed intensity white light and change in light intensity. The light source was moved up or down the track to adjust intensity with a variable speed, 12 V, electric motor connected to a gearbox so as to reduce speed of revolution and increase torque. Initial light intensity was adjusted with neutral density filters or by changing the wattage of the quartz halogen globe.



The effect of incident angle of light source was tested by comparing larval distributions in a testing chamber angled directly towards the angled light source with a testing chamber oriented vertically (Fig. 2). The angle of light incident on the testing chambers was 45° to vertical after refraction through the water surface. Trials conducted to compare the effect of incident angle of light source were conducted simultaneously for the vertical and angled testing chambers.

Table 1. Change of light intensity during natural sunset compared with experimental rates of intensity decline.

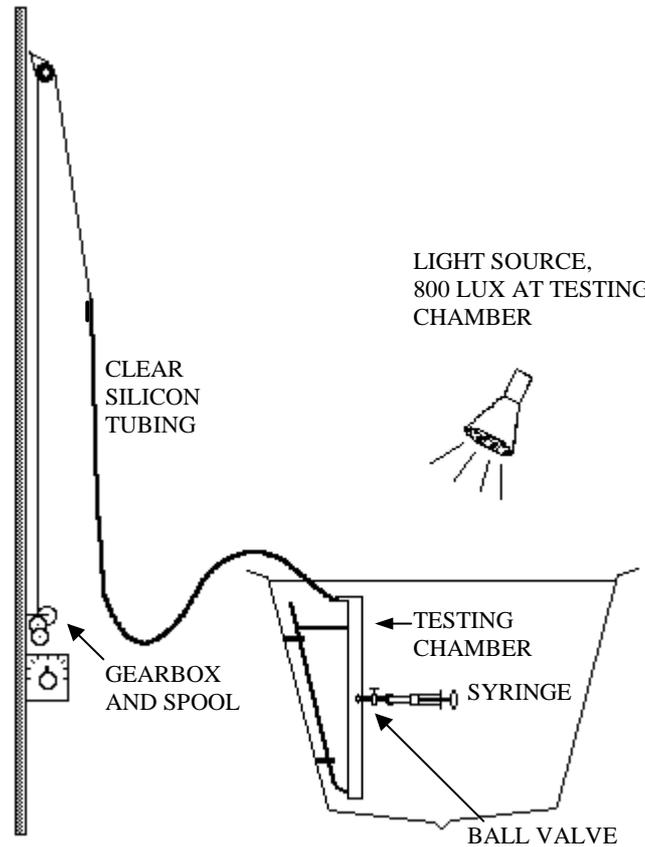
Initial Intensity (lux)	Natural ^a (lux/min)	Simulated change in intensity	
		Slow (lux/min)	Rapid (lux/min)
15	2.8	3	4.5
230	20.9	60	95
900	57.5	175	395
2000	103.9	450	875

^a Values for natural change in intensity at sunset are derived from a regression fitting recorded intensity changes.

Barokinesis

As with phototaxis experiments, the testing chamber was submerged in a water filled, blackened tank (Fig. 3). The testing chamber was orientated vertically and the response of larvae to change in pressure was measured in darkness and also with 800 lux lighting, angled at 45°. Larvae were introduced to the testing chamber through a ball valve which could be closed to seal the chamber. Silicon tubing was connected to the testing chamber and filled with seawater so that the water was continuous with that in the testing chamber. Pressure was then altered by raising or lowering this tubing with a variable speed electric motor and the rate of pressure change recorded as vertical cm per second. This method of recording change in pressure allowed pressure change to be directly related to potential larval movement in surface waters.

Figure 3. Experimental apparatus used to measure larval response to rate of change in pressure. Pressure in the testing chamber was regulated by the height of the silicon tubing, as the water in the tubing was continuous with that in the testing chamber. Change in pressure was achieved by raising or lowering the silicon tubing at different rates with a variable speed, 12 V, electric motor. Lighting was with a 50 W, quartz halogen floodlight. The ball valve was opened to introduce larvae into the testing chamber, with the syringe, and then sealed for pressure trials. The syringe and testing chamber supports were orientated away from the light source to prevent shadowing.

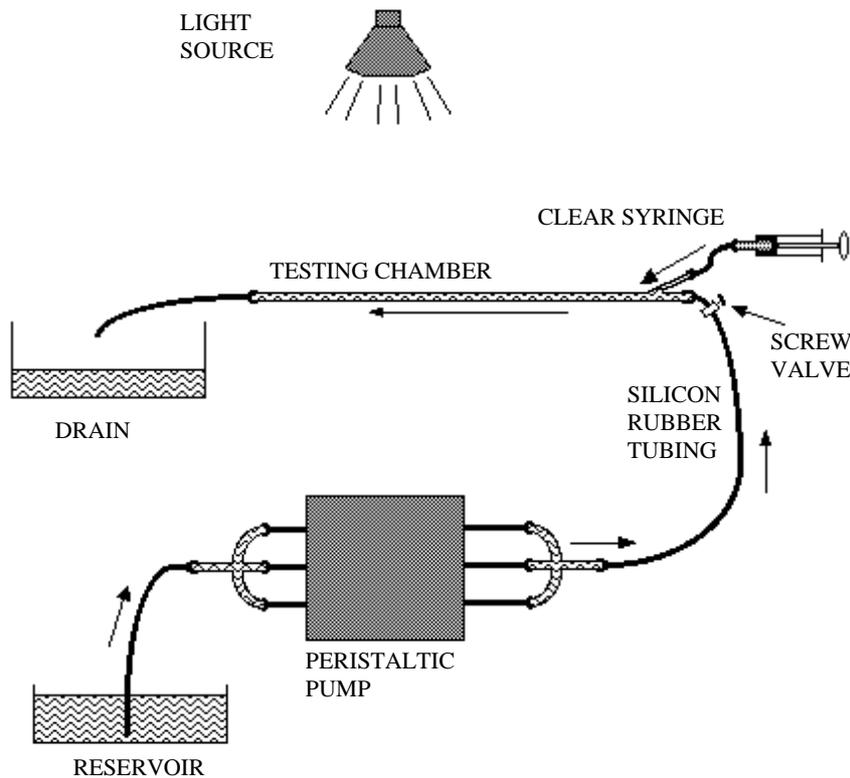


Rheotaxis experimental method

Rheotactic responses of individual zoeas were measured within a 10 mm internal diameter glass tube connected to a peristaltic pump to provide current (Fig. 4). Larvae were introduced to the apparatus with a 60 ml syringe and pulsation was largely removed by constricting the 1.5 m length of expandable silicon tubing, feeding from the pump, with a screw valve. Current speed was adjusted with the peristaltic pump and measured by recording the speed of passage of bubbles through the apparatus. The rheotactic responses of larvae were tested for current speeds from 0.35 – 1.87 cm/s. Lighting was at 90° to the current flow and oriented horizontally to produce an intensity of 80 lux incident on the testing chamber. A positive rheotaxis response was recorded when larvae actively oriented

themselves and swam into the current or maintained position; a negative response was recorded if the larvae were swept along the testing chamber or swam indifferently to the current. At least thirty larvae were used for each current speed tested.

Figure 4. Apparatus used for rheotaxis experiments. Pulsation in flow from the peristaltic pump was reduced by including 1.5 m of silicon tubing between the pump and the glass testing chamber and then constricting tubing immediately before the testing vessel. This caused the silicon tubing to expand and contract, which removed pulsation. Lighting was from a 50 W quartz halogen floodlight, 2.5 m distant and angled at 90° to the testing chamber to produce 80 lux incident on testing chamber.



Results

Swimming and sinking speeds

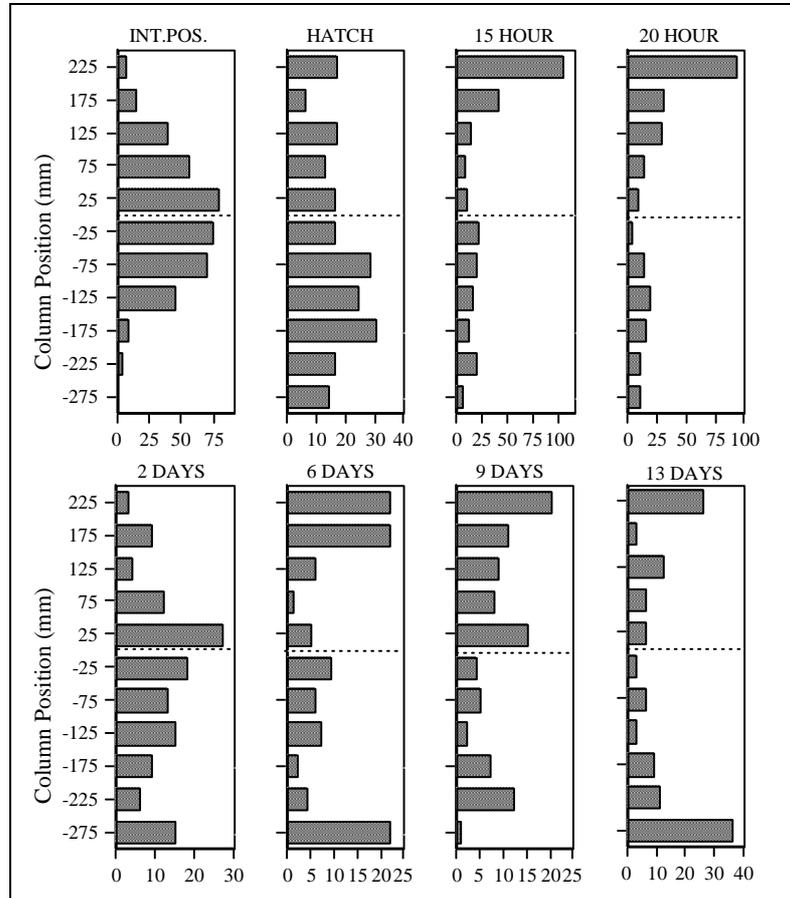
The mean vertical upwards swimming speed of larvae, without pausing, was 1.61 (± 0.38 s.d., $n=30$) cm/s. Assuming that larvae did not pause in swimming and also chose to swim vertically upwards, this rate of swimming would enable larvae released at 350 m depth to

reach the surface waters in about 6 hours. Larvae swam with the dorsal spine foremost and sank in the opposite manner, with the dorsal spine trailing. The average sinking rate for anaesthetised larvae was 0.61 (± 0.084 s.d., $n=30$) cm/s.

Geotaxis

There was a tendency for stage 1 zoeas to be negatively geotactic but this appeared to be influenced by the age of the larvae, or possibly time of day (Fig. 5). Immediately after hatching the larvae did not exhibit any clear geotactic response. Larvae exhibited strong negative geotaxis in tests at 15 h and 20 h but the strength of response declined in older larvae. The mean response of larvae tested at 2 d and 13 d was positively geotactic although the pattern of movement for all larvae tested was not clear with some larvae moving upwards in the column and others downwards.

Figure 5. Ontogenetic change in geotactic response of stage 1 and 2 zoeas. "Int. Pos." is the distribution of larvae immediately after introduction to the testing column; the mean of this distribution was used to define the zero position of column height in the subsequent geotactic response plots. "Hatch" plot is for larvae collected and tested within 10 minutes of release.



Phototaxis

Fixed intensity

In the experimental situation shown in Figure 1 (results, Fig. 6.), downward movement is positive phototaxis. There was strong phototaxis to blue light of 2 lux while larvae failed to exhibit a strong phototactic response when exposed to red light of 11 lux (Fig. 6). In the experimental situation shown in Figure 2 (results Fig. 7), a positive phototactic response will result in upwards movement. Larvae were phototactic to all intensities tested for white light (3-40000 lux, Fig. 7) and at no intensity were larvae induced to swim or sink downwards. There were no significant differences between larval responses to any intensities tested ($P < 0.05$), in both the angled and the vertical columns.

Figure 6. Vertical migration of Z1 larvae in response to white, red (617 nm) and blue (478 nm) light directed from below the vertical testing column. The response of larvae in darkness was measured and has been plotted as the lowest intensity reading for the white light series. This point has been artificially allocated an intensity of 0.5 lux so that light intensity could be plotted on a logarithmic scale. Negative values indicate positive phototaxis i.e. movement towards the light below the testing chamber (see Fig. 1).

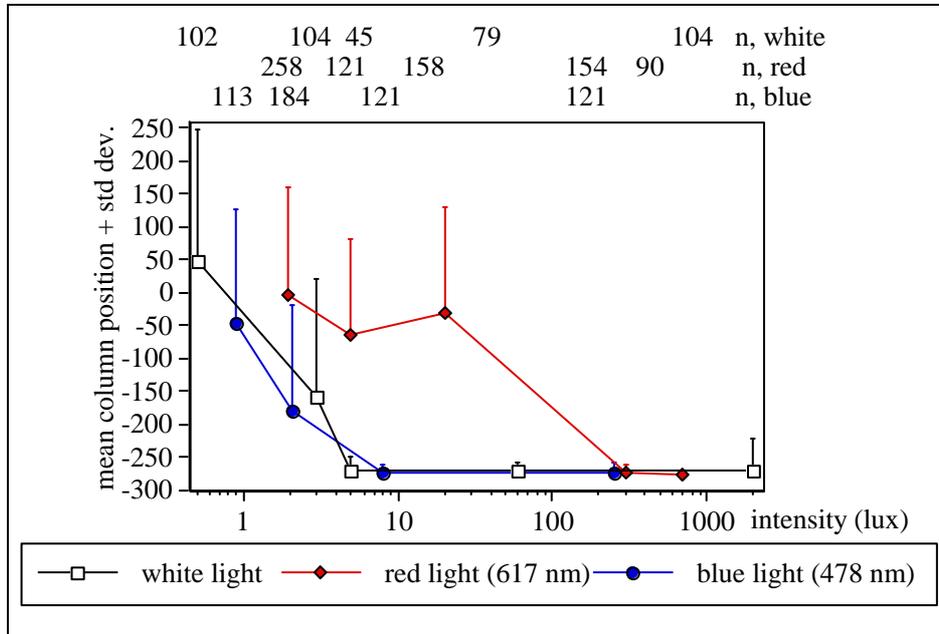
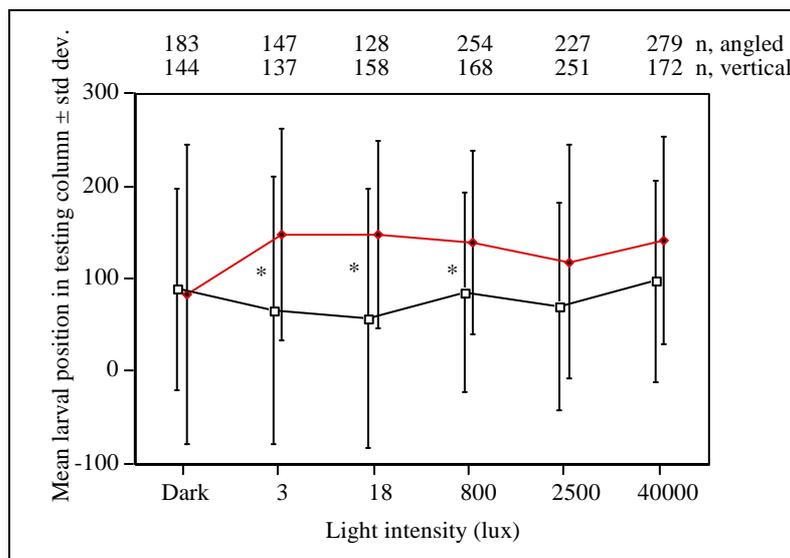


Figure 7. Migration response of Z1 larvae, in vertical and angled testing columns, to light of different intensities. Means have been artificially displaced sideways to prevent overlap of error bars. Significant differences between distributions of angled and vertical tests are denoted by * at $P < 0.05$. Squares - angled column, diamonds - vertically orientated column. Positive values indicate positive phototaxis i.e. movement towards the light above the testing chamber (see Fig. 2).



Effect of change in light intensity

There was a significant ($P < 0.01$) effect of change in light intensity on column position of larvae (Table 2). Downward movement (negative phototaxis, Fig. 2) was most evident in larvae exposed to changes in intensity between 9-15 lux and 6-15 lux (Figs. 8 to 10). In the vertical column, there was a significantly greater movement away from the light source at slow rates of changes in intensity than at faster rates ($P < 0.05$; Fig. 8). There appeared to be no effect of direction of light change (increasing or decreasing intensity) on larval movement (Fig. 9).

Table 2. Statistical comparisons of the effect of different light intensity changes under different regimes of: rate of change; increasing or decreasing intensity; and angled or vertical testing column. Light intensity changes are ranked from lowest to most positive phototactic response, left to right respectively. Bars beneath intensity ranges denote significance by joining non-significant tests ($P < 0.01$). See Fig. 2 for experimental apparatus. "n" = number of larvae per treatment level.

Treatment	Intensity range (lux)				n
Rapidly increasing intensity (angled)	6-15	40-230	250-2000	110-900	1098
Rapidly decreasing intensity (angled)	15-6	230-40	900-110	2000-250	728
Slowly increasing intensity (angled)	9-15	130-230	550-900	1100-2000	838
Slowly decreasing intensity (angled)	15-9	230-130	2000-1100	900-550	938
Rapidly increasing intensity (vertical)	6-15	40-230	110-900	250-2000	1001
Rapidly decreasing intensity (vertical)	15-6	230-40	2000-250	900-110	1012
Slowly increasing intensity (vertical)	9-15	130-230	550-900	1100-2000	985
Slowly decreasing intensity (vertical)	15-9	230-130	2000-1100	900-550	860

Figure 8, a-d. Effect of rate of change of light intensity on vertical migration in Z1 larvae exposed to different varying light intensities. The effect of fast and slow rates of change were tested in combinations of decreasing/increasing intensity and with the testing column orientated directly towards the angled light source (angled) or else orientated vertically (vertical). Plot symbols have been artificially displaced sideways to prevent overlap of error bars. Positive values indicate positive phototaxis (see Fig. 2). * indicates significance at $P < 0.05$.

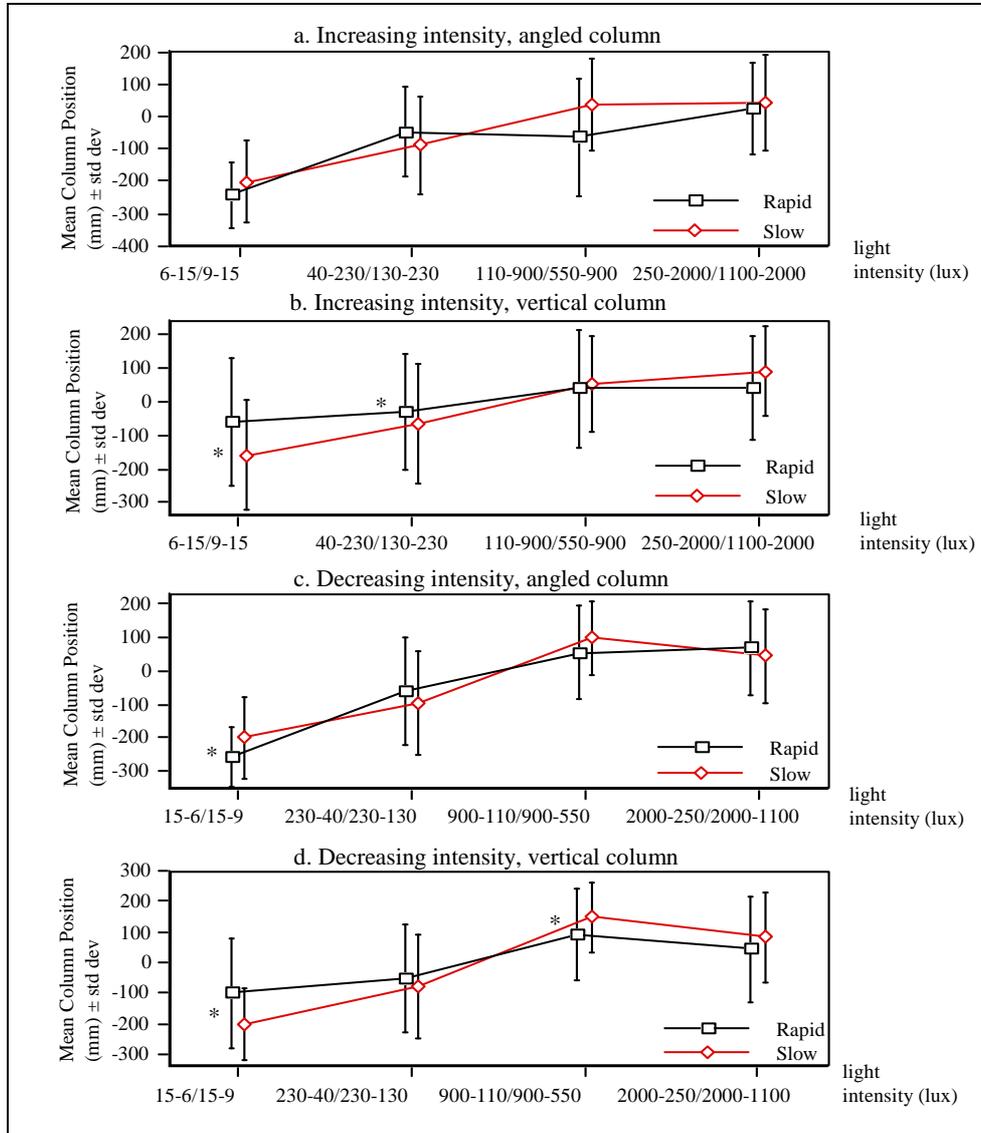


Figure 9, a-d. Effect of direction of change (increasing or decreasing) of light intensity on vertical migration in Z1 larvae exposed to different varying light intensities. The effect of direction of change was tested in combinations of rapid/slow change in intensity and with the testing column orientated directly towards the angled light source (angled) or else orientated vertically (vertical). Plot symbols have been artificially displaced sideways to prevent overlap of error bars. Positive values indicate positive phototaxis (see Fig. 2). * indicates significance at $P < 0.05$.

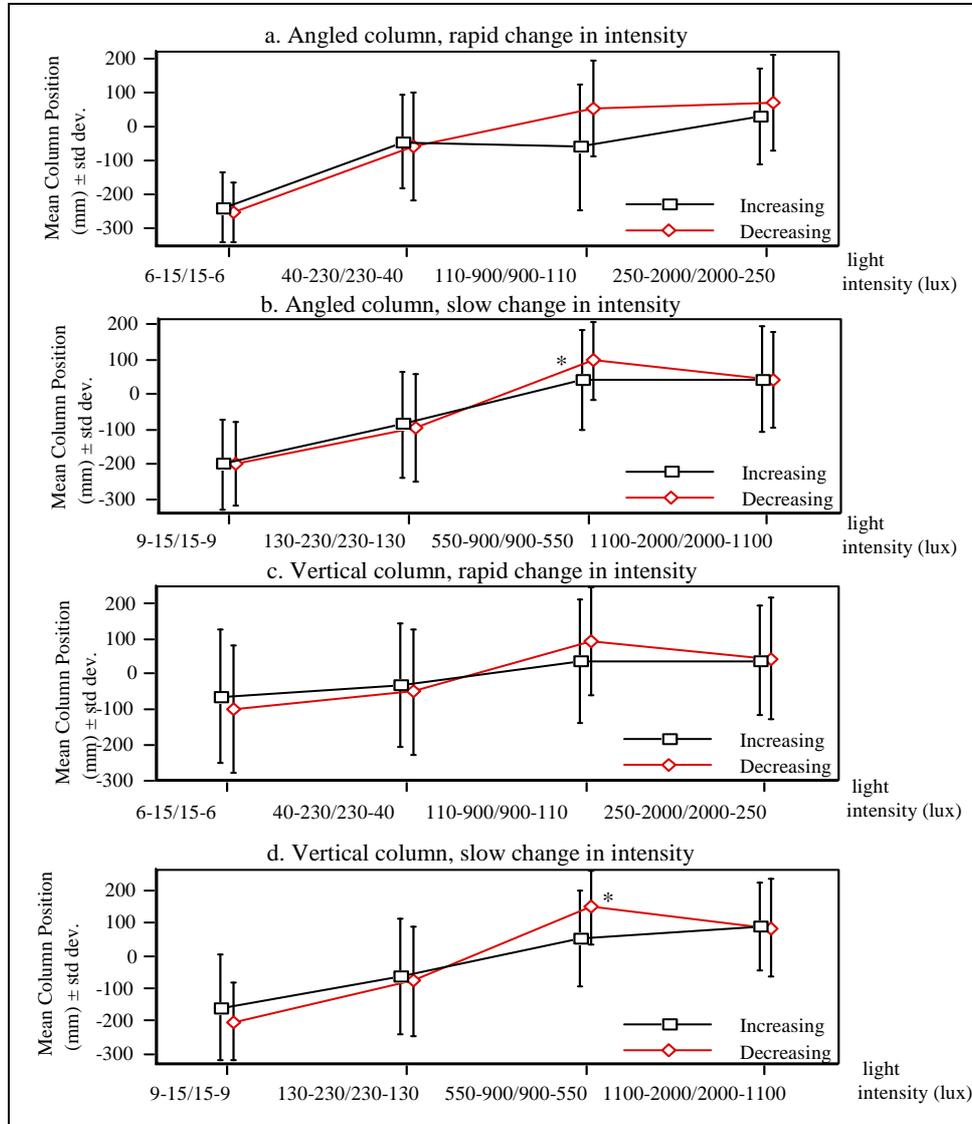
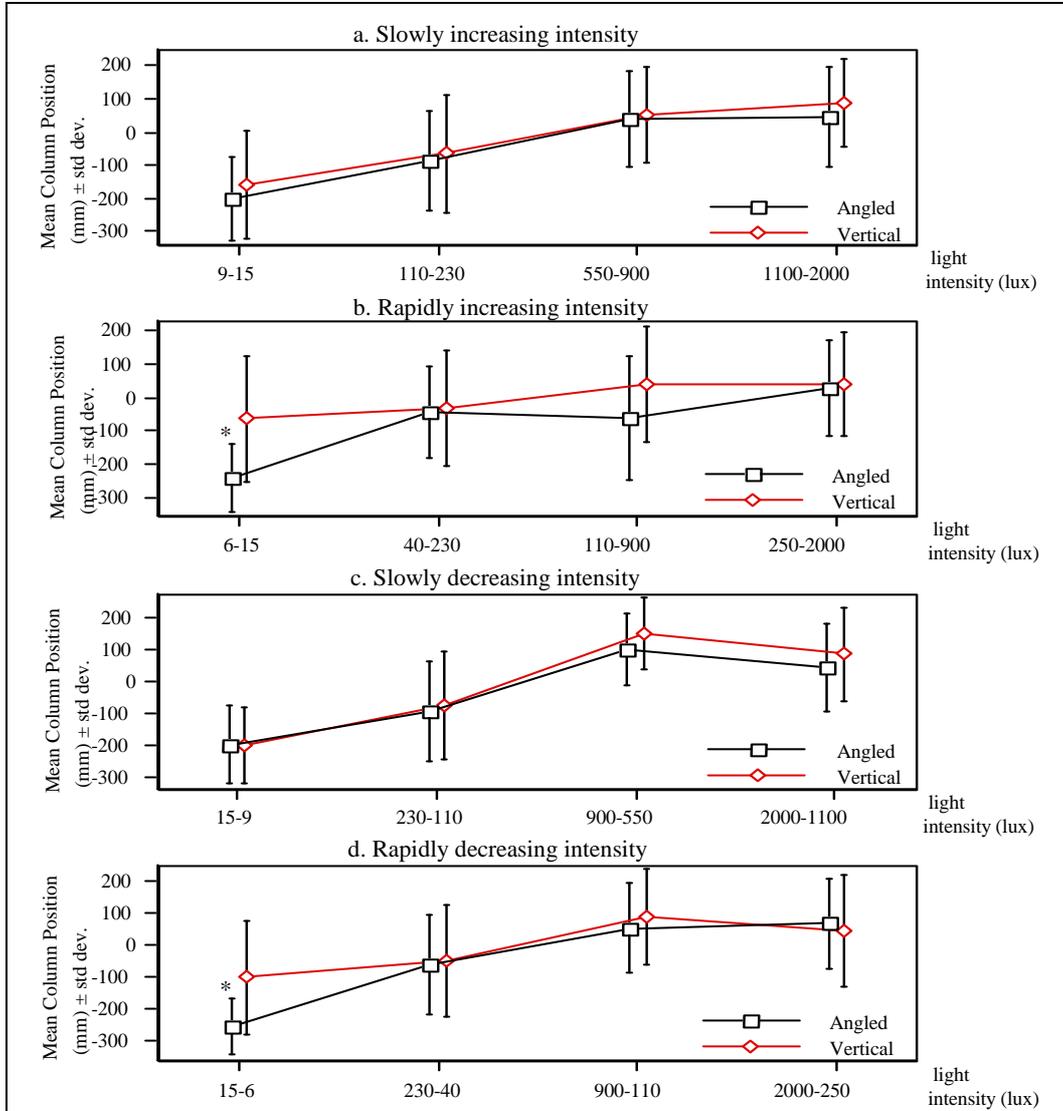


Figure 10, a-d. Effect of orientation of testing column to light source on vertical migration in Z1 larvae exposed to different varying light intensities. The effect of orientation was tested in combinations of decreasing/increasing intensity, at rapid/slow rates. Plot symbols have been artificially displaced sideways to prevent overlap of error bars. Positive values indicate positive phototaxis (see Fig. 2). * indicates significance at $P < 0.05$.



Effect of orientation of light source.

The effect of orientation of light source was investigated by exposing the larvae to an angled light source and comparing the distribution of larvae in a vertical and an angled testing chamber (Fig. 2). This was trialed with larvae exposed to white light of fixed intensity (Fig. 7) and also with varying intensity (Fig. 10). In the fixed light intensity experiments, there was significantly greater movement of larvae along the column in the vertically oriented column, than in the column angled directly towards the light source

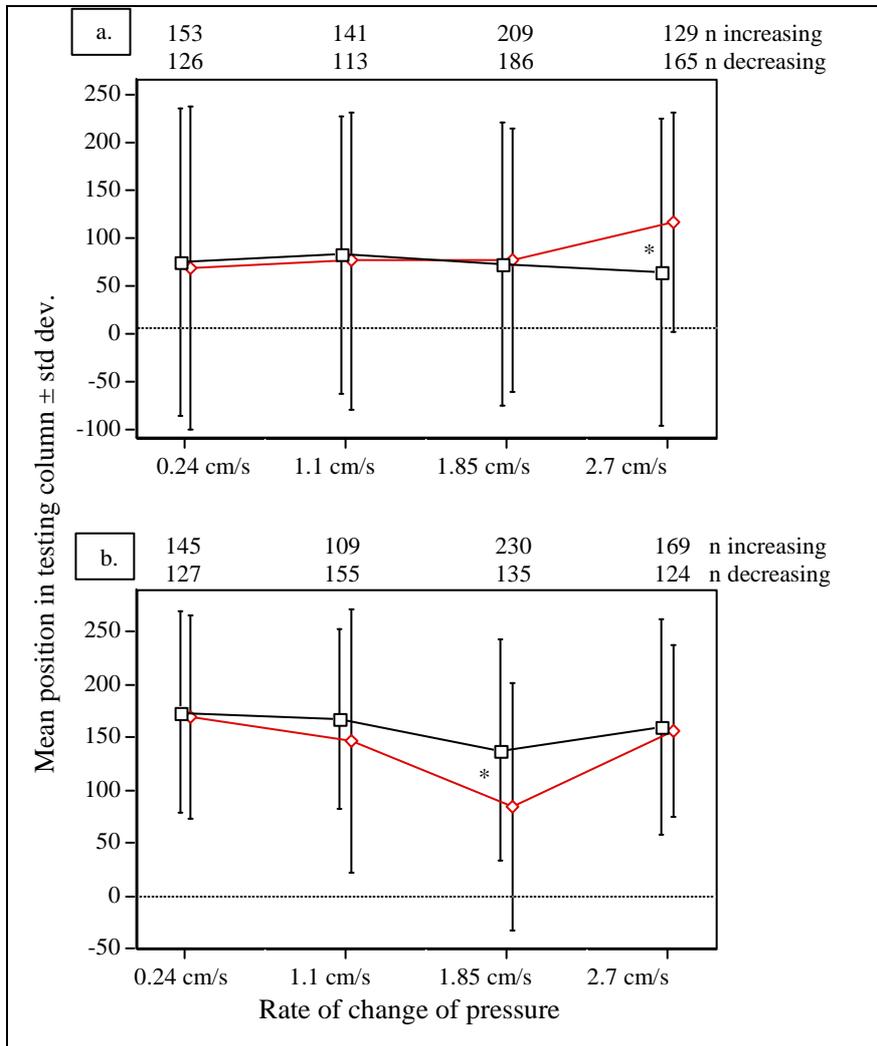
($P < 0.05$; Fig. 7). This trend was not significant at the higher light intensities measured, 2500 and 40000 lux or in darkness ($P > 0.05$).

In the varying light intensity experiments, significantly different movement, between the vertical and angled columns, only occurred when larvae exhibited negative geotaxis in response to rapidly changing light intensity ($P < 0.05$; Fig. 10).

Barokinesis

Larvae did not clearly respond differently to increasing or decreasing pressure under conditions of total darkness or with 800 lux overhead lighting (Fig. 11). Although significant differences were observed, these were considered inconclusive as there was no consistent effect of the rate of change of pressure on larval movement. However, it is noteworthy that the mean upward movement of larvae when exposed to overhead lighting was smallest, although still upwards, when the water column was lowered (decreasing pressure) at 1.85 cm/s (Fig. 11b). This rate of change was the rate closest to the observed swimming speed of stage 1 zoeas, 1.61 cm/s.

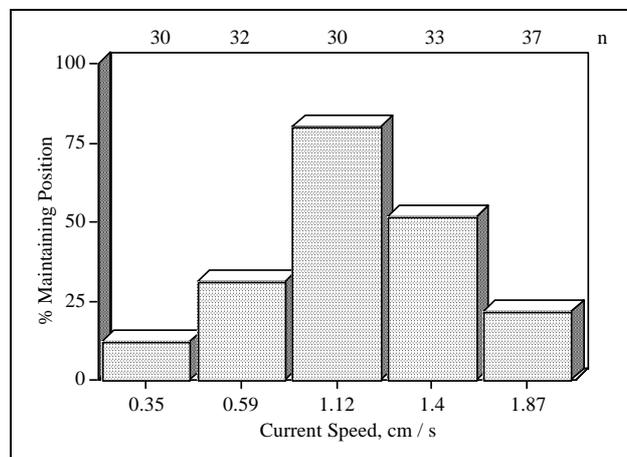
Figure 11, ab. Comparison of the effect of increasing and decreasing pressure of different rates on the vertical migration of Z1 larvae held in darkness (a) or with 800 lux overhead lighting (b). Means have been displaced sideways to prevent overlap of error bars. Increasing pressure - squares, decreasing pressure - diamonds. *



Rheotaxis

Larvae exhibited positive rheotaxis with the greatest proportion of larvae responding at a current speed of 1.12 cm/s (Fig. 12). At lower current speeds (0.35 & 0.59 cm/s), many larvae appeared to be unaware of the current and swam back and forth along the testing column apparently in response to the lighting. At current speeds greater than 1.12 cm/s (1.4 & 1.87 cm/s) larvae tended to swim into the current but failed to maintain position.

Figure 12. Rheotactic response of stage 1 larvae to current velocities of 0.35-1.87 cm/s. Values represent percentage of total larvae responding to stimuli so no measure of error is presented.



Discussion

The pattern of vertical swimming resulting in upward movement was the same as that described by Sulkin (1984); larvae are negatively buoyant which is countered by upwards orientation and locomotion to produce upwards swimming. Passive sinking rates of *P. gigas* (\bar{x} =0.61 cm/s) were similar to that of stage one zoeas of several other brachyuran species: *Cancer magister*, 0.64 cm/s (Jacoby, 1982); *Ebalia tuberosa*, 0.60 cm/s (Schembri, 1982); and *Hemigrapsus oregonensis*, 0.67 cm/s (Arana and Sulkin, 1993). The upwards locomotory force of these species is sufficient to counter sinking and produces similar mean upwards swimming speed to that of *P. gigas* (1.61 cm/s): *Cancer magister*, 0.95 cm/s (Jacoby, 1982);

Ebalia tuberosa, 0.96 cm/s (Schembri, 1982); and *Hemigrapsus oregonensis*, 1.78 cm/s (Arana and Sulkin, 1993). As discussed by Sulkin (1984), depth regulation relies on the interaction between passive sinking, active swimming, and orientation. Orientation and the speed of swimming are then adjusted in response to external stimuli, such as gravity, light and pressure, to induce depth regulatory response.

Geotaxis

The observed pattern of geotaxis suggests that larval swimming is not clearly affected by gravity immediately after hatching. After this initial period the larvae exhibit strong negative geotaxis which suggests that larvae migrate upwards to the surface waters. The speed of larval swimming suggests that this would be accomplished in a period of around 6 hours if the larvae were released at 350 m and did not rest. A negative geotactic response was also observed in the first zoeal stage of the deep sea crab, *Geryon quinquedens* (Kelly et al., 1982).

The strong negative geotactic response observed for *P. gigas* decreased in older larvae and it may be that the primary function of the geotactic response is to induce upward migration from the deep-water site of release to the prey-rich surface layers. An ontogenic change in geotactic behaviour between instars has also been reported for shallow water species *Leptodius floridanus* and *Panopeus herbstii* (Sulkin, 1973), *Callinectes sapidus* (Sulkin et al., 1980), and *Ebalia tuberosa* (Schembri, 1982). However, the changes in geotaxis observed in *P. gigas* differ from these reports as there was a decline in geotactic response within stage 1, rather than between instars.

Although the pattern of geotactic response up to 20 hours appears clear, the results of trials from 2 to 13 days are more difficult to interpret and they may be confounded by at least two factors. Diel cycles have been shown to persist in the laboratory which may have influenced results from these trials (Cronin and Forward, 1986). Attempts were made to only test healthy larvae by selecting active larvae, however, less fit larvae may have been selected for testing and may have compromised results.

Phototaxis

The spectral sensitivity of larvae in this study was essentially the same as that described for several other species in more detail by Forward (1987), and Forward and Cronin (1979). Larvae were more sensitive to shorter blue wavelengths, which have better penetration, than red wavelengths.

Light adapted larvae were not induced to descend in response to overhead white light of any intensity tested. The experimental design used in this study was intended to demonstrate the approximate field conditions where sinking may have been induced by light. Consequently, the testing chamber was oriented vertically so that negative phototaxis would be required to counter negative geotaxis in order to produce net downward movement (light-induced positive geotaxis). Comparisons between other studies where phototactic response was examined with horizontally oriented columns should be made cautiously.

Where phototaxis has been examined independently of geotaxis by the use of horizontal testing columns, larvae tend to exhibit negative geotaxis at low intensities and positive phototaxis at high intensities. This pattern has been observed in: *Rhithropanopeus harrisi* (Forward et al., 1984); *Cancer gracilis*, *Lophopanopeus bellus bellus*, *Hemigrapsus oregonensis* (Forward, 1987); and *Paralithodes camtschatica* (Shirley and Shirley, 1988). Forward (1987) attributed this pattern to predator avoidance.

Avoidance behaviour, or negative phototaxis at low light intensity, is not clearly demonstrated in vertically oriented columns where the natural behaviour of negative geotaxis is incorporated. Both Schembri (1982, *Ebalia tuberosa*) and Jacoby (1982, *Cancer magister*) tested the response of crab larvae to different light intensities in vertical columns and observed only upward movement. It is tempting to infer that the positive phototactic response of *P. gigas* (Fig. 7) indicates that the larvae congregate at the surface during the day. However, Forward (1985 & 1988) considers the absence of negative phototaxis in vertically oriented columns to be a laboratory artefact in most studies. For example, Stearns and Forward (1984) found that the copepod *Acartia tonsa* was positively phototactic to all light intensities although the natural migration pattern is nocturnal. Simulated natural underwater lighting distribution is difficult to achieve, so the observed response of *P. gigas* may be nothing more than a laboratory artefact despite attempted simulation of natural light distribution.

Larvae exposed to change in intensity at low light levels responded by downward movement. Conversely, larvae exposed to change in intensity at higher light levels were unaffected. This response was more pronounced at slower rates of change in intensity (in the vertical column only) but was not affected by the sign of intensity change (increasing or decreasing).

Light-induced downward movement in response to change in light intensity, regardless of whether intensity is increasing or decreasing, has not been previously reported. This response may be a variation of the predator avoidance or shadow response proposed by Forward (1986) where negative phototaxis was induced by a rapid decrease in intensity. The shadow response proposed by Forward (1986) was only initiated by rapid decreases in intensity and not increases as was observed in this study. Forward (1986) noted that the change in intensities which resulted in negative phototaxis were too rapid to simulate dusk or dawn. Because of this, he believed that the larval responses did not represent a typical behaviour relevant to diel vertical migration. The simulated rates of intensity changes in this study were also greater than that at dawn or dusk (Table 1), suggesting the response in *P. gigas* was a predator avoidance, shadow response. The response of stage 1 *P. gigas* zoeas to change in light intensity, only at low light levels, suggests that the larvae are adapted to respond to low levels; this supports the dismissal of the results of fixed light intensity trials as a laboratory artefact.

There was significantly greater ($P < 0.05$) upward movement of larvae in the vertically oriented column exposed to fixed intensity of light, compared with the column angled directly towards the light source (45°). This suggests that geotaxis is the orienting cue while photokinesis controls locomotory activity. Forward (1985) observed this same interaction in larvae of the xanthid crab *Rhithropanopeus harrisi*. Geotaxis appeared to be less important in the larval response to changes in light intensity at low light levels. When downward movement occurred in response to rapidly changing intensity, the larval distribution was further from the origin in the angled column. This suggests that larvae were actively swimming from the light, rather than passively sinking. In this case, the response is a true negative geotaxis rather than light-induced, positive geotaxis.

Barokinesis

Larval detection and response to small changes in pressure have been used to explain vertical migration patterns, as it is considered that pressure response provides a negative feedback on vertical movement (Knight-Jones and Morgan, 1966). Stage 1 zoeas of *P. gigas* did not appear to respond to small pressure changes; this has been reported elsewhere for species where the larvae occupy water of considerable depth: *Callinectes sapidus* (Sulkin et al., 1980); *Geryon quinquedens* (Kelly et al., 1982); and *Hemigrapsus oregonensis* (Arana and Sulkin, 1993). Barokinesis has been shown to change dramatically with ontogeny (Bentley and Sulkin, 1977; Wheeler and Epifanio, 1978) so older larvae of *P. gigas* may possess greater pressure sensitivity.

Rheotaxis

Stage 1 zoeas of *P. gigas* were able to detect currents and actively swam against them. Larvae appeared to be unable to detect slight currents below 1.12 cm/s and they were swept along by currents slower than their maximum swimming speed (1.4 and 1.61 cm/s respectively). The combination of the ability of larvae to detect currents, and then to swim against them, resulted in a narrow window within which larvae could maintain position. This suggests that rheotaxis may not be important in larval dispersal. Rheotaxis has also been observed in estuarine species such as the megalopas of *Cancer magister* (Fernandez et al., 1994) and is thought to assist in movement to and from the estuary. With open ocean species, the function of rheotaxis is less obvious as the environment is more homogeneous. Shirley and Shirley (1988) also observed rheotaxis in an oceanic species, *Paralithodes camtschatica*, and suggested that the function of rheotaxis in the oceanic environment may be important for zoeal feeding and predator avoidance.

Conclusions

Based on observed negative geotaxis, first stage zoeas of giant crab probably ascend to the surface waters after hatching. This initial upwards migration is probably the main function of the initially strong geotactic response of larvae. As the gravity-initiated strong upward swimming declines with age, the role of geotaxis may be to orientate larvae rather than to

induce migration. This would produce vertical movement in response to angled light stimuli as appeared to occur in this study. The observed negative phototaxis of larvae to increases and decreases in low light intensities has not been observed in estuarine species and may be a variation of the previously reported shadow response. However, assigning function to this unusual response is speculative and further research is required if the function is to be clarified. Sensitivity of larvae to intensity changes at only low light levels suggests that larvae are adapted for low light conditions. The presence of a rheotactic response is surprising in an oceanic species and may affect the dispersion of larvae by currents. Although rheotaxis may affect dispersal, the biological function of the response is likely to be otherwise in an oceanic environment, perhaps predator avoidance or prey capture.

Behavioural responses of oceanic, deep-water species have been investigated in only a few studies. Understanding the nature of these responses to oceanic conditions may assist in understanding other mechanisms such as survival and dispersal.

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*Effects of temperature
and thermoclines on
larval behaviour and development*



Abstract

The behavioural responses of giant crab *Pseudocarcinus gigas* larvae to temperature in the absence of light were analysed in experimental water columns. In addition, larvae were reared at 12 temperatures (in 1°C increments between 10.5 and 21.1°C) to determine the effects of temperature on instar duration, somatic growth and survival. Behavioural experiments were conducted with first and second instar zoeas and these readily penetrated experimental thermoclines of approximately 2, 5 and 10°C. Both stages had similar behaviour with upward swimming induced in water temperatures $\leq 12.7^\circ\text{C}$ and downward swimming induced by temperatures $\geq 16.2^\circ\text{C}$. Behavioural response to temperature appeared to constitute a negative feedback system which could contribute to depth regulation. Intermoult duration decreased with increasing temperature so that most rapid development through the five zoeal stages to megalopa was 41 d at 20.2°C. More rapid growth at higher temperatures was at the expense of somatic growth with smaller megalopas occurring in warmer treatments. Best overall survival was in treatments between 15.8 and 20.2°C with no larvae surviving to megalopa in those treatments below the threshold temperature where upward swimming was induced in behaviour trials (10.5 and 11.7°C). Many megalopas died shortly after moulting from zoea 5, particularly in treatments with rapid growth ($>16.8^\circ\text{C}$). Optimal survival of megalopas was in treatments below temperatures where downward migration of stage 1 and 2 zoeas was induced. These results suggest that temperature is an important factor in determining larval vertical migration, in the absence of light, and larval rearing studies indicate that this provides metabolic advantages.

Introduction

Temperature profoundly influences development of decapod larvae and numerous studies have demonstrated effects on instar duration, morphology, feeding rate, size, incidence of deformity, and survival (Johns, 1981; Shirley et al., 1987; Minagawa, 1990). These effects are species specific and this chapter presents results of trials to assess the effect of temperature on larval development of the giant crab *Pseudocarcinus gigas* (Lamarck, 1818).

Crustacean larvae do not experience temperature passively but regulate their vertical migration behaviour, and thus depth, in response to both absolute temperature and rates of temperature change (Forward, 1990). Sulkin (1984) reviewed the effect of temperature on vertical migration and noted that in most cases there is a direct relationship with swimming speed. The orientation of swimming responses may also be affected so that the geotaxis response is reversed (Forward, 1990). Forward (1990) considered that larval response to temperature constituted a negative feedback system so that depth was regulated relative to temperature.

In nature, these responses of organisms to temperature are compounded by thermoclines which influence the vertical distribution of a range of planktonic animals (Harder, 1968). Evidence from laboratory studies with crab larvae indicates that thermoclines do not have an inhibitory effect on vertical migration although results from few species have been reported (*Geryon quinquedens*, Kelly et al., 1982; *Eurypanopeus depressus*, Sulkin et al., 1983; *Callinectes sapidus*, McConnaughey and Sulkin, 1984). Unlike most species whose larval behaviour has been studied, *P. gigas* is a relatively deep water, oceanic species. This chapter presents results of studies on the behavioural response of *P. gigas* to absolute temperature and temperature change (thermoclines).

Despite improved understanding of the mechanisms of the behavioural response of crab larvae to temperature, understanding of the function of the response remains vague. Haney (1988) discussed a range of hypotheses to explain the adaptive advantage of diel migration of planktonic organisms and these include predator avoidance, avoidance of damaging solar radiation, tracking of prey items, and metabolic advantages. As Haney (1988) noted, there has been relatively little experimental support for proposed metabolic advantages. Comparisons between the behavioural and developmental responses of *P. gigas*

larvae were intended to provide a method of assessing if the behavioural response to temperature had metabolic advantages.

Materials and methods

Trials were conducted to investigate the effect of temperature on behaviour and on development. Behavioural responses were monitored for the first two zoeal stages while larvae in development trials were reared through to megalopa.

Source of larvae

Ovigerous females for all trials were collected from depths of 300-380 m off the east coast of Tasmania, Australia (41°17'S; 148°40'E) in June 1995 (development trials) and July 1996 (behavioural trials). Females ranged in size from 2.2-4.5 kg and were held communally in 4 m³ flow-through tanks receiving unfiltered seawater. Temperature in broodstock tanks ranged from 8 to 14°C and the lighting regime was approximately 10 h light.

For development trials, larvae were collected from two tanks to ensure that larvae were not from a single parent; further mixing probably occurred as several females were releasing larvae in each tank. For behaviour trials, 8 females were separated into 4 tanks before larval release so that larvae could be collected separately. Each of these tanks yielded a replicate group of larvae which were reared as 200 l upwelling cultures at 14°C (range $\pm 0.3^\circ\text{C}$) for 18 d, through to the second zoeal stage. The cultures were maintained in a reverse circadian cycle with 12 h light (i.e. light phase from 7 pm to 7 am) as experimentation was conducted in darkness. Water for these 200 l cultures was recirculated through a shared sump and biofilter to minimise variation from tank effects. Zoea larvae for both trials were fed a mix of Protein Selco™ enriched rotifers (*Brachionus plicatilis*) and artemia instar II nauplii for the first two zoeal stages (after which behavioural trials were terminated) and enriched artemia only thereafter.

System design for behavioural experiments

The response of *Pseudocarcinus gigas* larvae to thermoclines was investigated with an experimental system modified from McConnaughey and Sulkin (1984) to produce thermoclines in vertical columns (Figs. 1 and 2). Testing columns (450 x 50 x 50 mm) were surrounded by heated or chilled, upper and lower water baths, separated by a 10 mm insulated layer. Water in the lower bath was recirculated through a sump with a heat-chill unit while water in the upper baths was heated with aquarium heaters and circulated by aeration. Temperature of the upper water bath was increased relative to the lower water bath to produce thermoclines in the testing columns of approximately 10°, 5°, and 2°C.

Trials to assess the preferred temperature range of stage 1 and 2 zoeas were conducted with 5 different temperatures in the lower water bath which were intended to increase in 2°C steps (actual values approximately 11°, 12.7°, 14.5°, 16.0°, and 18.4°C) although there was slight variation between tests on stage 1 and stage 2 zoeas (for precise values see Fig. 3 and 4 results). In control chambers at the above temperatures, the regime was the same in both the upper and lower water bath so that no thermocline was generated.

Trials were run in darkness for 15 min and columns were then illuminated, to record the position of larvae, with red light of 617 nm wavelength (Kodak™ gelatine filter #25) at 10 lux which does not induce phototaxis (Forward, 1990; Gardner, 1996; Chapter 5). Light was directed perpendicular to the testing chambers so any phototaxis of larvae would not result in vertical movement along the column. No trials were run with simulated natural lighting due to the difficulty of avoiding laboratory artefacts (Haney, 1988).

Figure 1. Experimental apparatus used to generate thermoclines in testing columns (T) measuring 50 x 50 x 450 mm. The testing column was surrounded by two water baths separated by 10 mm; the upper (U) was maintained at a higher temperature than the lower (L). Larvae were introduced to the testing column at the top or through a ball valve (V) at the base of the column. In the upper chamber, experimental temperatures were maintained with a heater (H) and circulation was achieved with aeration. Experimental temperatures were maintained in the lower chamber by pumping water (P) from a sump (S) connected to a heat/chill unit (H/C). Water was returned to the sump by gravity via a standpipe to regulate pressure. In the control testing chamber (right), water was circulated from the lower chamber to the upper so that no temperature gradient was created.

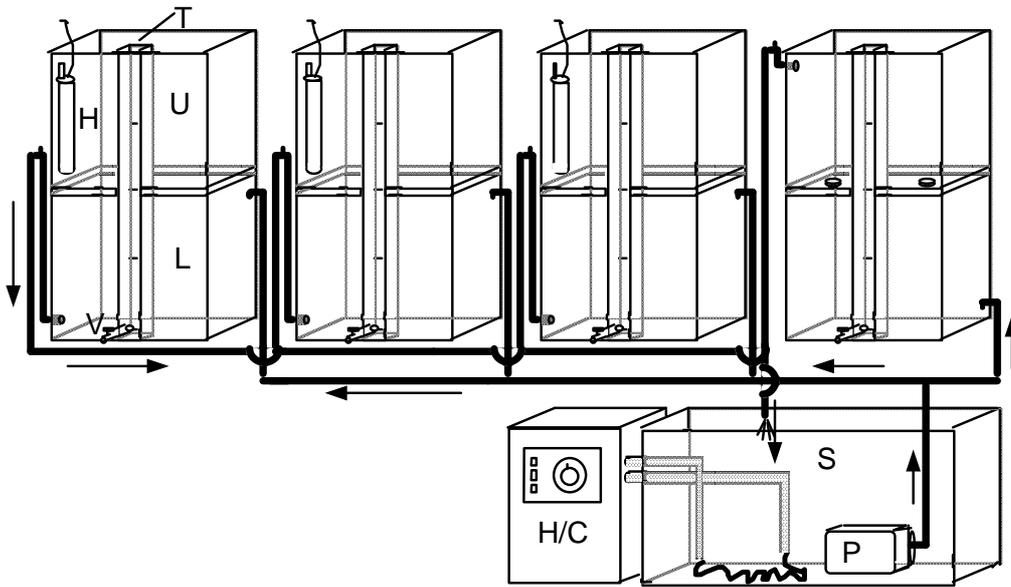


Figure 2. Vertical temperature profiles generated with the experimental system. Temperature profiles were recorded after introducing 15 ml of seawater at the base of the column to simulate the introduction of larvae.

Experimental protocol for behaviour experiments

In each trial, between 20 and 35 larvae from each of 4 replicates were introduced to the testing column by syringe after acclimatising to the experimental temperature for 5 minutes. Each trial involved the use of one control column and up to three experimental

columns operated simultaneously. At the conclusion of each experiment (15 min), the position of larvae in each of 4 divisions along the testing column was recorded. Larvae were never reused in any behaviour trial.

The ability of larvae to penetrate thermoclines was analysed by comparing the larval distribution following introduction through the top or the bottom of the testing chamber. Larvae were introduced to the chamber with a syringe and this was attached to a ball valve to introduce them at the base. Experiments to test response to thermoclines were conducted with the coldest lower bath temperature configuration (11.1° and 10.8°C for Z1 and Z2 larvae respectively) as this induced upward swimming. The proportion of larvae in the upper half of the chamber was arc-sine square root transformed and the difference between the distribution of larvae introduced to the column at the base or the top compared by one tailed, paired student's *t*-tests (Sokal and Rohlf, 1981). A significant difference indicated inhibition of larval movement by the thermocline.

Additional experiments were conducted to determine the preferred temperature as indicated by vertical distribution. These trials used the same apparatus as described for testing the response to thermoclines, except that larvae were introduced at the top of the column only and a range of lower water-bath temperatures was tested. Several studies have demonstrated that decapod larvae will sink passively to avoid temperatures above a preferred range (Ott and Forward, 1976; Yule, 1984). Consequently, the accumulation of larvae in the lower half of the testing chamber indicated that the temperature above the thermocline was above the preferred range. Statistical analyses were by students *t*-test, as described for thermocline experiments, to test the null hypothesis that larvae were distributed evenly in the testing column, that is, half were in the upper chamber. Both sets of experiments were conducted with zoeas 1 and 2 to assess ontogenic changes.

System design and protocol for development experiments

The effect of temperature on larval development was assessed by culturing larvae in 12 temperature regimes separated by approximately 1°C increments (10.5, 11.7, 12.8, 13.8, 14.8, 15.8, 16.8, 17.8, 18.6, 19.4, 20.2, and 21.1°C). The 12 temperature treatments were created with an aluminium temperature gradient plate constructed from a large aluminium block (800 x 400 x 50 mm) with channels at each end for circulating heated or chilled water

(Edwards and Van Baalen, 1970). This created an even temperature gradient along the aluminium block and 50 ml culture vessels were placed into 12 rows of holes bored into the block. Six vessels were maintained at each temperature; three replicate larval cultures and three vessels for preheating water prior to daily transfer of larvae (n=10 per replicate) into new water (0.2 μm filtered seawater).

Larval development in each of the 12 temperature treatments was monitored by recording the number and stage of dead larvae and exuviae. Many larvae moulted through to megalopa but died shortly afterwards. Those larvae which survived 24 h after moulting to megalopa were scored as viable. All megalopas were sacrificed at this stage then rinsed with distilled water and dried at 80°C for 24 h to determine dry weight.

Statistical analysis of development data

The effect of temperature on the timing of moults was tested by one way ANOVA at each zoeal stage using log transformed time (days; Hayes, 1949). Changes in survival due to temperature was tested by Kaplan-Meier survival analysis (Miller, 1981) to enable censoring of viable megalopas. Few larvae survived through to megalopa (n=81) so it was not possible to retain replicates for analysis of the effect of temperature on weight and viability of megalopas. Consequently, standard least squares regression of weight against temperature was used to test the null hypothesis that the slope = 0. The effect of temperature on the number of viable megalopas in each treatment was assessed with a Kolmogorov-Smirnov test (Sokal and Rohlf, 1981) by comparing the observed number of viable megalopas against the predicted number of viable megalopas if there was no temperature effect (that is, predicted frequency = initial number of megalopas in treatment \times (total viable megalopas/total initial number of megalopas)).

Results

Response of larvae to thermoclines

There was no indication of inhibition of larval movement by the experimental thermoclines as larvae introduced at the base of the column had distributions that were not significantly different from those introduced at the top ($P > 0.05$; Figs. 3 and 4, first two columns). This pattern was observed with both stage 1 and 2 zoeas. Two comparisons were almost statistically significant: the 8.5°C thermocline with stage 1 zoeas (11.1 and 19.6°C; $P = 0.09$), and the 4.3°C thermocline with stage 2 zoeas (15.1 and 10.8°C; $P = 0.08$). However, these were caused by trends in vertical movement which were opposite to that which would occur if the thermocline had inhibited larval movement.

Vertical migration

Larvae in control treatments tended to swim actively upwards at temperatures $\leq 12.6^\circ\text{C}$ and 12.7°C , while sinking was induced at $\geq 16.2^\circ\text{C}$ and 18.3°C for stage 1 and 2 zoeas respectively (Figs. 3 and 4, upper row). A similar pattern was detected in treatments where two alternative temperatures were separated by a thermocline. The response of stage 2 zoeas to the $16.3^\circ\text{C} / 14.0^\circ\text{C}$ treatment indicates that stage 2 zoeas will also descend at temperatures $\geq 16.3^\circ\text{C}$. Although a significantly higher proportion of zoea 1 larvae were found in the upper half of the $16.7^\circ\text{C} / 12.6^\circ\text{C}$ treatment, this appeared to be due to larvae swimming upwards from the lower temperature as most larvae were found immediately above the thermocline. Larvae placed in treatments where both the upper and lower temperature alternatives were outside the apparent preferred range tended to congregate around the thermocline ($19.6^\circ\text{C} / 11.1^\circ\text{C}$ and $21.3^\circ\text{C} / 12.6^\circ\text{C}$ in zoea 1, and $17.9^\circ\text{C} / 12.7^\circ\text{C}$ in zoea 2).

Figure 3. Response of stage 1 zoeas to experimental thermoclines. Bars represent mean percentage of larvae (\pm SE, $n=4$) in each of the 4 divisions along the length of the testing columns. Values are the temperature ($^{\circ}$ C) in the upper and lower halves of testing columns. Upper row represents response of larvae in control experiments without a thermal gradient. Significant deviation from 50% of larvae in the upper half denoted by * ($P<0.05$).

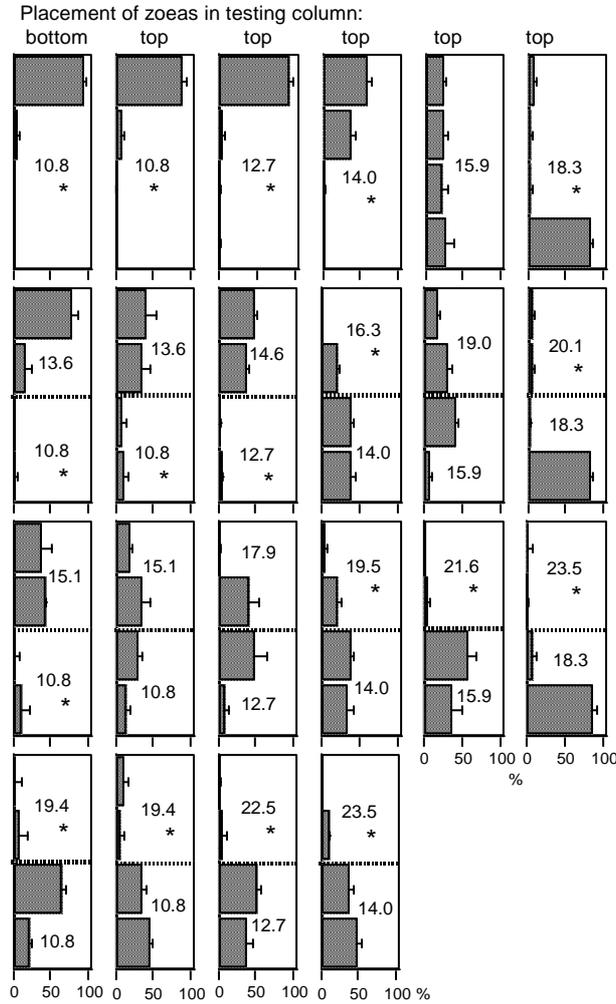
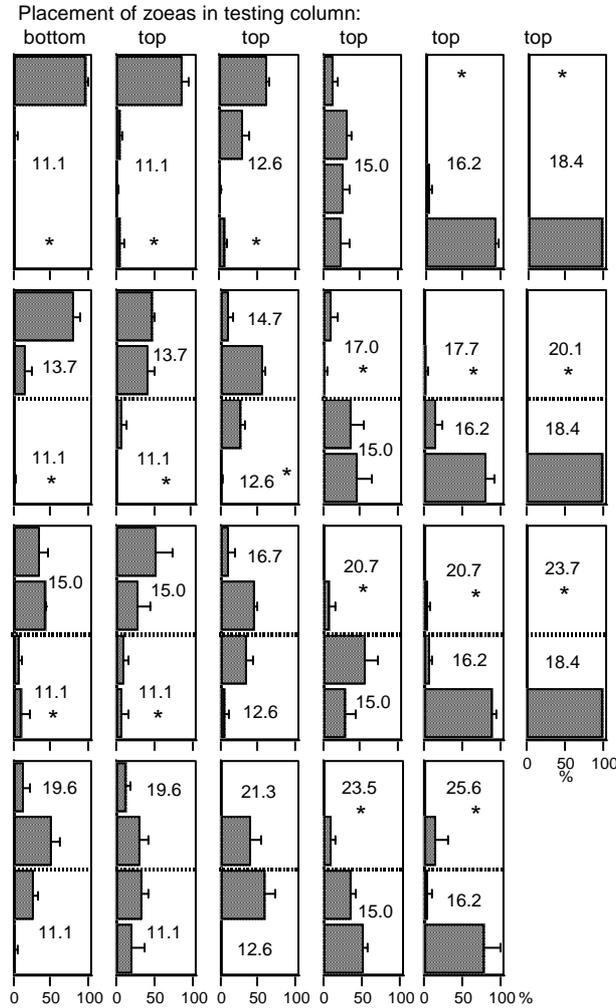


Figure 4. Response of stage 2 zoeas to experimental thermoclines. Bars represent mean percentage of larvae (\pm SE, $n=4$) in each of the 4 divisions along the length of the testing columns. Values are the temperature ($^{\circ}$ C) in the upper and lower halves of testing columns. Upper row represents response of larvae in control experiments without a thermal gradient. Significant deviation from 50% of larvae in the upper half denoted by * ($P<0.05$).



Growth

Intermoult duration decreased with increasing temperature at each instar (N=3; P<0.0001; Fig. 5). Larvae reared at 10.5°C failed to develop past zoea 2 and mean duration of the first zoeal stage was 32 days (\pm 0.33; N=3). Most rapid development through to megalopa was 41 days (\pm 0.41; N=3) at 20.2°C. The plot of log time against temperature was not linear which indicates that the decrease in intermoult duration with increased temperature is not simply exponential as occurs in chemical systems by van't Hoff's rule (Hayes, 1949; Garside, 1966). The more rapid development of larvae at higher temperature appeared to be at the expense of somatic growth as dry weight of megalopas declined significantly with increasing temperature (P<0.01; Fig. 6).

Figure 5. Effect of temperature on the timing of moulting of the planktonic larval stages of *Pseudocarcinus gigas*. Zoeal stages listed are the stages that zoeas moult into. Shift upwards indicates delay in the development of larvae. Missing points are due to complete mortality. Values are the means of three replicates.

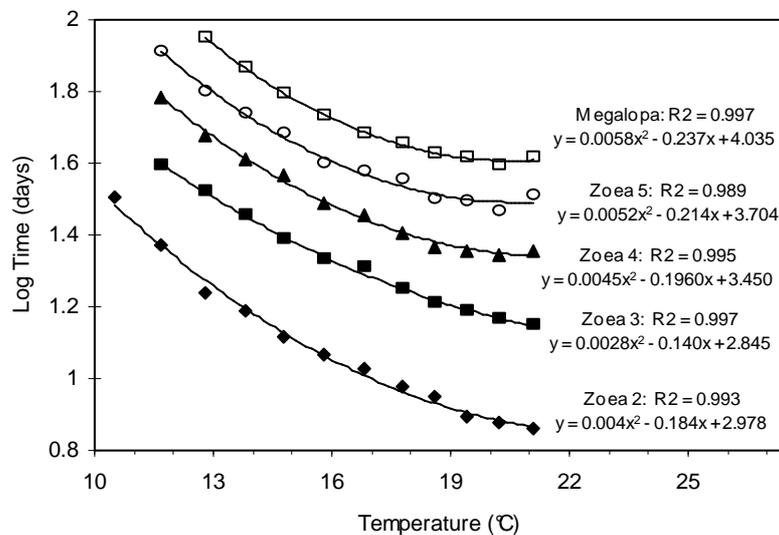
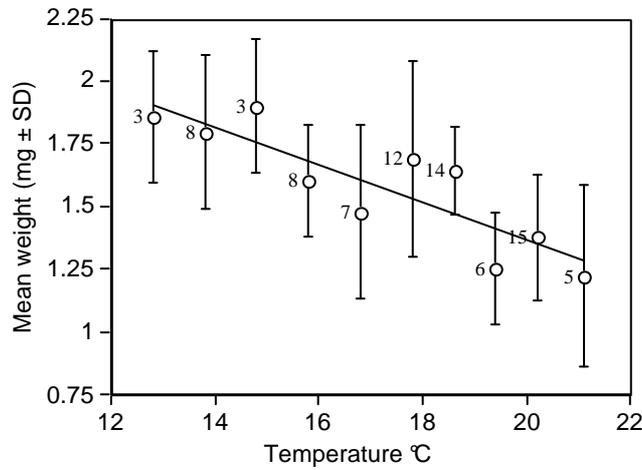


Figure 6. Effect of temperature on dry weight of megalopas. Values are means of individual larvae pooled from all replicates. Labels next to the means are N. The regression was derived from raw data rather than plotted means.



Survival

Survival increased with higher temperatures ($P < 0.001$) so that best survival was obtained with treatments 15.8-20.2°C (Fig. 7). Most mortality occurred at zoea 1 and at megalopa (Fig. 8). Although survival to megalopa was low (24%), the proportion of viable megalopas (those alive after 24 h) appeared to be affected significantly by temperature. The largest unsigned difference in the Kolmogorov-Smirnov test was at 15.8°C which indicates that viability of megalopas was highest at this temperature (Fig. 9).

Figure 7. Effect of temperature on survival of larvae, measured by survival to each instar with censoring of viable megalopas. Replicates in temperatures 14.8 and 19.4 suffered atypical mass mortality so the values plotted are drawn from single replicates. The regression was derived from raw data, rather than plotted means.

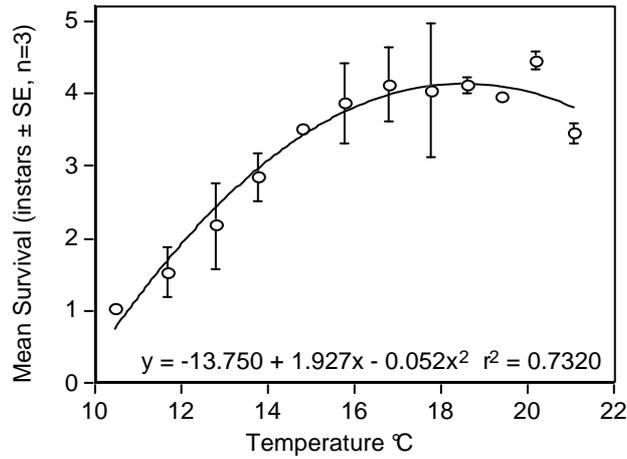


Figure 8. Patterns of mortality in larval rearing at different temperatures; temperatures were paired and survival averaged to show general trends. "M" is number of stage 5 zoeas moulting to megalopa, "M+" is megalopas active after 1 day.

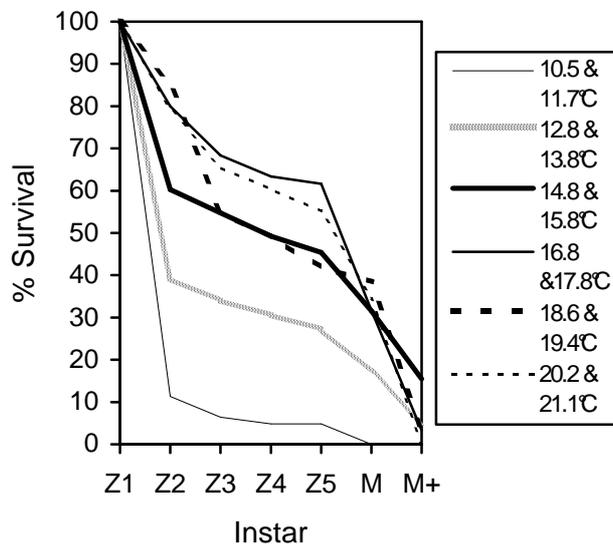
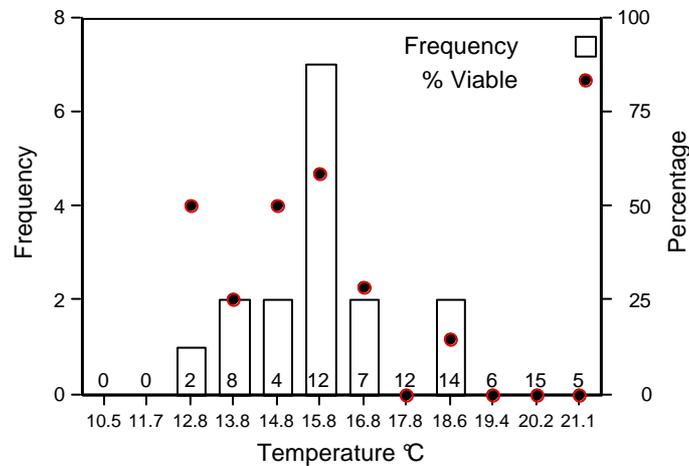


Figure 9. Viability of larvae reared at different temperatures. Megalopas often died after moulting, those that moulted successfully and were active after 1 day were scored as viable. Larvae did not survive to megalopa in all treatments (*n* total megalopas per treatment at base of column).



Discussion

Behavioural responses to temperature

Sulkin (1984) described the mechanism for vertical migration of brachyuran larvae as a combination between cues to orientate swimming, and cues to influence locomotor responses, either through passive sinking or active swimming. The principal factor influencing locomotion and orientation during daylight hours is light and there is potential for this to be important to *Pseudocarcinus gigas* larvae even at the depth of hatching, around 300 m (Beebe, 1934⁶; Clarke, 1970; Levings et al., 1996). However, phototactic responses do not account for larval migration patterns during the night. In an early paper, Sulkin

⁶ The light at 230 m depth was described by William Beebe following the first bathysphere descent, 6th June, 1930 “We were the first living men to look out at the strange illumination: and it was stranger than any imagination could have conceived. It was of an indefinable translucent blue quite unlike anything I have ever seen in the upper world, and it excited our optic nerves in a most confusing manner. We kept thinking and calling it brilliant, and again and again I picked up a book to read the type, only to find I could not tell the difference between a blank page and a coloured plate.... It actually seemed to me to have a brilliance and intensity which the sunshine lacked”.

(1973) proposed that decapod larvae regulate depth in the absence of light by a combination of responses to hydrostatic pressure and gravity. Since then, the response of larvae to temperature has been studied in greater detail and it appears that thermokinesis is also critical in maintaining depth (Ott and Forward, 1976; Kelly et al., 1982). *Pseudocarcinus gigas* zoeas do not appear to respond to change in hydrostatic pressure (Gardner, 1996; Chapter 5) so the thermokinesis observed in these trials may be an alternative mechanism for regulating locomotion. Although it is not possible to speculate on the effect of temperature on the response of *P. gigas* larvae during daylight hours, the observed temperature response indicates that the vertical position of stage 1 and 2 zoeas at night would be maintained within water of 12.6 and 16.3°C. This implies that where the surface temperature is less than 16.3°C, *P. gigas* larvae will migrate to the surface.

Behavioural responses to the thermocline

The distribution of planktonic organisms in nature is often strongly influenced by the presence of thermoclines with organisms restricted to one side, or found in greatest abundance around this layer (Keifer and Kremer, 1981; Southward and Barrett, 1983; Harding et al., 1987). Various mechanisms have been proposed for this phenomenon including: aggregation around areas of greatest prey density associated with the nitrite maxima at the thermocline (Keifer and Kremer, 1981), an inability to penetrate density gradients (Harder, 1968), and a negative feedback system of depth regulation based on rates of temperature change (Forward, 1990; Boudreau et al., 1991). It is important to note that the last of these proposed mechanisms, response to rates of temperature change, involves larvae detecting changes in temperature in relation to time, rather than responses to absolute temperatures. Forward (1990) demonstrated that this response was exhibited by *Rhithropanopeus harrisi*, although no response was detected with stage IV zoeas of *Neopanope sayi*.

The thermoclines resulting from the system designed by McConnaughey and Sulkin (1984), and reproduced here, are very abrupt and more extreme than that observed in the field where thermoclines of 5°C may occur through a depth of 5 meters (eg. Gray, 1996). Consequently, extrapolation from crab larval response in the experimental system to the natural environment may be flawed, especially where larval movement appears inhibited by the experimental thermocline. Experimental results indicating no effect are more readily

transferred to modelling of the natural system - if the larvae do not respond to the abrupt temperature changes generated experimentally, then they are unlikely to be influenced by the more gentle changes occurring in nature.

Stage 1 and 2 *P. gigas* zoeas were not influenced by the experimental thermoclines produced in this trial and this has also been reported for three other crab species, *Geryon quinquedens* (Kelly et al., 1982), *Eurypanopeus depressus* (Sulkin et al., 1983), and *Callinectes sapidus* (McConnaughey and Sulkin, 1984). This absence of an inhibitory effect of thermoclines on zoeal movement suggests that there is no physical barrier to restrict larval movement.

Rather, *P. gigas* larvae appeared to migrate vertically in response to absolute temperature so that they accumulated either above or below the thermocline. A similar response was observed with *Callinectes sapidus* where larvae failed to penetrate a thermocline only when the upper absolute temperature was extreme (McConnaughey and Sulkin, 1984). These observations indicate that the primary factor influencing crab larval accumulation around a thermocline is depth maintenance to avoid temperature extremes, while the magnitude of natural temperature gradients across thermoclines would not impede migration. Response to absolute temperatures may still result in accumulation of larvae on one side of a thermocline as reported with *Homarus americanus* (Harding et al., 1987).

Grey (1996) reported that thermoclines formed in waters at the northern end of the range of *P. gigas* in New South Wales (McNeill, 1920) did not appear to influence the broad scale distribution of larval fishes. Similar results would be expected with *P. gigas* although absolute temperature profiles are likely to be critical for modelling larval distribution.

Comparison between the effect of temperature on development and behaviour

Depth maintenance is used by estuarine species to regulate dispersal (Epifanio et al., 1984; Cronin and Forward, 1986) but this role is unlikely to be important for oceanic species where suitable sites for settling are more widespread and current flow is not bi-directional with tides. In *P. gigas*, vertical migration behaviour in response to temperature is likely to be directed towards optimising physiology and this was supported by observations on larval development at different temperatures.

The general pattern of a retarding effect of low temperature and accelerating effect of high temperature on intermolt duration was the same as that reported for numerous other

decapod larvae (Anger, 1983; Sulkin and McKeen, 1994; Goncalves et al., 1995). The relationship between log time and larval instar was not linear so van't Hoff's rule for chemical reactions was not met (Johansen and Krough, 1914; Garside, 1966) and larval development almost ceased in the 10.5°C treatment with no larvae surviving beyond zoea II. This implies that the thermal requirements for growth of *P. gigas* larvae have an optimal range and are not entirely cumulative (degree days), but involve some component of threshold phenomena (Waddy and Aiken, 1996).

No larvae survived to megalopa in temperatures <12.8°C which is in the order of the absolute temperature initiating upward migration of stage 1 and 2 zoeas (12.6 and 12.7°C respectively). While there appeared to be close similarity between vertical migration patterns and larval survival at lower temperatures, overall survival was relatively high in treatments above temperatures where downward vertical migration behaviour was induced (>16.2°C). Best overall survival, averaged across instars, appeared to be between 14.8 and 20.2°C (inclusive) although there is potential for this to be confounded by ontogenetic changes in thermal tolerance (Sulkin and McKeen, 1989; Rasmussen and Tande, 1995) which was not possible to assess in the trial with *P. gigas*.

Despite the high overall survival (cumulative for all instars) in treatments greater than 16.8°C, few larvae moulted successfully to viable megalopas and this is similar to the situation where downward vertical migration of stage 1 and 2 zoeas was initiated (16.2 and 16.3°C respectively). Larval response to temperature is known to change during development in other species (Forward, 1990) so the behavioural response of stage 1 and 2 larvae is not directly applicable to the final zoeal stage. Nonetheless, it is noteworthy that the optimal treatment for viability of stage 5 zoeas moulting to megalopa (15.8°C) was within the range that stage 1 and 2 larvae would migrate to in the absence of light and was the control temperature with uniform larval distribution.

Sulkin and McKeen (1989) considered the final zoeal stage of *Cancer magister* to be the most sensitive to temperature stress and *P. gigas* appears to be similar as mortality was highest at this stage. Incidence of deformity increases at extreme high temperature in most organisms (Battle, 1930) and this may have accounted for the low survival to megalopa which is typically reduced by high temperatures (Minagawa; 1990; Chaoshu and Shaojing, 1992; Okamoto, 1993). Johns (1981) noted that larval size of *Cancer irroratus* was greatest in the mid-range of thermal tolerance although larval weight more typically declines with

increased temperature as was observed with *P. gigas* (Shirley et al., 1987; Minagawa, 1990; Sulkin and McKeen, 1994). Low weight of larvae reared at higher temperatures may be indicative of reduced energy stores (Minagawa, 1990) and this could also have contributed to the poor survival to megalopa in treatments greater than 16.8°C.

Implications of temperature on distribution of Pseudocarcinus gigas

Very few *P. gigas* larvae have been obtained from plankton tows (Chapter 4) so there is insufficient information to assess distribution and development of larvae in nature. Consequently, studies of larvae in the laboratory provide the best indications of the effect of temperature on development of *P. gigas* although compounding factors such as egg incubation temperature will inevitably influence results (Laughlin and French, 1989). Several studies on brachyurans have demonstrated close relationships between laboratory results and observations from plankton sampling as temperature has a profound effect on development (Anger, 1983; Shirley et al., 1987).

Factors limiting the distribution of a species fall into three groups: the introduction or historical presence of a species in a location; abiotic factors; and biotic factors. Kinne (1963) considered that temperature was the principal abiotic factor influencing distribution and it also influences biotic factors by increasing exposure to predation when instar duration is extended (Jamieson and Armstrong, 1991). Given the low survival of *P. gigas* larvae in treatments less than 14°C, it is likely that zoeal larvae have a plankton period of less than 3 months and hence there may be little potential for long distance dispersal (Thorson, 1961). *P. gigas* has not colonised nearby New Zealand (McLay, 1988) while other species with longer larval duration, such as *Jasus edwardsii* (12-24 month larval duration; Phillips and Sastry, 1980), are found in both locations. Low survival of *P. gigas* larvae in treatments below 14°C would also indicate low survival in southern Tasmania which is confirmed by the fishery, as only occasional crabs are caught in this region. Likewise, poor survival to megalopa in treatments above 16.8°C would limit the northern range of the species explaining the rarity of specimens from the state of New South Wales (most northerly record, 34°25'S; McNeill, 1920).

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*Effect of photoperiod and
light intensity on larval survival,
development and cannibalism*



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Gardner, C. and Maguire, G.B., in press. Effect of photoperiod and light intensity on survival, development and cannibalism of larvae of the Australian giant crab *Pseudocarcinus gigas* (Lamarck). *Aquaculture*.

Abstract

Pseudocarcinus gigas larvae were reared to megalopa under two light intensities (2 and 500 lux) in five photoperiod regimes (0, 6, 12, 18, and 24 h light). Survival was not significantly affected by photoperiod or light intensity ($P>0.05$) although other effects were observed which are discussed in relation to swimming activity and feeding. Larvae had shorter intermoult duration in treatments with longer photoperiods and brighter light with most rapid development to megalopa in the continuous light, 500 lux treatment (49.2 d). Size (measured as telson width) of stage 4 zoeas was affected by photoperiod with smallest zoeas in the continuously dark treatment, whereas all other treatments were similar. Cannibalism was strongly influenced by lighting with greater damage to the dorsal spine occurring with increasing photoperiod and also in dimmer (2 lux) treatments. Lowest incidence of cannibalism was observed in continuous darkness. Viability of larvae after metamorphosis to megalopa was variable and no treatment effect was observed although viability was lowest in the two continuous light treatments. Results of this trial indicate that continuous light or dark regimes should be avoided. Optimal light intensity for culture was less clear and is discussed in relation to intermoult duration and cannibalism.

Introduction

In an attempt to optimise larval rearing of *Pseudocarcinus gigas* (Lamarck) the effects of photoperiod and light intensity were assessed. Light is one of the major factors influencing swimming and feeding behaviour in decapod larvae (Sulkin, 1984; Minagawa and Murano, 1993) and the effect of photoperiod on larval survival has been studied in several species (e.g. Sandoz and Rogers, 1944; Dalley, 1980; Radhakrishnan and Vijayakumaran, 1986; Nakanishi, 1987; Minagawa, 1994). Changes in survival and development of decapod larvae in different lighting regimes are often simply attributed to effects on feeding. However, other aspects of behaviour and physiology may be influenced by lighting including: swimming activity and thus metabolism (Gardner, 1996); cannibalism (Hecht and Pienaar, 1993); entrainment of physiological processes to circadian cycles (Dalley,

1980); initiation of ecdysis (Waddy and Aiken, 1991); and endocrine control of metamorphosis (Eagles et al., 1986).

Due to the potentially complex nature of effects of light on larval development, research on this topic tends to involve measurement of a wide range of responses (Aiken et al., 1981; Minagawa, 1994). This study examined the effect of photoperiod and light intensity on larval survival, intermoult duration, size, cannibalism, and metamorphosis to megalopa. Results are directly applicable to culture of *P. gigas* but information of potential relevance to decapod culture in general was also gained. As noted by Minagawa (1994), the effects of continuous light treatments has seldom been examined. The effect of light intensity on larval development has also received little attention despite the importance of intensity on swimming behaviour (Sulkin, 1984).

Materials and methods

Source of larvae

Ovigerous females were collected from depths of 300 – 380 m off the east coast of Tasmania, Australia (41°17'S; 148°40'E) in June 1995. Females ranged in size from 2.2 – 3.5 kg and were held communally in 4 m³ tanks with flow through, unfiltered, seawater. Temperature in broodstock tanks ranged from 8 to 14°C and the lighting regime was approximately 10 hours light per day. Larvae were collected at dusk from two tanks to ensure that larvae were not from a single parent; further mixing probably occurred as several females were releasing larvae in each tank.

Culture methods and experimental design

Newly hatched larvae were rinsed in 0.2 µm filtered seawater (32‰ salinity) and 100 were transferred to each of 36 (9 treatments x 4 replicates), 1.8 l, black, rectangular static culture vessels. Zoeas were maintained in a temperature control room at 15.5°C and were fed a mix of Protein Selco™ enriched rotifers (*Brachionus plicatilis*) and instar II artemia nauplii for the first two instars and artemia only thereafter. Larvae were pipetted into fresh, 0.2

µm filtered seawater daily (32-34‰ salinity). Although four replicates were used for all treatments initially, this was reduced to three in some treatments by high mortality not associated with treatment effects (chlorine contamination of one replicate in both the 24 L, 500 lux and 18 L, 500 lux treatments). Consequently, 1 replicate was randomly deleted from the remaining treatments to produce 3 replicates for all treatments. Incidence of cannibalism was assessed on stage 1 and 2 zoeas in all four replicates. The trial ran for 99 days after which all live animals were counted and censored in survival analyses.

Larvae were cultured under five photoperiod regimes with light phases of 0, 6, 12, 18, and 24 h per day (hereafter: 0, 6, 12, 18, and 24 L). Daily transfer of larvae to new culture vessels took around 10 min per container; therefore 0 L treatments actually received a short light phase. With the exception of the 0 L treatment, the photoperiod regimes were further divided into 2 light intensity treatments: 2 and 500 lux. Lighting in the 500 lux treatments was by 12 V, 20 W quartz halogen globes suspended 1.5 m over the culture vessels. The 2 lux treatments were conducted in separate compartments next to the 500 lux treatments with small gaps in the adjoining wall. These gaps allowed light to pass through which was then reflected onto the culture vessels. Gaps in the adjoining walls also allowed circulation of air to reduce potential temperature difference between treatments. Temperature was monitored between different treatments and no effect of warming from the light source was detectable at a resolution of 0.1°C. Light intensity was recorded with a Profisix™ lux meter.

Response data collected

The effects of photoperiod and light intensity were monitored daily by recording the number and instar of exuviae and mortalities to calculate survival and time to each moult. Larval size was measured on exuvia of stage 4 zoeas as treatment effects should be more readily apparent than in earlier stages. Only exuvia were measured to ensure the sample included only healthy, actively moulting zoeas. The carapace splits during ecdysis so it was impossible to measure carapace length on exuvia. Consequently, size was measured as telson width between the inner angles of the lateral spines on exuvia (Fig. 1). All measurements were made by image analysis using NIH-Image™ 1.6 software.

Extent of cannibalism was measured by a semi-quantitative scale modified from Minagawa (1994) by ranking damage to the dorsal spine into 4 categories (Fig. 2). Only stage 1 and 2

zoeas were assessed for cannibalism as spine regeneration by moulting may have obscured patterns in later stage larvae.

Most of the mortality of *P. gigas* larvae occurred within a short period after the moult to megalopa. The effect of treatments on megalopa viability was tested by rating any megalopas which survived for a period of longer than 1 day as viable.

Figure 1. Larval size was measured on exuvia as telson width, measured between the inner angles of the lateral spines.

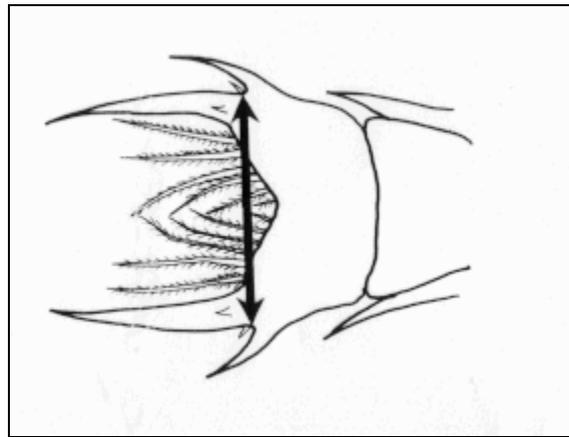
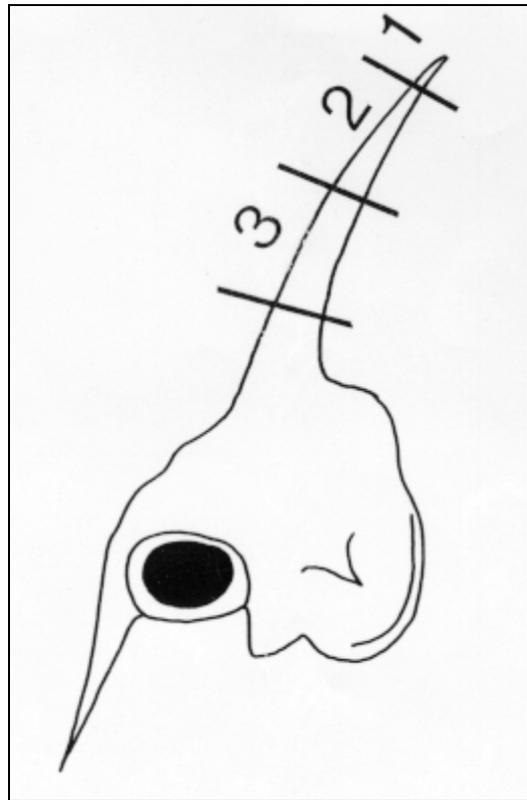


Figure 2. Cannibalism was measured by a semi-quantitative scale based on damage to the dorsal spine. Undamaged spines were ranked 0 and increasing damage was ranked 1-3 with increasing damage.



Statistical analysis

Treatment effects were analysed in three ways to incorporate the 0 L treatment which could be classed as neither bright (500 lux) nor dim (2 lux) intensity. First, the 0 L treatment was excluded and the effects of photoperiod, intensity and interactions were assessed for the 6, 12, 18 and 24 L treatments. The effect of photoperiod only was then assessed by including the 0 L treatment and analysing data from the bright and dim intensity treatments separately.

All statistical analyses were performed with JMP 3.0™ software (SAS Institute). Development of *P. gigas* megalopas to crab 1 has been accomplished when ongrown artemia were supplied (Gardner and Gardner, 1996). No ongrown artemia were supplied in this trial which may have affected megalopa survival, so only mortalities up to the final zoeal stage (5) were included in survival analyses. The significance of the effects of photoperiod and light intensity on survival were tested with the semi-parametric, Cox's proportional-hazards model (Miller, 1981) as it was not possible to model accurately the

hazard function parametrically. Data for survival analyses were obtained by recording the instar of all mortalities throughout the experiment. Survival to each zoeal stage, rather than through time, was then assessed by Cox's proportional-hazards model with censoring of megalopa.

The effects of light intensity and photoperiod on moult timing were tested by repeated measures analyses with significance determined with Wilk's lambda (Mardia et al., 1979). Effects of treatments on size of stage 4 zoeas, cannibalism of stage 1 and 2 zoeas, the proportion of megalopas that were viable, and survival to stage 5 zoea were tested by two-way ANOVA. Where a treatment effect was observed, means were compared by Tukey Kramer HSD (Sokal and Rohlf, 1995).

Results

Survival rate

Survival was assessed in two ways: by Cox's proportional-hazards to assess the survival of larvae throughout development (Fig. 3); and by two way ANOVA to test the effects of treatment on survival to the final zoeal stage (stage 5; Table 1).

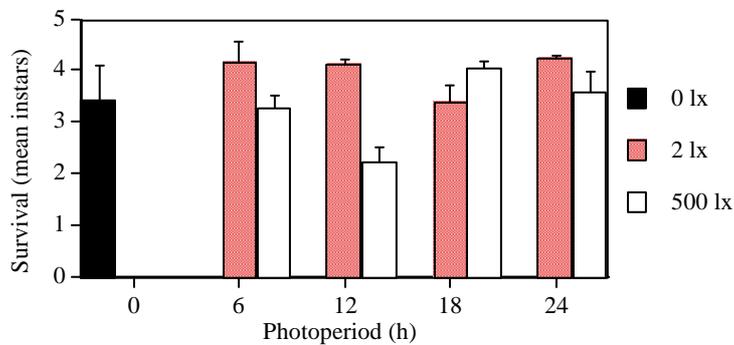
When survival was assessed throughout development, there was considerable variation between treatments although there was no significant effect of photoperiod or light intensity (Fig. 3; $P > 0.05$; Table 1). Survival of larvae throughout development was not significantly different ($P > 0.05$) between the two extreme photoperiods: 0 and 24 L.

Survival of larvae through to the final zoeal stage is presented in Table 1 and there was no significant effect of photoperiod or intensity on survival although survival tended to be higher in low intensity treatments ($P > 0.05$). Survival to megalopa was low so information presented in Table 1 is intended to provide general information on survival and no statistical analysis is presented.

Table 1. Percentage survival (\pm SE; n=3) of larvae to the final zoeal stage (Z5) and to megalopa in each of the lighting treatments. Survival to the final zoeal stage was not significantly affected by either photoperiod or light intensity ($P>0.05$).

Photoperiod (h)	Intensity (lux)	Z5	Megalopa
0		50.7 (13.7)	5.0 (1)
6	2	52.3 (25.7)	7.7 (7.2)
	500	25.3 (18.4)	3.3 (1.7)
12	2	70.0 (4.2)	9.3 (8.3)
	500	2.0 (1.5)	1.3 (0.9)
18	2	44.3 (7.5)	9.0 (3.5)
	500	64.7 (15.2)	10.7 (5.3)
24	2	74.3 (1.5)	10.3 (6.0)
	500	47.7 (11.3)	7.0 (3.1)

Figure 3. Mean survival (instars \pm s.e., n=3) of *Pseudocarcinus gigas* larvae cultured under different photoperiods and light intensities. Larval development involves 5 zoeal stages, therefore, a mean survival of 5 instars implies that all larvae survived through the zoeal stages.



Intermoult period and accumulated zoeal duration

Intermoult period was significantly affected by both photoperiod and light intensity ($P<0.05$; Fig. 4). Larvae cultured under 2 lux lighting tended to have longer intermoult period than 500 lux treatments and there was a general trend of increasing intermoult period with decreasing photoperiod. The interaction term between photoperiod and intensity was also significant ($P<0.05$). This relationship was influenced by the 12 L

treatment and was no longer apparent when this treatment was excluded (survival was lower in the 12 L 500 lux treatment than in any other treatment).

The most rapid development through the zoeal phases to megalopa was in the 500 lux 24 L treatment (mean = 49.2 d). Relative to larvae in the 500 lux 24 L treatment, dimmer light intensity appeared to delay development by around 4 days (mean = 53.1 d; 2 lux, 24 L) and shorter photoperiod delayed development by about 10 days (mean = 59.6 d; 0 L).

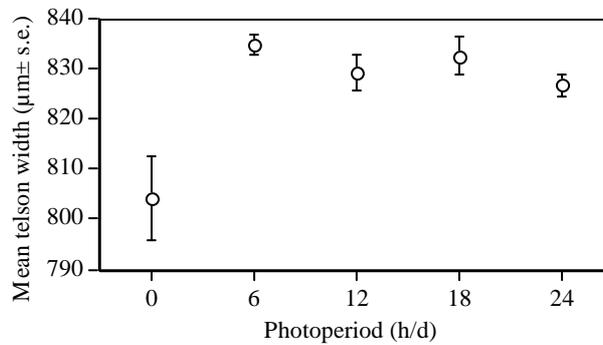
Figure 4. Effect of photoperiod and light intensity on duration of intermoult period in Pseudocarcinus gigas larvae. Means from larvae reared at high light intensity (500 lux) are joined by solid lines; low light (2 lux) by dotted lines. A shift towards the right implies an increase in intermoult period.

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Size of stage 4 zoeas

Larval size did not appear to be affected by lighting intensity ($P>0.05$). There was a significant effect of photoperiod on larval size ($P<0.05$; Fig. 5) with larvae cultured under 0 L being significantly smaller than in all other treatments. Mean size of larvae in the 6, 12, 18, and 24 L treatments were similar and not significantly different ($P>0.05$). There was no significant interaction between intensity and photoperiod ($P>0.05$).

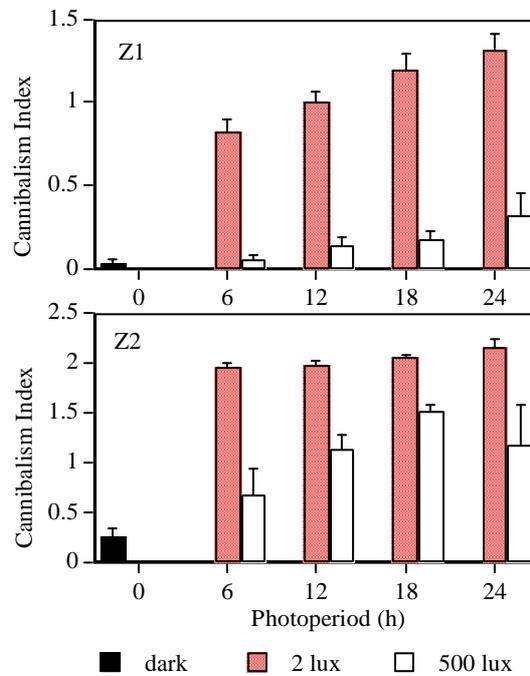
Figure 5. Effect of photoperiod on size of stage 4 *Pseudocarcinus gigas* zoeas, measured by width of telson. Light intensity appeared to have no effect ($P<0.05$) so treatments were combined. Consequently, the mean was derived from 3 replicates for the complete darkness treatment (0 h light/d) and from 6 replicates for all other treatments.



Cannibalism

Patterns of cannibalism were the same for both stage 1 and 2 zoeas although incidence increased with development (Fig. 6). Evidence of cannibalism was rarely observed in the 0 L treatment but increased significantly and proportionally with photoperiod ($P < 0.001$). Larvae cultured under 2 lux lighting suffered dramatically higher cannibalism damage than those cultured at 500 lux ($P < 0.0001$). There was no significant interaction between photoperiod and intensity ($P > 0.05$).

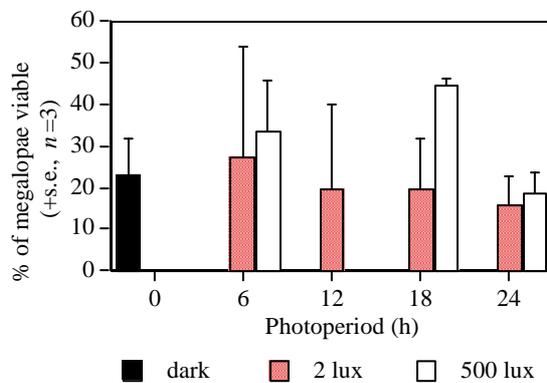
Figure 6. Effect of photoperiod and light intensity on extent of cannibalism in *Pseudocarcinus gigas* zoeas, measured by the extent of dorsal spine damage as "cannibalism index" (+s.e., $n=4$ replicates per treatment). Cannibalism index was measured on exuvia of stage 1 zoeas (upper) and stage 2 zoeas (lower).



Viability of megalopas

Very few larvae reached megalopa in the 12 L, 500 lux treatment so these results were not included in analyses; in all other treatments at least 15 larvae moulted from the last zoal phase in each replicate. There was large variation between treatments (range 16-24%; Fig. 7) and there was no significant effect on the proportion of megalopas which were viable ($P>0.05$).

Figure 7. Effect of photoperiod and light intensity on the viability of *Pseudocarcinus gigas* megalopas. Larvae which died during moulting to megalopa or which failed to survive for longer than 1 day were classed as unviable. All replicates include data from at least 15 individuals. No information is presented for the 12 h, 500 lux treatment as survival was too low to allow meaningful analysis.



Discussion

Survival throughout the zoal phases was not significantly affected by photoperiod yet within treatment variation was relatively small. This indicates that *Pseudocarcinus gigas* larvae are tolerant of various photoperiod regimes in culture. There was no significant effect of light intensity on survival although there appeared to be a trend of improved survival at low intensity. Only two treatments were examined and further research is required to clarify the effect of light intensity on larval survival, especially at higher intensity than that used in this trial. Although the effects of light on overall survival appeared to be minor, light intensity and photoperiod were observed to affect both growth of zoeas and incidence of cannibalism. It is probable that these effects resulted from changes in either feeding or swimming behaviours which are strongly influenced by light in *P. gigas* (Gardner, 1996).

Effects of photoperiod due to behaviour

Brachyuran larvae tend to migrate vertically in response to light by active upward swimming and passive sinking (Sulkin, 1984). In culture situations, this may result in larvae attempting to rise by actively swimming at the surface, or passively resting on the base in an attempt to migrate to greater depths. These behaviours become less apparent when larvae are mixed in a culture system such as plankton-kreisels (Charmantier-Daures and Charmantier, 1991), although energetic costs of increased swimming may still affect growth. This increased activity in response to lighting is known to retard development in teleost larvae (Bolla and Holmefjord, 1988; Liu et al., 1994). *Pseudocarcinus gigas* larvae in this trial were considerably more active during dark phases, as are *Callinectes sapidus*, due to negative geotaxis (Sulkin et al., 1979); this increased energy expenditure may have resulted in the smaller larvae in the 0 L treatment. Although there was no trend of increased size with longer photoperiod, the rate of development did tend to proceed faster with increased photoperiod which may be attributable to the expenditure of less energy during the dark phase.

Passive sinking in response to light will cause larvae to accumulate at the base of culture vessels in relatively high density, which tends to cause higher cannibalism (Hecht and Pienaar, 1993). Larvae of *P. gigas* have been shown to exhibit different swimming patterns with changes between light of low (6-15 lux) and high (550-900 lux) intensity (Gardner, 1996) which may have contributed to the observed effect of intensity on cannibalism. Likewise, increased swimming during dark phases should result in less cannibalism in treatments with shorter photoperiods.

Effects of photoperiod due to feeding

An alternative hypothesis for the observed effects of lighting is that intensity and photoperiod influenced feeding activity. While total reliance on visual cues for feeding is common in teleosts (Miner and Stein, 1993; Hart et al., 1996), it has seldom been reported in decapod crustaceans. In one of the first studies of decapod rearing, Sandoz and Rogers (1944) found zoeal stages of *C. sapidus* starve when reared in 0 L. In most other decapod species, larvae are less severely affected by 0 L and are able to capture prey at a reduced rate (for example: *Panulirus homarus* (Radhakrishnan and Vijayakumaran, 1986), and *Ranina*

ranina (Minagawa, 1994)), or they may actually feed at a rate equal to or greater than in light (for example: *Homarus americanus* (Templeman, 1936; Eagles et al., 1986), *Pandalus borealis* (Wienberg, 1982), and *Paralithodes camtschaticus* (Nakanishi, 1987)).

Pseudocarcinus gigas zoeas appear to be able to feed in the absence of light as larvae in the 0 L treatment developed through to megalopa, albeit at a slower rate than larvae exposed to light. This implies a degree of feeding by either chemosensory detection or by random encounter, as starved controls of other experiments died at the first zoeal stage (Gardner and Northam, 1997). A pattern of feeding by random encounter in brachyuran larvae, akin to filter feeding, has been reported in other brachyurans (McConaughy et al., 1991; Minagawa and Takashima, 1994).

While *P. gigas* are not obligate visual feeders, feeding appears to be enhanced by light; larvae cultured with light periods were larger than those cultured in 0 L and duration of intermoult tended to decrease with longer photoperiods. This is similar to patterns in *P. homarus* and *R. ranina* where consumption of artemia and growth was enhanced by lighting (Radhakrishnan and Vijayakumaran, 1986; Minagawa, 1994).

Effect of light intensity on growth

Light intensity affected duration of *P. gigas* larval intermoult with shorter intermoult periods at brighter intensity, possibly caused by increased feeding. However, the dimmer light intensity used in this study (2 lux) is at the extreme lower limit of the visual capacity of *P. gigas* (Gardner, 1996) so bright light (e.g. 500 lux or more) is not necessarily optimal for feeding. The effect of light intensity on larval feeding and development has seldom been examined in decapod crustaceans. Survival of larval *Paralithodes camtschaticus* is enhanced at intensities as high as 2000 lux (Nakanishi, 1987), which would be expected given that *P. camtschaticus* are known to migrate towards the surface during the day (Shirley and Shirley, 1987). In *P. gigas*, the diel migration pattern and associated natural light intensities is less clear although it is likely that they migrate from the surface at day (Gardner, 1996).

Cannibalism

Use of visual cues for feeding may not only affect food consumption but also incidence of cannibalism. Minagawa (1994) found that increased cannibalism occurred with increased food consumption in *R. ranina*. Both photoperiod and light intensity are considered amongst the primary factors affecting cannibalism (Hecht and Pienaar, 1993) and they appeared to influence cannibalism of *P. gigas*. The observed effect of increased photoperiod on increased cannibalism in *P. gigas* is readily related to feeding: if larvae utilise visual cues to feed, longer photoperiods will provide greater opportunity to consume, or cannibalise, prey items. Two feeding-related hypotheses are suggested to explain the observed higher cannibalism at lower light intensity.

First, feeding rate may be enhanced by dim lighting (2 lux) resulting in increased capture of artemia and also increased cannibalism. Running contrary to this hypothesis is the observed longer intermoult duration of zoeas reared in dim light than in bright light. However, slower growth may be expected as dorsal spine damage was greater and this is known to increase incidence of disease (Armstrong et al., 1976). Under this hypothesis, optimal lighting for *P. gigas* culture would be long photoperiods at dim intensity (e.g. 2 lux) to optimise food consumption with some form of mixing, such as aeration, to reduce cannibalism.

A second hypothesis to explain the higher cannibalism in the 2 lux treatment is that a shift to larger prey may occur at dimmer light intensity. As the 2 lux treatment was at the extreme lower limit of the visual capacity of *P. gigas* (Gardner, 1996), visual detection of small prey items may have been impaired. Miner and Stein (1993) reported a shift of this nature in predation by larval finfish (*Lepomis macrochirus*) where larger prey were consumed at dimmer light intensities. Also, avoidance of predating zoeas may have been impaired. Under this hypothesis, optimal lighting for *P. gigas* culture would be long photoperiods at bright intensity (e.g. 500 lux) to optimise predation on artemia, rather than on zoeas.

Performance of larvae in continuous light or dark regimes

Development of *P. gigas* larvae in 24 L was relatively rapid but otherwise similar to larvae reared in light-dark regimes. This is similar to *Paralithodes camtschaticus* (Nakanishi, 1987) but contrasts to most other planktonic decapods where continuous lighting retards growth

(Starkweather, 1976; Dalley, 1980; Radhakrishnan and Vijayakumaran, 1986; Minagawa, 1994).

The hormonal pathways underlying metamorphosis to megalopa are not fully understood although a low titre of juvenile hormone is thought to be necessary (Christiansen, 1988). Low rates of metamorphosis have been observed in larval *H. americanus* and *R. ranina* reared in continuous darkness, despite relatively high growth, which suggests a neuroendocrine effect of continuous darkness (Eagles et al., 1986; Minagawa, 1994). Metamorphosis of *P. gigas* larvae did not appear to be affected by 24 or 0 L treatments, however this should be accepted cautiously as data were variable and lacked statistical power (Fig. 7). Given that mean metamorphosis was lowest in the two 24 L treatments, a cautious approach of avoiding continuous lighting is suggested.

Conclusion

Although lighting did not appear to influence survival of *P. gigas* in this trial, there does appear to be potential to influence growth and cannibalism. Larvae were able to feed in continuous darkness and had low incidence of cannibalism, however, growth was slow and larvae were smaller than in treatments with light periods. Longer photoperiods resulted in more rapid growth and are recommended, although 24 L should be avoided as viability of megalopa may be affected. Larvae grew more rapidly and suffered less cannibalism at brighter intensity (500 lux). Consequently, brighter light appears preferable for culture although research is needed to clarify the effect of light intensity on feeding and feeding-related cannibalism.

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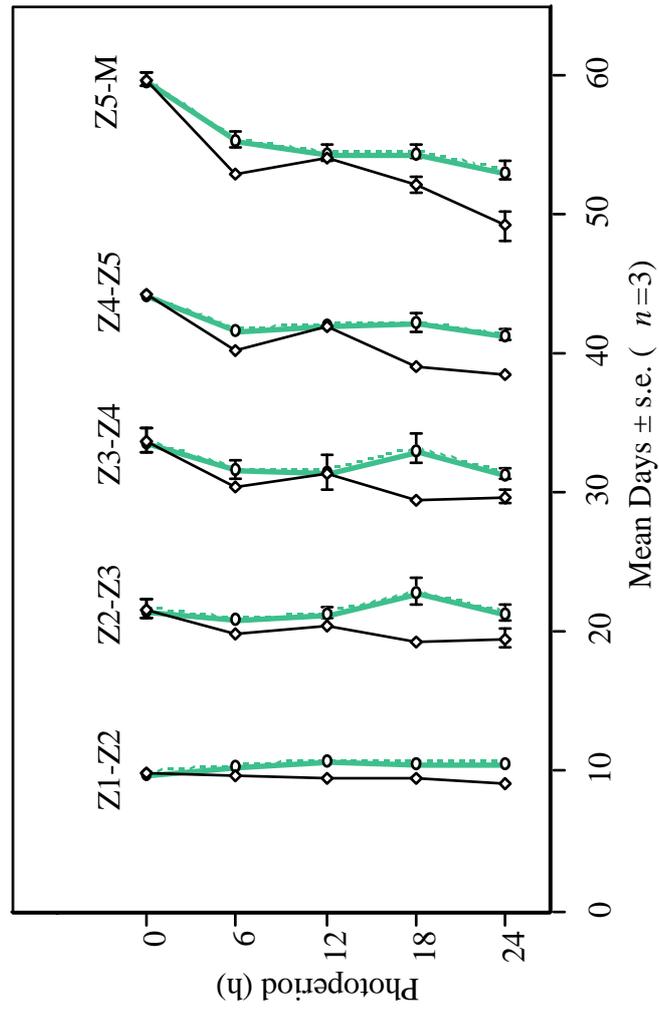
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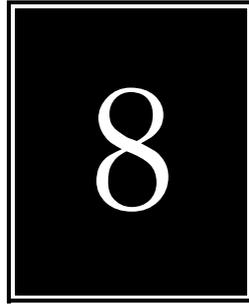
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Use of Prophylactic Treatments for Larval Rearing



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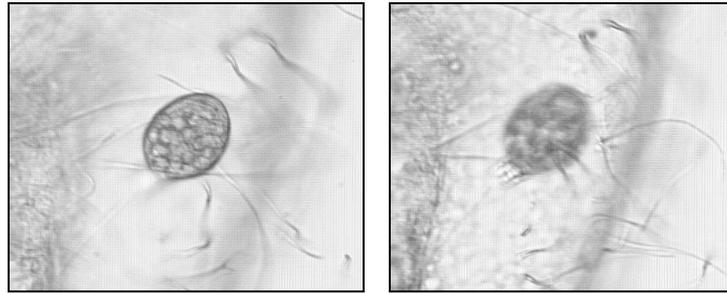
Abstract

Chemicals were screened for prophylactic treatment of epibiotic fouling and fungal mycosis in larvae of giant crabs *Pseudocarcinus gigas*. The following treatments were applied as indefinite baths: oxytetracycline, trifluralin, carbendazim, copper oxychloride, malachite green, and formalin. Most effective treatments for improving survival were oxytetracycline (25 mg l⁻¹, despite increased deformity), trifluralin (0.01 mg l⁻¹), carbendazim (0.001 mg l⁻¹), and copper oxychloride (0.05 mg l⁻¹). Three of these treatments affected size and shape of the megalopa carapace with relatively smaller megalopas developing in carbendazim and trifluralin, and relatively broader megalopas in copper oxychloride. Toxic effects, measured by increased mortality, deformity, death during ecdysis, or delayed ecdysis, were recorded with oxytetracycline (≥ 25 mg l⁻¹), trifluralin (≥ 0.03 mg l⁻¹), malachite green (≥ 0.1 mg l⁻¹), and formalin (for all concentrations tested: ≥ 2.5 mg l⁻¹).

Introduction

Optimising survival during larval rearing of crustaceans is important in aquaculture and is also of benefit in research of crustacean taxonomy, physiology, and fisheries biology. Laboratory cultures of crab larvae often suffer severe mortality from disease, particularly from epibiotic bacteria and larval mycosis (Armstrong et al., 1976; Ebert et al., 1983; Hamasaki and Hatai, 1993). Larval mycosis occurs when fungal hyphae, commonly *Lagenidium* and *Sirolopidium* species (Brock and LeaMaster, 1992), invade body tissues, developing zoospore discharge tubes which protrude through the crustacean's cuticle in the later stages (Fig. 1; Paynter, 1989). Infected larvae become immobilised and their surface develops a fouled appearance from the external processes of the fungi.

Figure 1. Fouling diseases on giant crab zoeas. A stalked fungal sporangium is present in the center and filamentous bacterial fouling is visible as fine threads.



In research involving culture of giant crab larvae, larval mycosis has consistently caused high mortality which prompted assessment of the various chemical controls available. The pelagic zoeal phase of *P. gigas* is relatively long, around 60 days, and larval mycosis occurs throughout this period, rather than just in early stages as is more typical in crustaceans (Brock and LeaMaster, 1992; Lightner, 1993). Consequently, prophylactic treatments must be administered for prolonged periods which can produce toxic effects, not evident in short term trials, such as delay in moulting (Caldwell et al., 1978) and deformity at megalopa (Ebert et al., 1983).

Prophylactic treatments are often assessed by separately establishing toxic levels of a given chemical for fungal zoospores and for the larvae to be treated (Armstrong et al., 1976; Lio-Po et al., 1982; Lio-Po and Sanvictores, 1986). While this is an invaluable technique for evaluating treatments rapidly, it can fail to assess the value of a treatment in culture situations. Adverse effects of the treatment may be underestimated because chronic toxicity can become apparent late in development and there may be interaction with bacterial species (Gil-Turnes and Fenical, 1992).

In this chapter results are presented from prolonged clinical trials with giant crab larvae comparing several chemicals reported to immobilise fungal zoospores: malachite green (Armstrong et al., 1976); trifluralin (Armstrong et al., 1976); and formalin (Hamasaki and Hatai, 1993). In addition, trials were conducted with two horticultural fungicides with low toxicity to insects: carbendazim and copper oxychloride. Larvae were also treated with the antibiotic, oxytetracycline, to control pathogenic bacteria.

Materials and methods

Source of larvae

Ovigerous females were collected from depths in the range of 300 to 380 m off the east coast of Tasmania (41°17'S; 148°40'E) in June 1995. Females ranged in size from 2.2 to 3.5 kg and were held communally in 4 m³ tanks with flow through, unfiltered water supply. To ensure that only freshly hatched larvae were used, the tanks were thoroughly flushed prior to collecting larvae for the trial. Although attempts were made to collect larvae from several females, most of the larvae used for this trial appeared to be from one female weighing 2.6 kg.

Culture methods and experimental design

Newly hatched larvae were rinsed in 0.2 µm filtered seawater (34‰ salinity) then transferred to 1.8 l vessels. Fed controls, starved controls, and chemical treatments were randomly allocated to vessels. Fifty larvae were placed in each vessel and were maintained in a temperature control room at 16°C with 700 lux, 8 l :16 d photoperiod. Zoeas were fed Protein Selco™ enriched artemia nauplii but megalopas required larger, 10 day old artemia. Larvae were pipetted into fresh, 0.2 µm filtered seawater every 2 days. The concentrations for each chemical tested to control fouling were: carbendazim (Hoechst and Schering™), malachite green, and trifluralin (DowElanco™) at 0.001, 0.003, 0.01, 0.03, and 0.1 mg kg⁻¹; copper oxychloride (ChemSpray™) at 0.025, 0.05, 0.1, 0.2, and 0.4 mg kg⁻¹; formalin at 2.5, 5, 10, 20, and 40 mg kg⁻¹; and oxytetracycline (Norbrook™) at 10, 25, 50, 100, and 200 mg kg⁻¹. Four replicates were used for each concentration. Treatments were run as indefinite baths for 115 days after which all live animals were censored in survival analyses.

Response data collected

The effect of different concentrations was monitored by recording the number and instar of exuvia and mortalities which allowed calculation of survival and time to each moult. All

mortalities were also classed into possible causes of death: fouled (severe fouling of exoskeleton), moulting (died during ecdysis), deformed, and normal (where no cause was apparent). Larvae were classed as fouled when at least 50% of the external surface was covered. Epibiotic bacterial fouling increased rapidly after death, so the fouling index only gives a general indication of disease. When dead larvae could be ascribed to more than one possible cause of mortality, such as moulting and fouled, each cause was counted and treated as independent. Although wet preparations and microbiology were performed, the cause of fouling was not determined in all cases; animals classed as fouled may have had fungal or bacterial infections. Microbiological cultures were made on TCBS, blood, and Ordal's medium.

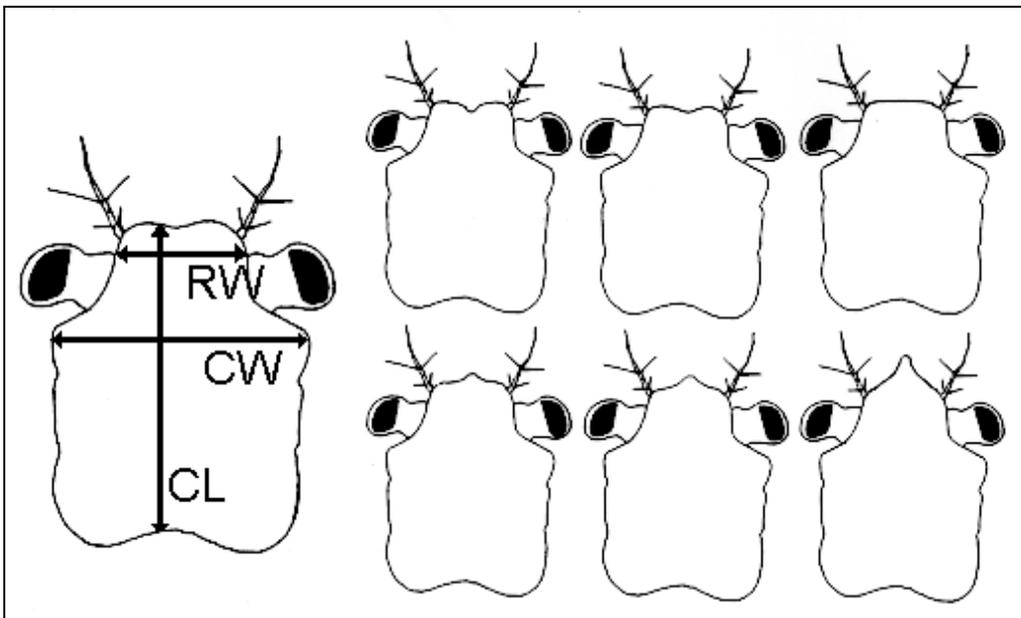
In normal development, giant crab larvae pass through 5 zoeal stages (Gardner and Quintana, 1998; Chapter 3). In this study, many larvae developed to a sixth zoeal stage, which was intermediate between zoea 5 and megalopa (termed 5-a-lopae by Ebert et al., 1983; Fig. 2). Chelae were present and the pleopods bore setae, while the carapace retained the form of the zoea. None of these larvae survived and they were classed as deformed.

The effect of treatments on size of dead megalopas or megalopa exuvia was assessed by measuring carapace length, carapace width, and rostrum width (Fig. 3). The shape of the rostrum varied between individuals so carapace length measurements were made slightly off centre. All measurements of megalopas were made by image analysis using NIH-Image™ 1.6 software.

Figure 2. Giant crab larva intermediate between zoea 5 and megalopa. As with a megalopa, the chelae are differentiated and the pleopods are setose, however zoeal characters such as the dorsal carapace spine and bifurcated telson have been retained. These larvae were classed as deformed.



Figure 3. Morphological measurements taken to assess the effect of treatment on megalopa size. RW = rostrum width, CW = carapace width, CL = carapace length. Note that there was considerable variation in the form of the rostrum so CL was measured slightly to the left of center.



Statistical analysis

Statistical analysis was performed with JMP 3.0™ software (SAS Institute). Where comparisons between treatments and controls are presented, the controls are the fed group. The effect of concentration on moult timing was tested with repeated measures analysis. As survival was poor in some treatment replicates, analysis was restricted to data collected from the start of the trial to the moult from zoea 3 to zoea 4. Data were arranged for multivariate analysis and significance determined for between effects (concentration) with Wilk's lambda (Mardia et al., 1979). Where a significant effect of concentration was found, comparisons were made between concentrations by analysis of the canonicals (Mardia et al., 1979).

Survival data were analysed by the Kaplan-Meier method with significance between groups determined by Wilcoxon's test (Miller, 1981). To prevent an increase in type 1 errors, a Bonferroni adjustment was made to alpha for comparisons between concentrations, so that comparisons were only treated as significant where $P < 0.003$ (Sokal and Rohlf, 1995). The effect of treatments on size of megalopas and cause of mortality was assessed by one way ANOVA. Cause of mortality data were arc-sine square-root transformed to produce normality and remove heteroscedasticity. Where a treatment effect was observed, means were compared by Tukey Kramer HSD (Sokal and Rohlf, 1995). Percentage survival to crab 1 data are presented although low survival prevented meaningful statistical analysis.

Results

Larvae classed as fouled appeared to be afflicted with several types of infection. Peritrich ciliates were present in low numbers and were not regarded as pathogenic. Apart from larvae cultured in oxytetracycline, mixed bacterial flora consisting predominantly of *Vibrio* spp were isolated from all cases of fouling. Fouling appeared to be predominantly from two causes: the terminal vesicles of fungal infections (tentatively identified as *Lagenidium* spp based on method of sporogenesis; Lightner, 1993) and filamentous bacterial infection (colourless, gram-negative and short cylindrical bacteria forming unbranched filaments, tentatively identified as family Leucothricheae; Lightner, 1993). In all chemical treatments and controls, some of the mortalities classed as fouled had larval mycosis.

Larvae in starved controls remained alive for a mean time of 18.8 days although all were dead by day 20 (survival data from starved and other treatments are presented in Appendix 8). A small number of these successfully moulted to zoea 2 (1.5%). Larvae from both starved and fed controls had low level surface infections of mixed *Vibrio* spp. The proportion of larvae which died with high levels of fouling were significantly higher ($P < 0.001$) in fed controls than in starved controls: 45.5% compared with 10.5%.

Detrimental effects

Some treatment concentrations had significantly lower survival than fed controls which is attributed to toxicity: 100 and 200 mg l⁻¹ oxytetracycline, and 20 and 40 mg l⁻¹ formalin ($P < 0.003$; Figs. 4 and 5). Chronic toxicity was evident in 25 and 50 mg l⁻¹ oxytetracycline treatments with significantly increased incidence of deformity ($P < 0.001$). Both of these oxytetracycline treatments had significantly higher moulting mortality ($P < 0.001$) as did treatments 0.03 and 0.1 mg l⁻¹ trifluralin ($P < 0.01$), and 10 mg l⁻¹ formalin ($P < 0.05$).

Survival was also reduced in the 10 mg l⁻¹ oxytetracycline treatment which was associated with significantly higher fouling relative to controls ($P < 0.0001$; Figs. 4 and 5). This fouling was gelatinous, rather than filamentous as in other treatments, and was caused by a single *Vibrio* species which produced pale yellow colonies in TCBS media.

Sub-lethal toxic effects were observed in the 100 mg l⁻¹ oxytetracycline and 0.1 mg l⁻¹ malachite green treatments as moulting was significantly delayed (Fig. 6; $P < 0.001$). Consequently, the mean time to reach megalopa was delayed by 51 days and 13 days respectively.

Enhanced survival

Although toxic effects were observed at some concentrations, survival was enhanced by the best concentrations of all treatments relative to controls ($P < 0.003$). This improved survival was associated with significantly lower fouling only in oxytetracycline, for concentrations ≥ 25 mg l⁻¹ (Fig. 4; $P < 0.0001$). In no other treatment was there a significant reduction in fouling to account for the improved survival although trends were apparent, such as in copper oxychloride and malachite green treatments. Because there was large

variation in the incidence of fouling in controls, the experiment had low power to discern differences; as percentages, the mean incidence of fouling in controls was 45% while standard error was 20.1%.

Ranking of the “best” concentrations of each treatment in increasing survival (quantified by the Kaplan-Meier method) was: oxytetracycline (50 mg l⁻¹) > carbendazim (0.001 mg l⁻¹) > trifluralin (0.1 mg l⁻¹) > malachite green (0.03 mg l⁻¹) > formalin (10 mg l⁻¹) > copper oxychloride (0.05 mg l⁻¹). There was no significant difference between treatments except between oxytetracycline and all other treatments (P<0.0001).

Larvae survived to crab 1 in several treatments. Ranking of treatments for survival to crab 1 was: 25 mg l⁻¹ oxytetracycline (7%) > 50 mg l⁻¹ oxytetracycline (3.5%) > 0.4 mg l⁻¹ copper oxychloride (1.5%) > 0.05 mg l⁻¹ copper oxychloride (1.0%) > 0.025 mg l⁻¹ copper oxychloride, 0.001 and 0.003 mg l⁻¹ carbendazim (all 0.5%). No larvae survived to crab 1 in controls or trifluralin, malachite green, and formalin treatments.

Figure 4. Effect of treatment concentration (mg l^{-1}) on larval survival and cause of mortality. Mean survival for each concentration is the mean number of instars to which larvae survived, eg. mean survival of 2.9 implies that the mean survival was almost to Z3, while a mean of 1.1 implies that most larvae died at Z1 before moulting. Letters denote significantly different pairs of means ($P < 0.003$). Symbols indicate major causes of mortality (accounting for >20% of total during moulting, >30% other causes) for each concentration: ♣ - moulting; ▽ - fouling; ♠ - deformity; ◆ - normal.

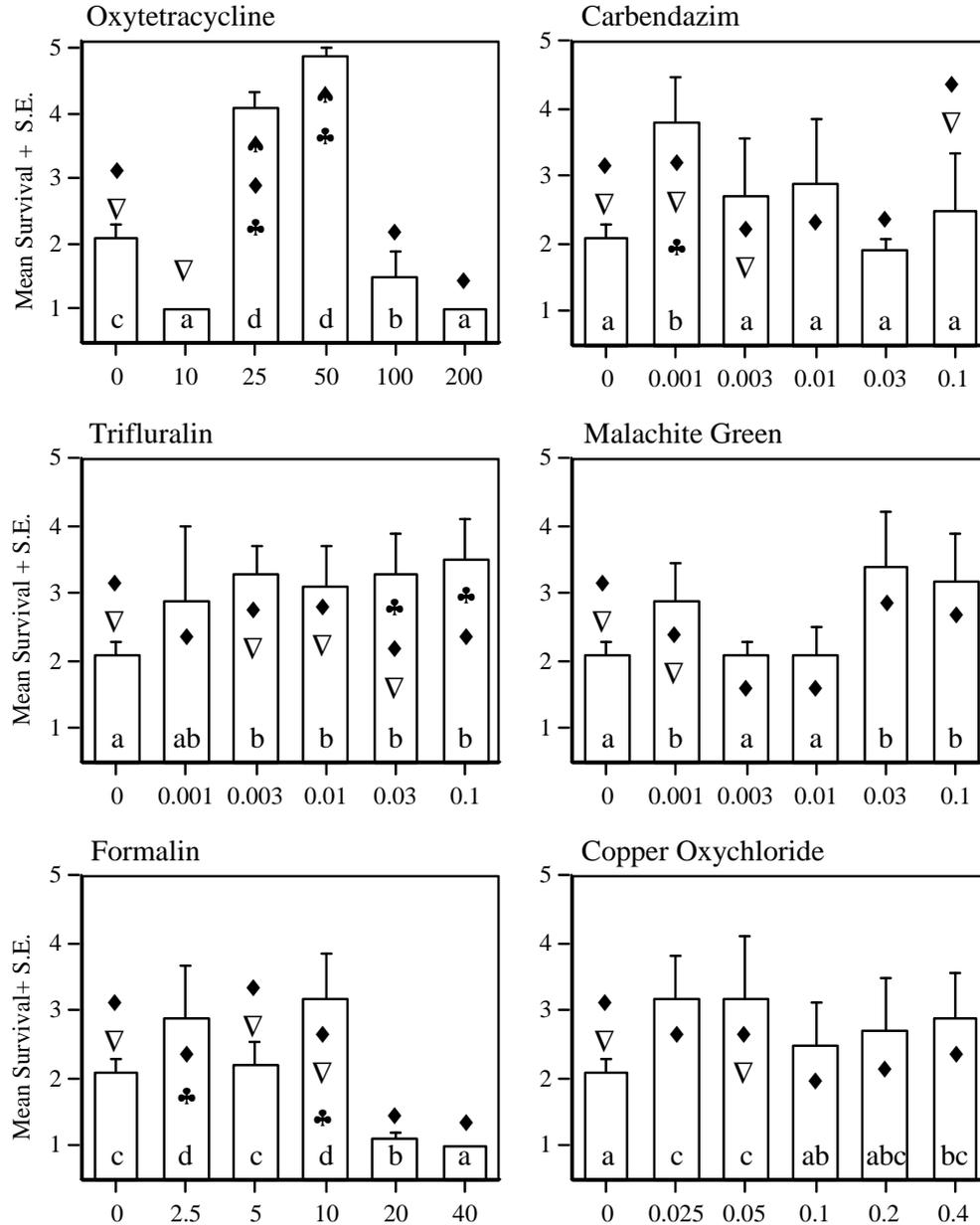


Figure 5. Survival of larvae in oxytetracycline treatments to each larval stage.

Z1...Z5 = zoea 1...zoea 5, M = megalopa, and C = crab 1. Mean survival is the mean number of instars that larvae survived to, eg. mean survival of 2.9 implies that the mean survival was almost to Z3. Superscripts denote significantly different pairs of means ($P < 0.003$).

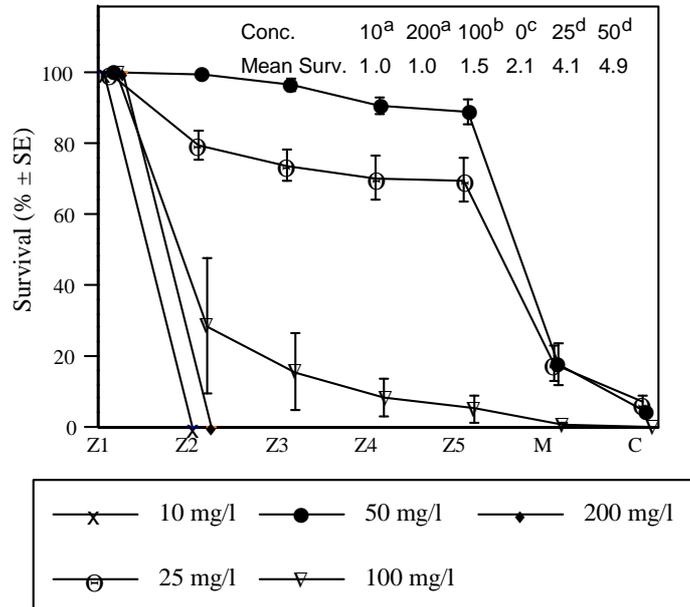
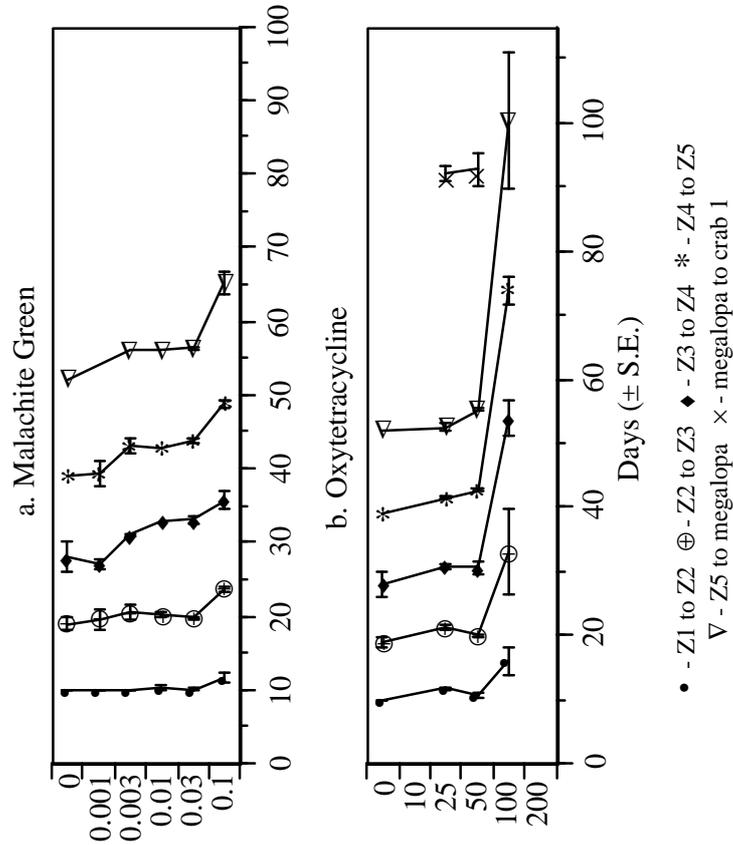


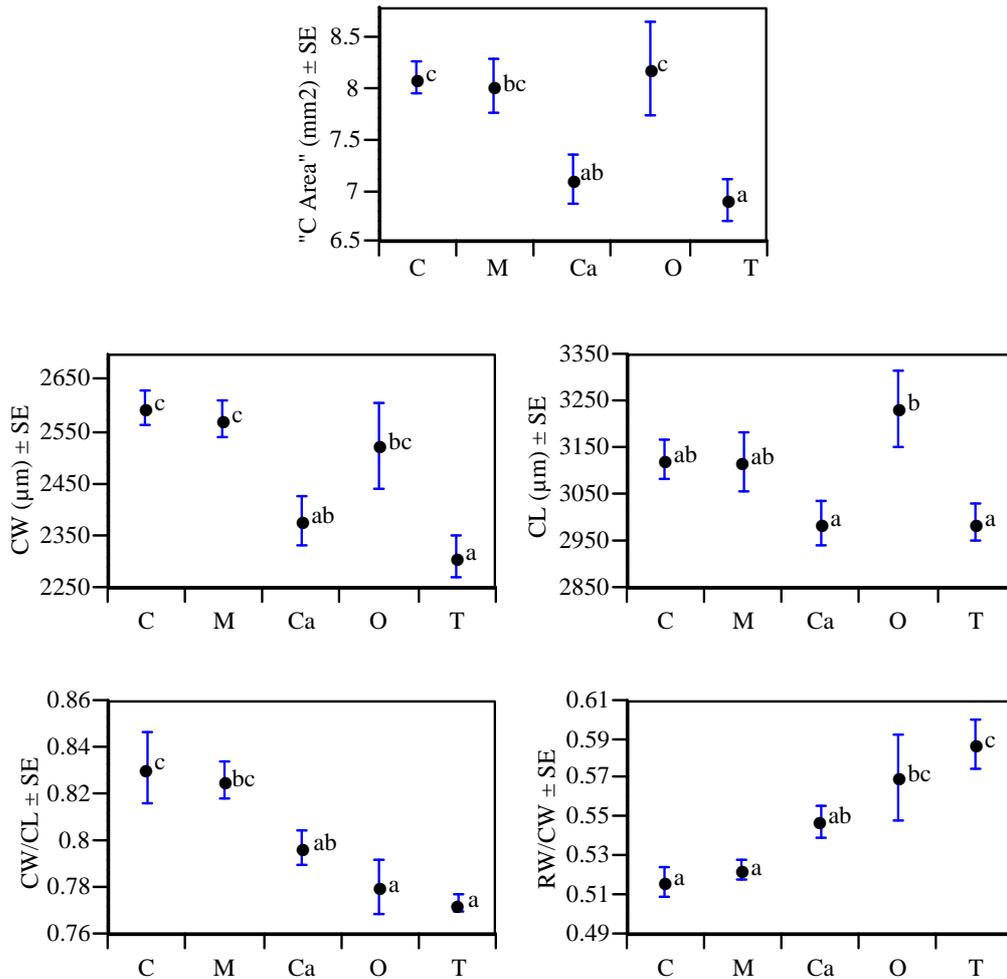
Figure 6, a & b. Effect of treatments on the timing of moults. Shift towards the right suggests that the treatment has delayed the development of the larvae. Points plotted for moult stages more advanced than the moult to Z4 should be interpreted with caution, as replication was frequently reduced in these later samples to less than 4. Treatment concentrations given on the X axes are in mg l^{-1} . Missing points are due to complete mortality.



Effects on megalopas

Megalopa size and morphology was affected by treatment (Fig. 7; $P < 0.05$). Megalopas from copper oxychloride, malachite green, and oxytetracycline treatments were larger than those from carbendazim and trifluralin treatments as measured by carapace area, carapace width, and carapace length. Opposite trends between the carapace width/carapace length and rostrum width/carapace width ratios were observed. This suggests that treatment type influences the shape of the carapace by increasing curvature or flexion of the lateral margins of the carapace. Megalopas from copper oxychloride and malachite green treatments were relatively broader.

Figure 7. Effect of treatments on size of megalopas. C = copper oxychloride, M = malachite green, Ca = carbendazim, O = oxytetracycline, T = trifluralin. Treatment had a significant effect ($P < 0.05$) on size for all measures tested: "carapace area" (C Area = $CW \times CL$); carapace width (CW); carapace length (CL); and the ratios between carapace width/carapace length (CW/CL) and rostrum width/carapace width (RW/CW). Higher mean values for CW/CL implies a relatively broader carapace and higher values for RW/CW implies a relatively broader rostrum. Labels next to means indicate significance of means comparisons. Formalin and control measurements were not included due to poor survival. Mean values are from all concentrations of each treatments. Larvae did not develop to megalopa in most replicates so the number of replicates contributing to the mean varied: C, $n = 6$; M, $n = 5$; Ca, $n = 6$; O, $n = 7$; T, $n = 8$.



Discussion

General observations on prophylactic treatments

Larval *P. gigas* were successfully reared to juvenile crabs with prophylactic treatment. This has not been achieved in numerous other trials, even with high standards of hatchery hygiene and water quality.

Both larval mycosis and filamentous bacteria disease were associated with mortality of larvae in this trial. Possible sources of fungal infection were split into three options by Ebert et al. (1983): parents; food source (artemia); and incoming water. No spores or bacteria should have entered this trial via the incoming water as filtration was absolute to 0.2 μm and *Lagenidium* spp. spores are 7 by 5 μm in size (Armstrong et al., 1976). The higher incidence of fouling in fed controls than in starved controls suggests that artemia contributed to disease, by acting as a source of infection or by contributing to water quality deterioration. Infection risk from artemia could be lowered by avoiding overfeeding and by prophylactic chemical treatment of artemia.

Oxytetracycline

The good performance of larvae cultured with oxytetracycline indicates the importance of bacterial disease on larval mortality. There are indications that oxytetracycline also reduced fungal infection because no cases of larval mycosis were found in 50, 100, and 200 mg l^{-1} oxytetracycline treatments. Possible explanations for this are: disease resistance improved as a result of general improvement in larval health (Anderson, 1989); possible deactivation of fungal zoospores; prevention of bacterial septicaemia following dorsal spine damage, which is known to increase risk of fungal infection (Ebert et al., 1983; Anderson, 1989); and an interaction between bacteria and fungal populations so that reduction of bacterial fauna resulted in a reduction in the incidence of fungal infection. In regards to the last hypothesis, the opposite trend is usually observed with bacterial competition preventing fungal infections (Gil-Turnes and Fenical, 1992) so that antibiotics, such as oxytetracycline, increase the risk of larval mycosis (Nogami and Maeda, 1992).

Lightner (1993) suggested oxytetracycline in concentrations of 10 to 90 mg l⁻¹ for use as indefinite baths to control *Leucothrix mucor*. For rearing giant crab larvae, this range is too broad. Bacteria proliferated at 10 mg l⁻¹ and chronic toxicity resulted in high levels of deformity, similar to those reported by Ebert et al. (1983) with streptomycin sulphate, at all concentrations where larvae survived better than controls (≥ 25 mg l⁻¹). Of the oxytetracycline concentrations tested, 25 mg l⁻¹ would be most suitable due to lower chronic toxicity. The prophylactic use of oxytetracycline is clearly effective in culturing giant crab larvae although the widespread use of antibiotics is discouraged to prevent development of resistant strains (Anderson, 1989).

Carbendazim and trifluralin

Highest survival in prolonged fungicide treatments, without chronic toxic effects, was observed in carbendazim (0.001 mg l⁻¹) and trifluralin (0.003 mg l⁻¹). Both of these chemicals would be expected to degrade between water changes. Williams et al. (1986) reported the half-life of trifluralin in water could be as short as 30 minutes although the manufacturers, DowElanco, list the half-life as 6 days in aquatic systems. Survival of larvae may have been improved further by more frequent application of chemicals. Despite the apparent lack of toxic effects at higher concentrations, highest survival in carbendazim treatments was at only 0.001 mg l⁻¹. The apparent absence of toxic effects at all concentrations tested (up to 0.1 mg l⁻¹) for carbendazim suggests that further trials are warranted. Although the implications on survival and growth are unclear, it is worth noting that megalopas from both carbendazim and trifluralin treatments were smaller than in other treatments.

Toxic effects of trifluralin on larvae were observed at only 0.03 mg l⁻¹ which is within the range suggested for prophylactic treatment of crustacean larvae by Lio-Po and Sanvictores (1986) and Lightner (1993). This discrepancy is likely to be due to the prolonged period of treatment needed with giant crab larvae. The maximum acceptable trifluralin concentration determined in this study was 0.01 mg l⁻¹ as no toxic effects were observed. This concentration leaves some margin for toxicity to fungal zoospores as there was a significant improvement in survival at 0.003 mg l⁻¹. Also, Armstrong et al. (1976) reported effective control of mycosis with trifluralin at 0.0015 mg l⁻¹.

Copper oxychloride, malachite green and formalin

Copper oxychloride, malachite green and formalin treatments had lower survival than optimal concentrations of carbendazim and trifluralin although all significantly improved survival relative to controls. The action of copper oxychloride, malachite green and formalin is broad as they can also control epibiotic fouling bacteria and protozoa (Lightner, 1993). Consequently, this trial evaluated appropriate concentrations for prolonged treatment rather than the effect of treatments on specific pathogens.

Copper oxychloride appears useful as a prophylactic as it improved survival and no toxic effects were observed within the range tested, up to 0.4 mg l⁻¹. Although survival was less than in optimal concentrations of trifluralin or carbendazim, this difference was not significant (P>0.05). It is also worth noting that relatively large megalopas were produced in the copper oxychloride treatment, several of which developed to crab 1.

Malachite green at 0.03 mg l⁻¹ improved survival of giant crab larvae while higher concentrations delayed moulting. Malachite green has been used with crab eggs (Fisher, 1976) but was considered too toxic to zoeas to be a useful prophylactic treatment by Armstrong et al. (1976) and Fisher and Nelson (1977). Armstrong et al. (1976) found a *Lagenidium* sp. was resilient to concentrations of malachite green below 1 mg l⁻¹ which is far greater than the concentration which produced chronic toxic effects in this study (0.1 mg l⁻¹).

Formalin appeared to be unsuitable for prolonged prophylactic treatment as moulting mortality was significantly higher (P<0.005) where survival was improved, indicating poorer health (Fisher, 1983). Short term formalin baths as used by Hamasaki and Hatai (1993) or Kaji et al. (1991) may be beneficial when infection occurs for only a short period.

Conclusion

The effect of chemicals on toxicity and survival were evaluated in this trial as prolonged prophylactic treatments. Best results were obtained with oxytetracycline (25 mg l⁻¹) although use of antibiotics for commercial larval rearing is generally discouraged. Trifluralin (0.003 mg l⁻¹) and carbendazim (0.001 mg l⁻¹) improved survival and appear to be useful alternatives. Further trials with copper oxychloride and carbendazim may be

warranted as survival was improved with no apparent toxic effects, even at high doses. Concentrating prophylactic treatment around critical periods, such as moulting, may allow stronger concentrations to be used without causing toxic effects (Armstrong et al., 1976).

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General Discussion:
Larval Biology of the Giant Crab
Pseudocarcinus gigas



Taxonomy

The anatomy of the larval stages of *Pseudocarcinus gigas* was consistent with other members of the family Eriphiidae. Some unusual characters indicated affinity with crabs of the genus *Ozius*, such as a spine on the dorsal surface of the first abdominal somite of zoeas, and a spine on the cheliped ischium of the megalopa. *Ozius truncatus* and *O. deplanatus* are found across southern Australia in the same region as *P. gigas* which strengthens the proposed affinity (Edgar, 1997). Many life history traits of crab species, such as mating behaviour, are consistent within family taxa so comparison with similar species can be useful (Hartnoll, 1969). McLay (1988) reviewed published information on *O. truncatus* which is limited to observations on feeding and defence behaviour of adults, diet studies (Chilton and Bull, 1984; Skilleter and Anderson, 1986), and description of the larvae (Wear, 1968; Wear and Fielder, 1985). Unfortunately, no information has been published on the reproductive biology of this species although it would be useful for predicting strategies in *P. gigas* due to the similarity between the genera evident from larval morphology.

Taxonomically conservative aspects of the reproductive biology discussed in the next section of this thesis, such as soft/hard shelled mating of females, could be studied more easily with a small intertidal species like *O. truncatus*.

The megalopas of *Menippe* species are similar to *P. gigas* based on the characters used by Martin (1988), although this was considered to be mainly an artefact of the presence of 5 zoeal stages, rather than 4 as in other members of Eriphiidae. Nonetheless, the zoeas of *Menippe* and *P. gigas* were grouped together using a range of characters described by Martin (1984) for xanthoid crabs and this provides more legitimate evidence of taxonomic affinity. *Menippe* species are harvested commercially in the West Atlantic and the Caribbean and there have been several publications on the reproductive biology. These studies provide a guide to aspects of the reproductive strategies of *P. gigas* and are discussed in the next section.

Larval dispersal

Modern, three-dimensional hydrodynamic models permit the larval dispersal of species to be predicted provided accurate biological and oceanographic information is available (Keough and Black, 1996). These authors listed categories of biological data that they considered critical for modelling and these were the duration of the planktonic period, buoyancy, and larval taxes and swimming speeds.

An aim of the research on larval behaviour presented in this thesis was to collect this information so that dispersal modelling could be conducted in the future. The construction of three-dimensional hydrodynamic models of southern Australia is underway to investigate dispersal of southern rock lobsters *Jasus edwardsii* (CSIRO Australia) and it is anticipated that these models will be suitable for *P. gigas*. Model outputs of dispersal of *P. gigas* larvae should have higher precision than for *J. edwardsii* as rock lobsters have an extremely protracted planktonic stage of up to 24 months (Cobb et al., 1997) and are able to delay metamorphosis by mark-time moulting until conditions are suitable (Pollock and Melville-Smith, 1993; Baisre, 1994).

Planktonic period

The planktonic period of *P. gigas* is relatively long compared with other eriphiids, due to development through 5, rather than 4 zoeal stages (in eriphiids other than *Menippe* species), and the more temperate environment. *Ozxius truncatus* and *O. verreauxii* take 24-28 and 15 days to reach megalopa respectively, while *M. merveneria* takes 12-22 days (Wear, 1968; Ong and Costlow, 1970; Dittel and Epifanio, 1985; McConnaughey and Krantz, 1992). Larval duration of *P. gigas* was variable and became longer at lower temperature, lower light intensity, shorter photoperiod, and in response to toxicity at high concentrations of prophylactic chemical treatments. Feeding and nutrition was not researched but this can also influence larval duration, although larvae tend to grow larger, rather than faster, with improved nutrition (Minagawa and Murano, 1993; Tong, et al., 1997).

Temperature had a profound effect on planktonic period and the duration from hatch to megalopa ranged from 41 days at 21°C to 73 days at 13°C. As is often demonstrated in

crustacean aquaculture trials, rapid growth is not necessarily optimal growth, as larvae may fail to accumulate sufficient reserves to metamorphose successfully (Minagawa, 1990; Chaoshu and Shaojing, 1992; Okamoto, 1993). The size of *P. gigas* larvae decreased with increasing temperature which appeared to affect metamorphosis in larvae cultured at temperatures greater than 16.8°C. Results from larval behaviour trials indicated that larvae would sink to avoid these temperatures so that the typical temperature range of *P. gigas* larvae is likely to lie between 14°C and 16°C (established for stage 1 and 2 zoeas only). This is similar to water temperature in the upper 50 m along southern Australia during late Spring and early Summer (Fig. 1). The mean duration of zoeal stages at these temperatures ranged from 48 to 62 days.

The megalopa stage of brachyurans is also a planktonic dispersal stage although they regularly descend to the substrate (Lochmann et al., 1995). The duration of the megalopa stage in *P. gigas* was only determined in trials with prophylactic treatments for disease at 16°C where the mean duration was 38 days. Keough and Black (1996) considered that estimates of larval duration from laboratory studies were upper limits as most planktonic marine larvae require specific cues to settle, which are not usually present in the laboratory. This is unlikely to apply to brachyuran zoeas (which do not moult to a settling stage), although the duration of the megalopa stage in laboratory studies may be longer than in nature. There was some evidence of this in rearing of *P. gigas* as the megalopas ceased swimming around 10 days before metamorphosing to juveniles and moved entirely by walking. The abdomen was flexed under the cephalothorax which suggested that the musculature of the abdomen had altered, and that locomotion by swimming with the pleopods was no longer possible. The natural location of settlement of this species is likely to be at considerable depth so it is quite probable that appropriate settlement cues were not present and that metamorphosis was delayed.

Figure 1. Temperature maps of the upper 50 m of oceanic water across southern Australia in November, December and January (averaged for 7 years preceding 1996; maps supplied by CSIRO).

Buoyancy

All *P. gigas* larval stages were negatively buoyant and stage 1 zoeas sank at 0.61 cm s^{-1} . However, the mean upward swimming speed of stage 1 zoeas was 1.61 cm s^{-1} so larvae were readily able to maintain vertical position and some larvae maintained position in horizontal currents of up to 1.87 cm s^{-1} . Except in conditions of upwelling or downwelling, their ability to maintain position and vertical distribution would be determined by their response to environmental stimuli.

Larval taxes

Behavioural responses of zoea 1, *P. gigas* larvae to a range of environmental stimuli suggests that they migrate towards the surface after hatching, then live in relatively low light environments ($< 230 \text{ lux}$). The conclusion of upward swimming to the surface immediately after hatch is based on the strong, negative geotactic swimming behaviour of larvae during the first 24 h after hatch. Although only three specimens were captured, this is supported by the depth of *P. gigas* larvae obtained from plankton samples which were all in the upper 100 m of water.

An indication that larvae are adapted for low light environments is that the negative phototactic response of larvae to change in light intensity only occurred at low intensity below 230 lux (roughly similar to the light level in a forest on an overcast day). This was an unusual response as it involved active negative phototaxis, rather than merely slow swimming speed resulting in sinking. The sensitivity of larvae to changes in light at low intensity, but not at bright intensity, suggests that this may be the natural light environment of *P. gigas* larvae. Although positive phototaxis was observed at higher intensities, this may be a laboratory artefact which is difficult to avoid, even with the apparatus design described in Chapter 5 which was intended to simulate natural underwater light distribution (Stearns and Forward, 1984; Forward, 1985; Forward, 1988). Larvae were able to feed and grow through to megalopa in low intensity lighting (2 lux) or complete darkness which demonstrates that they would not need to be present in surface waters during the day to capture prey. The proposed diel migration pattern of *P. gigas* larvae is deeper distribution during the day and shallower distribution during the night.

Results of trials on the effect of temperature on larval behaviour indicate that temperature regulates depth distribution in the absence of light. As noted earlier, the typical temperature range of *P. gigas* larvae is likely to lie between 14°C and 16°C. This indicates that at night, larvae will accumulate at the surface in water of less than 14°C and at deeper depths in water of more than 16°C. Larvae readily penetrated thermoclines but their distribution may still be influenced by the absolute temperatures on either side of the boundary layer. For instance, it is predicted that larvae would accumulate at a thermocline with an upper temperature of 17°C and a lower temperature of 13°C.

Results from behavioural experiments were limited for several reasons and it is unrealistic to expect precise prediction of the natural distribution of larvae. Rather, these trials provide experimental evidence of mechanisms to explain larval distribution. Most experimentation was based on first stage zoeas and there is potential for ontogenetic change in the response of larvae to environmental stimuli (Forward, 1990). Also, larval behaviour is extremely complex and an attempt was made to isolate specific stimuli in most of the experiments reported here. Natural behavioural responses of brachyuran larvae involve complex interactions between separate stimuli, such as light and temperature (Anger, 1983; Sulkin, 1984). Accurate information on natural distribution could be obtained by plankton sampling, as described in Chapter 4, at different depths and in regions with higher density of adult crabs, such as in the north-west and north-east of Tasmania during October, November and December. This information would allow testing of the hypotheses on larval vertical migration presented in this thesis.

Aquaculture potential

Demand for small giant crabs of less than 3 kg has continued to grow since the inception of this project with an associated increase in beach price. The fishery appears to have peaked and be in decline after an initial fish-down of virgin stock, so the current high beach prices of \$(Aust)50/kg should be sustained and may increase. Most product is exported to Asian markets and prices have not been affected by recent economic problems in this region, which also indicates that current prices are robust. There is clearly market opportunity for small, aquaculture product.

Hatchery production

Research conducted on prophylactic treatments for larval disease demonstrated that hatchery production of juvenile giant crabs is possible (Fig. 2). Best performance was in treatments with oxytetracycline with survival in some tanks of 10% through to crab 1. Although oxytetracycline enhanced survival of *P. gigas* larvae, the prophylactic use of antibiotics in crustacean hatcheries is inappropriate as it promotes the development of resistant strains of bacteria (Anderson, 1989). Two other chemicals enhanced survival of *P. gigas* larvae and appear to be promising alternatives to antibiotics: copper oxychloride and carbendazim.

Figure 2. Hatchery-reared, first instar giant crab on an Australian 5 cent coin (around the size of a US 1 cent coin).



Survival through to the last zoeal stage was generally above 50% with most mortality at the moult to megalopa. This may indicate that conditions were inappropriate for the last zoeal stage, or more probably, that the larvae were lacking sufficient reserves from the zoeal stages to successfully metamorphose. This is a common problem in larval rearing of decapods and further research is required with *P. gigas* to improve survival to megalopa.

Two areas are suggested for further trials in hatchery production of *P. gigas*. First, cannibalism of spines and appendages appeared to cause mortality, primarily through weakening the larvae and exposing the larvae to bacterial septicaemia. Although this was reduced by culturing larvae in complete darkness, the size and growth rate of larvae was affected and continuous darkness

is not recommended. A more effective option may be improved tank design with greater mixing of larvae. High survival of over 70% to megalopa has been achieved with *Menippe mercenaria* cultured in Kreisel tanks which produce thorough mixing to minimise contact between larvae (Hughes et al., 1974; McConnaughey and Kranz, 1992). A second area for further research on hatchery production is to adjust environmental conditions through development to suit ontogenetic changes in environmental preferences. Temperature is likely to be especially important as behavioural tolerance of first and second stage zoeas was restricted to a narrow temperature window. Ontogenetic changes in temperature tolerance could be assessed by varying temperature through development experimentally, or by assessing instantaneous mortality rates of different stages maintained in static temperature systems. This was not possible in the trial presented in Chapter 6 due to low sample size.

Larval duration of *P. gigas* was extremely protracted relative to other species which have been considered as aquaculture candidates. For instance, both *Scylla serrata* and *M. mercenaria* take around 12 days to reach megalopa compared with 48 to 62 days in *P. gigas* (Chaoshu and Shaojing, 1992; McConnaughey and Kranz, 1992). This extended larval duration may prohibit production of *P. gigas* juveniles at a realistic cost and an economic assessment would be wise prior to any additional research. Estimates of survival and density could be based on those obtained with *M. mercenaria* in pilot scale hatchery production (72.2% and 30.7 megalopas/l respectively; McConnaughey and Kranz, 1992).

Grow-out of juvenile crabs

No trials on the grow-out of *P. gigas* juveniles are described in this thesis although some observations are relevant. Cannibalism has been a problem with the grow-out phase in culture of portunids, especially during ecdysis (Bardach et al., 1972), but was not observed with *P. gigas* juveniles or adults.

Growth of juvenile *P. gigas* was very slow for an aquaculture species with juveniles reaching a mean of 27 mm carapace width in 12 months in ambient flow-through water in Hobart (Fig. 3; Gardner, 1998). This is similar to *M. mercenaria* which take 12 months to reach 10 mm carapace width under normal conditions for Florida (Tweedale et al., 1993) and 2.5

years to reach marketable size of 100 mm carapace width (Bardach et al., 1972). An increase in temperature typically leads to shorter intermoult up to an optimal temperature for fastest growth (Kondzela and Shirley, 1993). Water temperature in the grow-out of *P. gigas* juveniles ranged from 7° to 17°C and faster growth of juveniles may be possible at warmer temperatures. However, growth is unlikely to compare with that of other aquaculture candidate species such as *Scylla serrata* which can reach marketable size within 9 months (C. Keenan, Pers. Comm., Queensland DPIF, 1997) or *Callinectes sapidus* which can reach marketable size in 4 months (Bardach et al., 1972). Although *S. serrata* and *C. sapidus* are worth far less than *P. gigas*, the slow growth of *P. gigas* juveniles remains a problem for aquaculture. Grow-out of *P. gigas* juveniles may not be viable except in combination with existing operations, such as abalone culture, or for stock enhancement.

Figure 3. Hatchery-reared juvenile giant crab, 12 months after hatching.



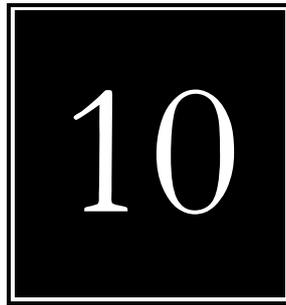
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General Introduction:
Reproductive Biology of the Giant Crab
Pseudocarcinus gigas



The need for research

The giant crab fishery grew rapidly after 1991 and fisheries managers initially had no biological information to assist in formulating management strategies. During this development period, the fishery was managed by a federal authority (Australian Fisheries Management Authority) and licences were issued with little attempt to restrain increase in effort. The lack of caution exercised by this authority was an anathema to most State fisheries management authorities and it was criticised in the wider community. At this time, the orange roughy *Hoplostethus atlanticus* fishery had just collapsed as information on growth and biomass had been collected only after intense fishing pressure was applied to stocks, rather than before. Scientists such as Tim Flannery (1994) considered that the orange roughy collapse could be repeated with giant crabs as they were also from deep water, were probably slow growing, and were subjected to high fishing pressure before research was conducted. Tsaamenyi and McIlgorm (1995) discussed the draft "FAO Code of Conduct for Responsible Fishing" and illustrated problems with current practice in Australia using the Tasmanian giant crab fishery as an example. This code of conduct suggests that exploitation of a previously unfished stock should not increase until a plan for rational exploitation has been agreed.

This illustrates the need for information on the biology of the giant crab which prompted research including that presented in this thesis. Information on larval biology was initially required to address concerns that dispersal may be limited. After having established that development was not abbreviated, research on the larval biology was directed towards providing input data for dispersal modelling. While this research can contribute to the long term viability of a giant crab fishery across southern Australia, information on the reproductive biology was required more immediately for establishing basic management rules, such as size limits and closed seasons.

Commercial management arrangements

Current management arrangements for the Tasmanian commercial fishery are listed in Table 1. There are also restrictions of the recreational harvest of giant crabs with a maximum daily catch limit of two crabs. This recreational limit is largely unnecessary as no recreational catch of giant crabs was recorded in a recent survey of recreational fishing in Tasmania (Lyle and Smith, 1998). Note that the commercial management arrangements are based on input controls to constrain effort and most of these are based on the rock lobster fishery for ease of enforcement (e.g. escape gaps and closed seasons). Of the existing controls, only the minimum size limit is linked to biological information and this was introduced as an interim measure until more extensive data became available.

The management system is currently under review and various alternatives have been proposed including the introduction of a total allowable catch, reduction of licenses, and the introduction of a maximum size limit. Information on the reproductive biology is clearly useful in this process.

Table 1. Current management arrangements for the Tasmanian giant crab fishery

Management zone:	one management zone for the State (since January 1997).
Limited entry:	106 licences (approximately 1/3 of the 321 rock lobster licences in the State).
Limited seasons:	18 th November - 21 st December; 3 rd January - 14 th February; 1 st March - 31 st August.
Limits of pots on vessels:	minimum of 15 pots, maximum of 50 pots.
Restrictions on setting pots:	pots cannot be set, or pulled, between two hours after sunset and two hours before sunrise. Pots must be hauled no longer than 5 days after being set. Pots may be deployed in long-lines of up to 10 pots.
Restrictions on pot size:	maximum size of 1250 mm x 1250 mm x 750 mm.
Escape gaps:	one escape gap at least 57 mm high and 400 mm wide and not more than 150 mm from the inside lower edge of the pot, or two escape gaps at least 57 mm high and 200 mm wide and not more than 150 mm from the inside lower edge of the pot (as designed for rock lobster).
Minimum size limits:	150 mm carapace length for both sexes (since 1993).
Ovigerous females:	taking of ovigerous females prohibited (since 1993).

Development of techniques for research

Two chapters in this section describe techniques developed for use in subsequent research on reproductive biology. The first reports the effect of various treatments tested for humanely killing or immobilising giant crabs. Crabs were difficult to subdue or kill using conventional methods such as chilling and they would often damage themselves struggling against claw ties. Aside from ethical considerations, the ability to immobilise crabs during procedures such as removing eggs was beneficial as crabs were easier to work with. Giant crabs are extremely strong, and sometimes fast, so techniques to immobilise them reduced the risk of human injury.

The second chapter on technique describes options for the non-lethal imaging of spermathecae. This was investigated for research on sperm storage in females held for several years in tanks (Chapter 14). It was intended that scanning spermathecae would allow images to be collected of the spermathecae without killing the crabs, should infertile clutches be produced. This never eventuated despite holding the female crabs isolated from males for the duration of the project, so the techniques described were never employed. However, the imaging techniques are useful as crab research methods and have been applied in research that is on-going from that described in this thesis. This ongoing research utilises computerised tomography scanning (CT) to assess gonad development and thus onset of sexual maturity. Important benefits from non-lethal imaging are that crabs can be sold after the images are collected (the market for giant crab is based on live animals with beach price ranging from \$50 to \$200 each), and crabs can be processed more rapidly than would be possible by dissection.

Objectives of research on giant crab reproductive biology

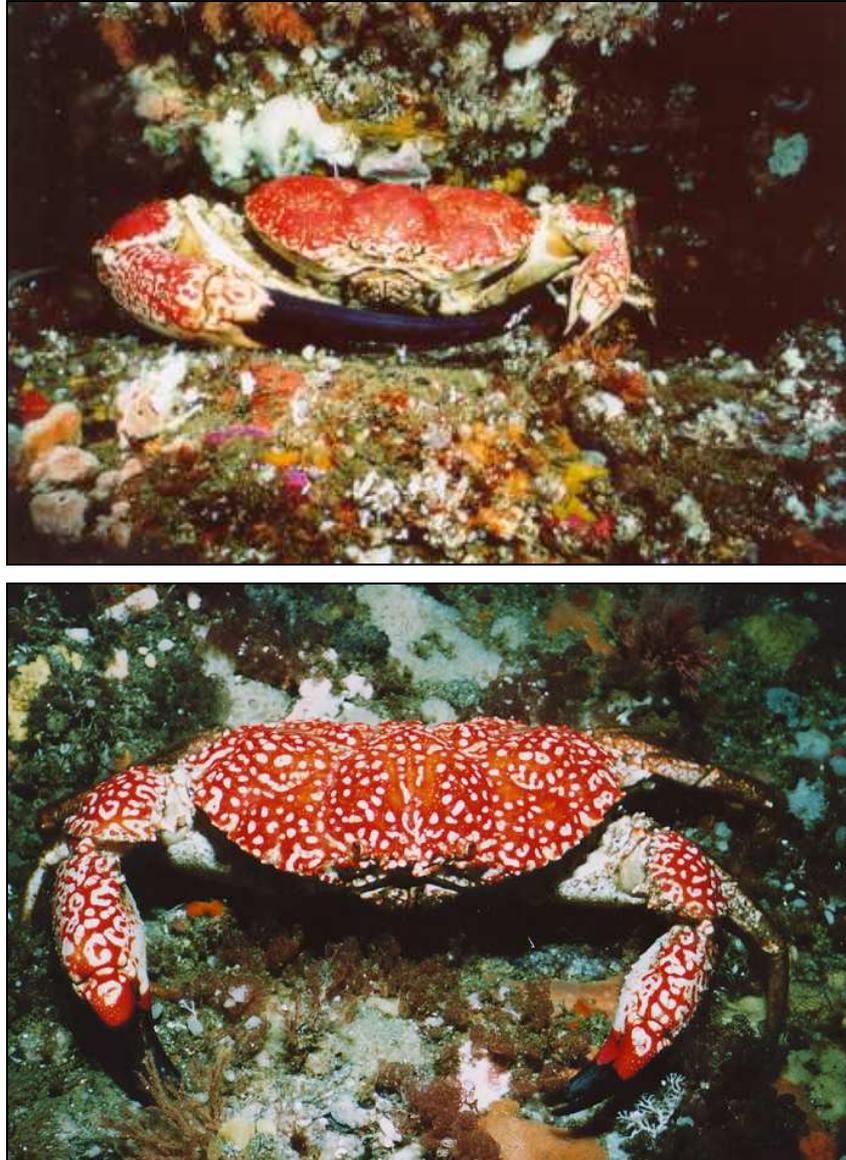
Research was conducted on the reproductive biology of both male and female giant crabs as both are available for harvest. Although both sexes are harvested, males may be subject to higher exploitation as they appear to grow faster and the same minimum legal size limit

is applied to both sexes (Pers. Comm., R. McGarvey, South Australian Research and Development Institute). Also, the open season is effectively reduced for females as they cannot be retained while ovigerous and egg extrusion occurs in May or June, while the season closes at the end of August.

Initial research on the reproductive biology of male crabs identified morphological stages in development which have been observed in numerous other species (Paul, 1992). Three morphological stages of development in male giant crabs were defined by development of the molariform chelae relative to carapace size (Figure 1). The reproductive maturity of these stages was the subject of additional research which involved a preliminary histological study on the formation of spermatophores. Results from this research are discussed in relation to current management of the resource.

Several aspects of the reproductive biology of female crabs was studied including the mating system, sperm storage, and cycles of ovarian development and interactions with the hepatopancreas. Egg production was studied in detail to assess the effect of female size on fecundity, individual egg size and egg composition. This information is required for modelling the impact of fishing on egg production of the population. Although information on the onset of sexual maturity in females is critical for management of the resource, this was not investigated in the current study as it was beyond the scope of available resources. However, where possible, data were collected from different regions of the fishery to provide some information on the extent of spatial effects. Taxonomic affinity between *Pseudocarcinus* and *Menippe* was demonstrated in Chapter 3 and this was useful for evaluating results of research on reproductive biology.

Figure 1. Male (upper) and female (lower) giant crabs *Pseudocarcinus gigas* showing sexual dimorphism in the development of the chelae. The male is large (11 kg) and is in the final morphological stage identified in Chapter 14, termed morphological maturity (photos by Karen Gowlett-Holmes).



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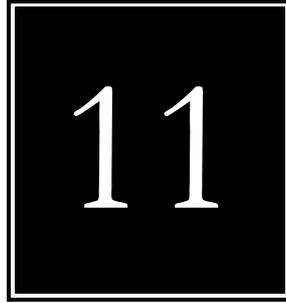
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Reproductive Biology of the Giant Crab
Pseudocarcinus gigas



Gardner, C. 1997. Options for humanely immobilising and killing crabs.
Journal of Shellfish Research. 16(1): 219-224; and as

Abstract

Trials were conducted on the Australian giant crab *Pseudocarcinus gigas* (Lamarck) to evaluate methods to: paralyse by injection (so that no muscular response is observed); paralyse by bath; humanely kill for scientific purposes; and humanely kill for human consumption. Treatments tested were: freshwater bath, chilling, heating, prolonged exposure to air, hypercapnic seawater bath (carbon dioxide addition), 2-phenoxy ethanol bath, magnesium sulphate bath, benzocaine bath, MS 222 bath, chloroform bath, clove oil bath, AQUI-S™ bath, xylazine-HCl by injection, and ketamine-HCl by injection. Xylazine-HCl (16 or 22 mg/kg) and ketamine-HCl (0.025-0.1 mg/kg), administered by injection, appear to be the best techniques for paralysing crabs for short periods. Where injection is impractical, crabs may be successfully paralysed within 30 min by a bath treatment of clove oil (≥ 0.125 ml/l) or AQUI-S™ (≥ 0.5 ml/l). Chloroform (1.25 ml/l; 1.5 h) and clove oil (≥ 0.125 ml/l; ≤ 60 min) baths appeared to kill crabs humanely and are useful options for scientific use, however, clove oil is preferred as chloroform poses a human health risk. Of the methods tested, only clove oil and AQUI-S™ appear promising as treatments for the humane killing of crabs for human consumption.

Introduction

Methods of paralysing crabs can benefit many research situations involving live crabs; procedures may be conducted more efficiently and trauma to the crab is reduced (Oswald, 1977). Where the application is prolonged or the dosage increased, humane killing may result which is desirable for research and commercial uses of crabs. In commercial situations, it is important that quality is not harmed by effects such as autotomy, and toxic chemicals cannot be used. Recent changes to Australian animal cruelty legislation have added another consideration to the commercial killing of crabs: that the crab be killed humanely.

Humane killing involves attempting to inflict as little pain as possible while killing the crab. Pain is a difficult, or perhaps impossible, aspect to measure in animals other than humans,

so it is usually inferred from changes in behaviour which seem to indicate distress (Chapman, 1992; Cook, 1996). These behavioural changes are not apparent when the muscular response is blocked by induced paralysis, so anaesthesia (blockage of pain) is not assured, despite an apparent lack of distress. Likewise, the absence of behavioural indications of distress does not necessarily indicate that killing is painless. Nonetheless, in the absence of methods to quantitatively measure pain, techniques for killing or immobilising animals where distress is apparently reduced are preferred to techniques which produce obvious distress. This study reports the results of trials in temporarily paralysing and killing the Australian giant crab *Pseudocarcinus gigas* (Lamarck) which is large and potentially dangerous, as large males are capable of fracturing a human wrist. Thus, techniques for immobilising giant crabs are also beneficial as they improve handling.

Although numerous methods of temporarily paralysing and killing crustaceans have been documented, many are slow, inconsistent, and appear to cause trauma (Brown et al., 1996). A range of physical and chemical treatments was tested on giant crabs to establish which treatments were effective and economical for this large species, and also to note apparent trauma from treatments. Treatments were evaluated for the following applications: paralysing by injection (appropriate for large crabs); paralysing by bath (appropriate for small crabs); killing for research (toxic chemicals acceptable); and killing for commercial use (safe for consumption).

Materials and methods

Adult giant crabs (*Pseudocarcinus gigas*) were collected from western Tasmania by commercial fishers and ranged from 1 to 7 kg with most between 2.5 and 3.5 kg. Crabs were held in 4 m³ tanks with flow-through seawater and were only used if they exhibited normal avoidance of capture. Treatments were first tested in producing paralysis; crabs were then allowed to recover in tanks with flow-through seawater and were monitored for two days to assess any ill effects. Where the treatment was effective and did not appear to cause pain (see below), further trials were undertaken to establish appropriate dosages for producing temporary paralysis and to assess the treatment for humane killing. In some treatments, the crabs appeared to be severely harmed by the paralysis trial and recovery was not assessed.

Criteria for assessing pain, paralysis, and death

Although pain is impossible to quantify, changes in behaviours of experimental animals have been used to infer perception of pain (Chapman, 1992; Cook, 1996). In these trials, the treatment was considered to have caused pain where crabs dropped limbs (autotomy), tore at their appendages or abdomens, became tensed and rigid, or appeared to have muscle spasms.

Paralysis was considered complete when the abdomen could be easily lifted and chelae (claws) could not be used defensively. Where recovery was to be assessed, crabs were removed from bath treatments before circulation of water over the gills ceased (externally observed by flow of water). Nervous systems of crabs have two centres, the cerebral and the posterior ganglia. Baker (1955) devised a simple system of testing if these centres were functioning, and thus if the crab was alive, by observing the response to stimuli applied to different appendages. Where no response was observed the crab was classed dead. Baker's (1955) system was modified in this study to avoid the use of optical stimuli as giant crabs are deep sea animals and their spectral sensitivity may have been impaired by surface level sunlight after capture (Cronin and Forward, 1988). Consequently, the following tests were used to assess if crabs were dead:

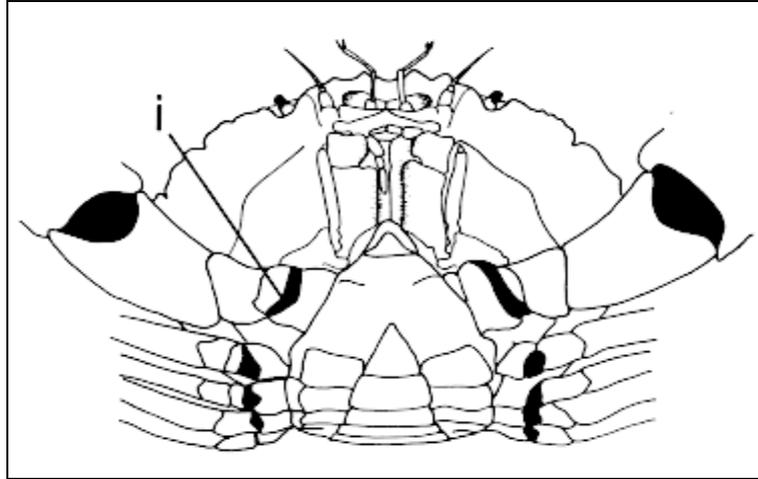
Antennal reaction: The crab does not retract the first antennae when the distal end is touched (cerebral ganglion).

Maxilliped reaction: The third maxilliped (mouth frame) can be moved outwards from the body and is not drawn back (posterior ganglion).

Treatment strategy

Bath treatments were conducted in individual tanks of 20 l with continuous aeration (except hypercapnic seawater treatment). These tanks were filled with water from the larger holding tanks so that salinity (35 ppt) and temperature (range 9-13°C) were not altered. Injections were made intravascularly through the coxal arthropodial membrane of a cheliped (Fig. 1). Doses by injection were made up to a maximum of 2 ml as volumes greater than this were considered difficult to administer.

Figure 1. Ventral surface of a giant crab showing the site of intravascular injections (i). Treatments were introduced with the needle tip only slightly below the joint membrane to avoid penetrating muscle tissue.



Physical methods tested for paralysing were: freshwater bath; chilling (5°, 2°, and -1.5°C); heating (17°, 18°, 20°, and 24°C); and prolonged exposure to air.

Chemical methods tested as baths were: hypercapnic sea water (CO₂ bubbled into bath through a graphite airstone); 2-phenoxy ethanol (maximum 1 ml/l); magnesium sulphate (35 g/l); benzocaine (0.08 and 0.24 g/l, stock solution of 40 g/l benzocaine in acetone); MS 222 (tricaine methane sulphonate; 0.5 g/l); chloroform (1.25 and 2.5 ml/l in water and agitated); clove oil (0.015-1.0 ml/l, dissolved in ethanol); and AQUI-S™ (Fish Transport Systems™ New Zealand)(0.015-1.0 ml/l).

Chemical methods tested by intravascular injection were: xylazine-HCl (0.6-22.0 mg/kg; as 2% solution, Rompun-Bayer™); and ketamine-HCl (0.01-0.05 mg/kg; as 10% solution, Ilium-Troy™).

Of these treatments, four were tried for humane killing: freshwater bath; chilling; chloroform; and clove oil. Chilling was achieved by the addition of ice slurry to 100 l tanks held in a refrigerated room. Heating was achieved by placing immersion heaters in 100 l tanks.

The number of crabs used for experiments varied (Table 1) as the response of individual crabs to some treatments was so poor at very high doses that further trials were not warranted. Other trials were conducted opportunistically with industry so large numbers

were used, such as with prolonged exposure to air where 55 animals were monitored. The opportunistic nature of the trials prevented concurrent experimentation.

Results

None of the physical methods appeared to be suitable for producing temporary paralysis as they were either ineffective or they appeared to distress the crab (Table 1). There were also practical problems with the physical methods that rendered them unsuitable. Crabs were only affected by cold water temperatures close to freezing. Consequently, regular monitoring was required for the entire 2 h period needed to partially paralyse crabs, to ensure the water did not freeze. Also, crabs revived when the temperature rose so they recovered rapidly during experimental procedures.

Table 1. Results of trials to assess the use of treatments for paralysing.

Method	Time to paralyse	Indication of stress / revival
Fresh water bath (n=10)	Immediately became rigid and easily handled	Motionless and rigid for 10 min then became very active. Autotomy occurred and crabs tore at their abdomens and walking legs. No revival was attempted.
Chilling: 5°, 2°, and -1.5°C (n=10, 10, and 60 respectively)	Unaffected after 14 h at 5° and 2°C. Mild paralysis in 2 h at -1.5°C (retained antennal, maxilliped, and limb movement).	Active at 5° and 2°C. Ice formed at -1.5°C so the last segments (propodus and dactylus) of the limbs to become frozen. All recovered within 45 min on return to 10°C and appeared healthy after 48 h. No effects of freezing were seen although tissue damage is likely.
Heating: 17°, 18°, 20°, and 24°C (n=3 for all treatments)	Appeared unaffected at all temperatures tested except 24°C. Mild paralysis at 24°C in 2 h.	Appeared uncomfortable and attempted to climb from the container as temperature rose. Although apparently paralysed at 24°C, limbs constantly twitched. Recovery was rapid and crabs appeared healthy after 48 h.
Prolonged exposure to air (n=55).	No effect at 4 or 8 hours. Less active after 14 h (8-12°C).	Crabs were vigorous after 14 h. Appeared healthy after 48 h in seawater.
Hypercapnic seawater (n=3)	Mean = 44 min (range 33-60 min).	Thrashed and crushed limbs. Although immobile, they were tensed and became rigid when returned to fresh seawater to recover. Some autotomy. Slow recovery, incomplete after 48 h.

Method	Time to paralyse	Indication of stress / revival
2-phenoxy ethanol: 1 ml/l (n=1)	No effect after 14 h in saturated solution.	No apparent effect. Healthy 48 h after return to seawater.
MgSO ₄ : 35 g/l in fresh water; 35 g/l in sea water (n=6)	No effect at 4 h	Active 48 h after return to seawater. Cost of chemicals made trials with higher doses unviable.
Benzocaine: 0.08 g/l (n=1), and 0.24 g/l (n=3).	2 h at 0.08 g/l, Mean = 45 min, range 20-55 min at 0.24 g/l	Apparent distress, tensed and rigid when immobilised. Autotomy occurred. Rapid recovery, crabs mobile within 10 min and healthy after 48 h.
MS 222: 0.5 g/l (n=1)	No effect after 4 h.	One tenth this dose (20 min) is used for killing finfish (Clark,1990). Trials at higher doses were unviable due to chemical costs.
Chloroform: 1.25 ml/l (n=3), and 2.5 ml/l (n=3)	60 min for all crabs	No apparent distress. Slow recovery, crabs still sedated after 24 h although apparently normal after 48 h.
Clove oil: 0.015 ml/l - 1.0 ml/l (Fig. 2; n=18).	Ineffective at 0.015 ml/l. Time at higher doses (≥ 0.03 ml/l) ranged from 85-16 min.	No apparent distress. Rapid recovery (at 0.125 ml/l) and active 2.5 h after return to seawater. Appeared healthy after 48 h.
AQUI-ST TM : 0.015 ml/l - 1.0 ml/l (Fig. 2; n=14).	Ineffective at ≤ 0.06 ml/l. Effective at ≥ 0.125 ml/l in 70-20 min.	No apparent distress. Rapid recovery and active 2.5 h after return to seawater. Appeared healthy after 48 h.
Xylazine-HCl: 0.6, 1.2, 5.6, 11.2, 16, and 22 mg/kg. (n=6)	Ineffective ≤ 11.2 mg/kg. Effective in 3-5 min at 16 and 22 mg/kg.	No apparent distress. Rapid recovery: 25 min at 16 mg/kg, and 45 min at 22 mg/kg. Appeared healthy at 48 h.
Ketamine-HCl: 0.01, 0.025, 0.05, and 0.1 mg/kg. (n=8)	Ineffective at 0.01 mg/kg; effective at 0.025, 0.05, and 0.1 mg/kg in 15-45 s at all concentrations.	Cheliped became rigid immediately after injection, the other cheliped was thrashed, then relaxed. Recovery took 8, 15, 25, and 40 min at 0.01, 0.025, 0.05, and 0.1 mg/kg respectively. Apparently healthy after 48 h.

None of the physical methods appeared suitable for humane killing (Table 2). Aside from ethical problems, prolonged exposure to air would take longer than 48 h and was not attempted. Heating appeared to cause distress to the crabs and killing by a gradual increase in temperature was not attempted. While a fresh water bath killed crabs, it did not appear to be a humane method. Chilling was ineffective.

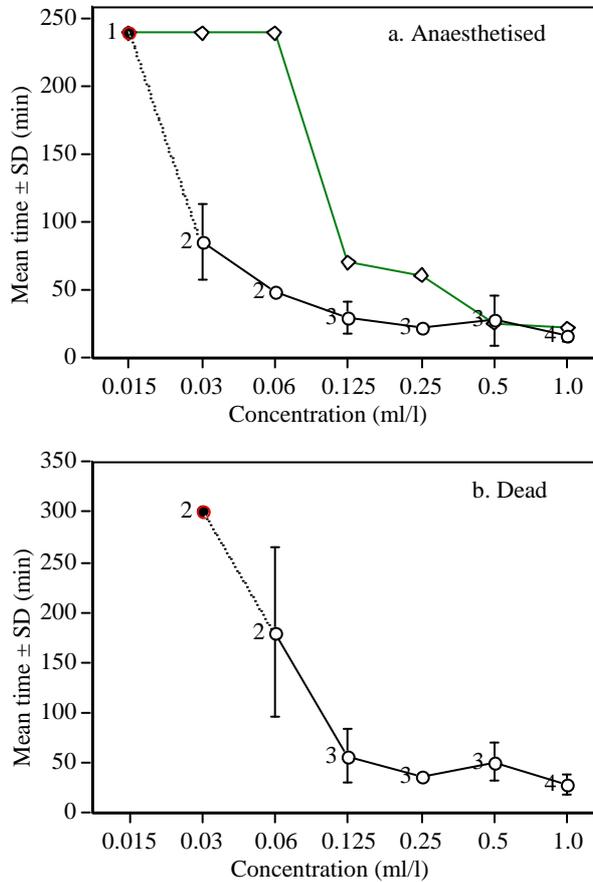
Table 2. Results of trials to assess the use of treatments for humane killing.

Method	Time to death	Comments
Fresh water bath (n=10)	Mean = 4.6 h (range = 3-5 h)	Apparent distress (see Table 1).
Chilling: 2° and -1.5°C (n=10 and 60 respectively).	2°C group alive at 24 h. -1.5°C alive at 6 h.	Appendage reactions occurred at -1.5°C and limb movement was retained. Activity increased rapidly on warming.
Chloroform: 2.5 ml/l (n=3)	1.5 h in all crabs.	No apparent distress.
Clove oil: 0.06 ml/l - 1.0 ml/l (Fig. 2; n=17).	180-28 min, varying with concentration.	No apparent distress.

Of the chemical bath treatments tested, only chloroform, clove oil and AQUI-S™ produced what appeared to be relaxed temporary paralysis. Crabs treated with chloroform had poor recovery after paralysis and took longer to die compared with clove oil treatments. Chloroform solutions become saturated at approximately 6.17 ml/l at 10°C which is considerably higher than the concentrations used in this study (1.25 and 2.5 ml/l). Consequently, the similar times for crabs to become paralysed in the two concentrations of chloroform cannot be attributed to saturation of solution. The optimal concentration of clove oil for both paralysing and killing was 0.125 ml/l as stronger doses did not produce faster effects (Fig. 2; Tables 1 and 2). Optimal concentration of AQUI-S™ for paralysing was 0.5 ml/l (Fig. 2).

Both of the temporary paralysis treatments administered by injection were effective and acted rapidly. Unlike xylazine-HCl, where paralysis appeared to be painless, ketamine-HCl appeared to produce distress in the crabs although this was only momentary as paralysis occurred within 45 seconds.

Figure 2. Effect of concentration of clove oil (circles) and AQUI-S™ (diamonds) on time taken to paralyse (upper) and to kill (lower) giant crabs *Pseudocarcinus gigas*. Solid symbols represent trials terminated before paralysis or death occurred. Value labels next to means are numbers of crabs used. Tests with AQUI-S™ used only two crabs at each concentration so no error values are presented.



Discussion

Several of the treatments tested in producing paralysis were rejected as they were ineffective or because the dose required was too large for practical purposes: prolonged exposure to air, 2-phenoxy ethanol, magnesium sulphate, and MS 222. MS 222 is used widely in paralyzing finfish (Clark, 1990) and was recommended by Ahmad (1969) for amphipods although several other studies have confirmed that it is ineffective in decapods (Foley et al., 1966; Oswald, 1977; Brown et al., 1996). MS 222 is believed to act at the nerve membrane affecting sodium conductance in finfish (Ryan, 1992) and the ineffectiveness of MS 222 in decapods may be related to the absence of acetylcholine at these terminals (Oswald, 1977). The large amount of magnesium sulphate required to

paralyse large decapods was considered impractical in this study and the same conclusion was drawn by Foley et al. (1966). For smaller animals, and thus smaller bath volumes, the technique may still have value (Gohar, 1937).

Temporary paralysis

Although many of the methods tested in this trial produced paralysis, some were only partially effective and others appeared to be unsuitable due to evidence of pain or distress during relaxation. Hypercapnic seawater has been recommended for paralysing reptantian decapods (Smaldon and Lee, 1979) and was also effective with *Pseudocarcinus gigas*.

However, the technique resulted in autotomy and thrashing of limbs in *P. gigas* which indicates paralysis was not painless. Smaldon and Lee (1979) report that *Crangon* spp. and *Palaemon* spp. also exhibit distress when placed in hypercapnic seawater. Oswald (1977) assessed the use of benzocaine to temporarily paralyse *Cancer pagurus* L. and *Carcinus maenas* (L.) by injection and observed no effect. Benzocaine is widely used to produce paralysis in finfish and abalone research where it is administered as a bath, as was done in this study with *P. gigas*. The bath solution of 0.24 g/l benzocaine produced paralysis in *P. gigas* although there was some indication of pain, as with hypercapnic sea water.

None of the physical methods tested produced relaxed paralysis in *Pseudocarcinus gigas*. A gradual increase in temperature was described as an effective and humane method of anaesthetising and killing large crustaceans by Gunter (1961) and was subsequently recommended by Smaldon and Lee (1979). This method was effective at paralysing *P. gigas* although animals showed signs of distress, contrary to the observations of Gunter (1961). Baker (1955) also tested the response of crabs to gradual increase in temperature and concluded that the method was unacceptable on humanitarian grounds as indications of distress, such as autotomy, occurred unless the crab was already in poor health. Following publication of Gunter's (1961) conclusion on the use of gradual heating, objections were raised to the method on the basis that there was no evidence of an anaesthetic effect (Baker, 1962; Schmidt-Nielsen, 1962).

Current Australian guidelines for the killing of crabs for scientific purposes recommend chilling as a humane method for paralysing crabs, which can then be killed by sectioning to destroy ganglia (Reilly, 1993). While chilling may be useful for reducing activity in tropical or warmer water species, it was ineffective as a paralysing technique for the temperate

Pseudocarcinus gigas. Freezing inevitably results in death in *P. gigas* but is of limited use in research as tissues are no longer suited for many applications, such as histology. Chilling has drawbacks which affect its use in all species, it is generally a slow and inconsistent technique (Brown et al. 1996) and it is ethically dubious as it involves subjecting the crab to conditions which it would normally avoid (Schmidt-Nielsen, 1962).

Killing crabs by freshwater bath is one of the most widely used methods in Australia; it is termed “drowning” and is popularly considered a humane technique. Of all the treatments tested for producing paralysis, “drowning” in a freshwater bath appeared to cause greatest trauma as crabs dropped most limbs. Similar conclusions were drawn by Baker (1955) for *Cancer pagurus*.

Both xylazine-HCl and ketamine-HCl were particularly effective and produced paralysis in less than 5 min in *Pseudocarcinus gigas*. Xylazine-HCl produces relaxation by central blockade of interneurons in the mammal (Oswald, 1977) but the mode of action in crustaceans is unknown. Although injection of ketamine-HCl appeared to cause localised excitation, the apparent distress was only momentary as most crabs became paralysed within 45 s. Ketamine-HCl is effective in paralysing crayfish *Orconectes virilis* (Hagen) although the reported dose rate by intramuscular injection (90 mg/kg body weight; Brown et al., 1996) was considerably higher than that required by *P. gigas* by intravascular injection (0.025 mg/kg). The duration of paralysis in *P. gigas* treated with ketamine-HCl (8-40 min) also differed from *O. virilis* (>1 h; Brown et al., 1996). Dose rates of xylazine-HCl required to temporarily paralyse *P. gigas* (22 mg/kg) were less than reported values for *Cancer pagurus* and *Carcinus maenas* (70 mg/kg; Oswald, 1977), although duration of paralysis was similar (around 45 min for all species). Two other chemicals, reportedly effective in other decapods, were not tested on *P. gigas* but warrant mention: procaine-HCl is reported to produce prolonged paralysis of 60 min in *C. pagurus* and *C. maenas* (Oswald, 1977); and lidocaine-HCl is reported to produce shorter-duration paralysis of 20-25 min in *O. virilis* (Brown et al., 1996).

Where the experimental animals are very small, injection is less practical than bath treatments; chloroform (>1.25 ml/l), clove oil (>0.125 ml/l), and AQUI-S™ (>0.5 ml/l) produced relaxed paralysis by this method. Chloroform has been used for many decades to kill decapods that subsequently remain relaxed for museum storage (Gohar, 1937; Mahoney, 1966), but the use of chloroform to produce temporary paralysis has been less well documented. Foley et al., (1966) attempted to paralyse *Homarus americanus* Milne

Edwards, by chloroform bath but concluded that too large a dose was required for practical purposes. The chloroform bath treatment was effective and inexpensive with *P. gigas* although there are important limitations: the time to onset of paralysis (60 min) and recovery from paralysis (>24 h) was protracted; and chloroform poses a serious health risk to humans due to its hepatotoxicity. Clove oil was the superior bath treatment in respect of both time to onset of paralysis (as rapid as 16 min) and recovery (2.5 h). Clove oil is inexpensive and is likely to be effective over a wide range of species given that it also produces paralysis in rabbitfish *Siganus lineatus* (Cuvier and Valenciennes)(Soto and Burhanuddin, 1995). AQUI-S™ produced similar results to clove oil but may have limited application in paralyzing crabs for scientific purposes as higher doses were required. A potentially useful observation from the clove oil trials is that embryos of ovigerous giant crab females did not appear to be harmed by the treatment and continued through development to hatch.

Bath treatments of clove oil and AQUI-S™ may have commercial application to improve seafood quality and to reduce mortality during live transport. Reduction of stress during transport and prior to harvest is known to increase quality of seafoods (Lowe et al. 1993) and to decrease transport mortality (Paterson et al., 1994).

Humane killing

Two of the treatments widely used in Australia for killing crabs were either ineffective or appeared to cause suffering: chilling; and freshwater bath. Both chloroform and clove oil were effective and crabs did not appear distressed by the treatments. As discussed earlier, chloroform has long been used to kill crustaceans for museum collections (Gohar, 1937; Mahoney, 1966), where it is important that the animal does not autotomise limbs. Chloroform is hazardous to humans due to its hepatotoxicity so it should only be used where all fumes can be removed.

Unlike chloroform, clove oil has potential to be used for killing animals destined for human consumption although the long term chronic effects on humans are not yet known (Soto and Burhanuddin, 1995). Cloves have been shown to delay rancidity of seafood (Joseph et al., 1989) although the oil has a strong smell which can alter the taste of the meat. AQUI-S™ is approved for use with food fish in New Zealand with zero withholding time; it produced paralysis in giant crabs although higher doses were required than with clove oil.

Unlike clove oil, AQUI-S™ does not have a strong odour so is less likely to affect the taste of the meat. Further trials are warranted to assess the use of AQUI-S™ in the killing of crabs and to assess the effect on meat quality utilising human sensory evaluation.

Conclusions

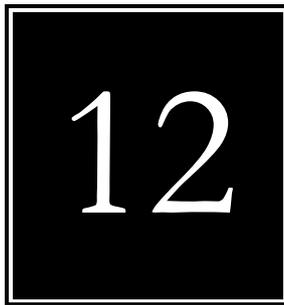
Several methods of paralysing and killing crabs are clearly suitable for research situations. The two injectable treatments, xylazine-HCl (16 or 22 mg/kg) and ketamine-HCl (0.025-0.1 mg/kg), have much potential in research to reduce trauma to the crab, increase work efficiency, and reduce risk to humans from the chelae. Xylazine-HCl and ketamine-HCl act rapidly so they can be readily applied in most research situations. Where injection is impractical, clove oil (≥ 0.125 ml/l) or AQUI-S™ (≥ 0.5 ml/l) baths were effective in paralysing crabs although they both required around 20 min to act at optimal doses. A clove oil (≥ 0.125 ml/l) bath appeared to kill crabs humanely and is a useful option for research; crabs did not appear to experience trauma by this method and there was no limb loss or other damage. Of the methods tested, only clove oil and AQUI-S™ appear promising as treatments for the humane killing of crabs for human consumption, however both required long periods (≥ 28 min) to act which may limit their commercial application. Baker (1955) described a method for killing crabs for human consumption by sticking, which involves piercing the nerve ganglia with an awl. This was not attempted with *P. gigas* as the sternum is exceptionally thick and difficult to pierce. However, in other species sticking is likely to be a useful, and rapid, technique.

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Non-lethal Imaging Techniques For Crab Spermathecae



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Abstract

Techniques for collecting information on spermathecae without dissection were evaluated on the giant crab *Pseudocarcinus gigas*. Techniques tested were: biopsy, ultrasound, conventional x-radiography, computerised tomography (CT) scans, and magnetic resonance imaging (MRI). Attempts at biopsy and ultrasound imaging were unsuccessful. Spermathecae were imaged by x-radiography, although resolution was poor, suggesting that it can be applied only to gain general information, such as determining whether mating has occurred. High resolution images were produced with CT and MRI. Resolution by MRI is of such detail that internal structure of spermathecae is imaged. Nonlethal techniques allow animals to be used repeatedly, which permits monitoring of changes during mating, sperm storage, and extrusion.

Introduction

The more advanced brachyuran crabs possess paired spermathecae which store sperm between mating and fertilisation. The function of these organs is of biological interest as layered storage of separate ejaculates can affect paternity of offspring (Koga et al., 1993; Sévigny and Sainte-Marie, 1996). Spermathecae have also been the subject of fisheries-oriented research where they provide a means of measuring the occurrence of copulation, which may be affected when males are harvested. The ability of females to utilise stored sperm to fertilise separate broods may buffer the impact of male-only fisheries (Paul and Paul, 1992). Examination of spermathecae for this range of research has traditionally involved dissection and analysis (e.g. histology) of the spermathecae, or assessment of the fertilisation rate after eggs are extruded.

This paper reports results of trials on a range of nonlethal techniques tested on the Australian giant crab *Pseudocarcinus gigas* (Lamarck). The objective was to develop techniques to examine spermathecae before ovulation, so infertility could be linked to either unviable sperm if spermathecae were full, or depleted reserves if spermathecae were empty. Nonlethal techniques allow for the repeated “sampling” of the

spermathecae from the same individuals. In this way, changes can be tracked from mating, through storage, to extrusion. Nonlethal techniques tested were: biopsy, ultrasound, conventional x-radiography, computerised tomography scan (CTS), and magnetic resonance imaging (MRI).

Materials and methods

Female giant crabs *Pseudocarcinus gigas*, greater than 3.0 kg, were collected by commercial fishers from depths in the range of 300–380 m off the east coast of Tasmania (41°15'S;148°40'E) in May 1994. Crabs used for trials with conventional x-radiography were alive and restrained by claw ties; in all other trials, crabs were killed in baths of clove oil in sea water (0.125 ml/l; Gardner, 1997). Following imaging, crabs were dissected to relate imaged structures with actual tissues. In the case of CT and MRI, crabs were frozen and sliced by band-saw (5 mm sections) to duplicate the orientation of the images which were collected as transverse slices.

Attempts were made to biopsy contents of spermathecae using Pipelle de Cornier™ human endometrial biopsy catheters on dissected females with the gonoduct exposed. This allowed the biopsy tube to be observed as it was passed through the gonopore and along the gonoduct. Conventional x-radiography images were taken with standard veterinary equipment (AtomScope™ 100P). Ultrasound imaging was attempted with an ATL-8™ ultrasound using 7, 5, and 3.5 Mhz transducers. Computerised tomography scanning was performed with a GE™ scanner and MRI was performed with a 1.5 Tesla, Picker™ magnetic resonance imager.

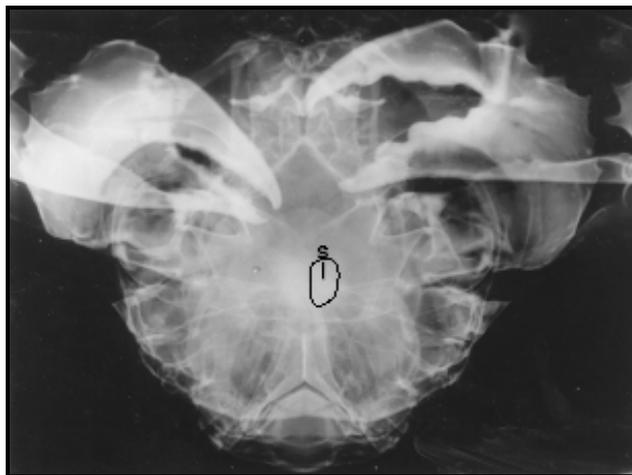
Results

The collection of stored sperm by biopsy was not possible without injuring the crab. Although the gonopore of giant crabs is large, it is calcified during intermoult and force was required to introduce a biopsy tube. Once within the lumen of the gonoduct, the tube tore through the delicate walls rather than bending with the curvature of the duct.

Ultrasound penetrated the exoskeleton, but there was insufficient definition to discern the spermathecae. Factors contributing to the poor results with ultrasound appeared to be the echoing from internal calcified plates and the lack of sufficient acoustic difference between the spermathecae and surrounding tissue.

Spermathecae were successfully imaged using conventional x-radiography, although images were very unclear (Fig. 1). Optimal exposure was relatively high in order to penetrate the carapace (3 LV, 0.1 SIC). Consequently, most tissue definition was lost and the boundary layer between the spermathecae and surrounding tissue became blurred.

Figure 1. Image of female *Pseudocarcinus gigas* produced by conventional x-radiography showing spermathecae (S) as a pale region.



High quality images of the spermathecae were made using both MRI and CT scans so that details of substructure could be detected, especially with MRI (Fig. 2). Spermathecae were dissected after imaging. The dark bands detected by imaging related to separate areas of seminal plasma which bounded areas of spermatophore deposit, possibly from separate ejaculates. The optimal MRI setting for giant crabs was considered to be scan protocol 14. Images produced by CTS were not as defined as those by MRI, but additional information can be gained by this technique, since internal calcified plates are detected (Fig. 3). Both MRI- and CTS- scanned images are collected as a series of “slices” through the specimen. Thus, 3-dimensional surface profiles can be composed and the resulting 3-dimensional images can be reconstructed, and cored or sliced along any plane (Fig. 4).

Figure 2. Dorsal (upper) and longitudinal (mid) images of a female *Pseudocarcinus gigas* produced by magnetic resonance imaging (MRI) showing high resolution of spermathecae (S). Schematic (lower) is of the longitudinal image produced by MRI. Separate regions (EJ1, EJ2, and EJ3) can be seen within the spermatheca which may relate to different ejaculates.

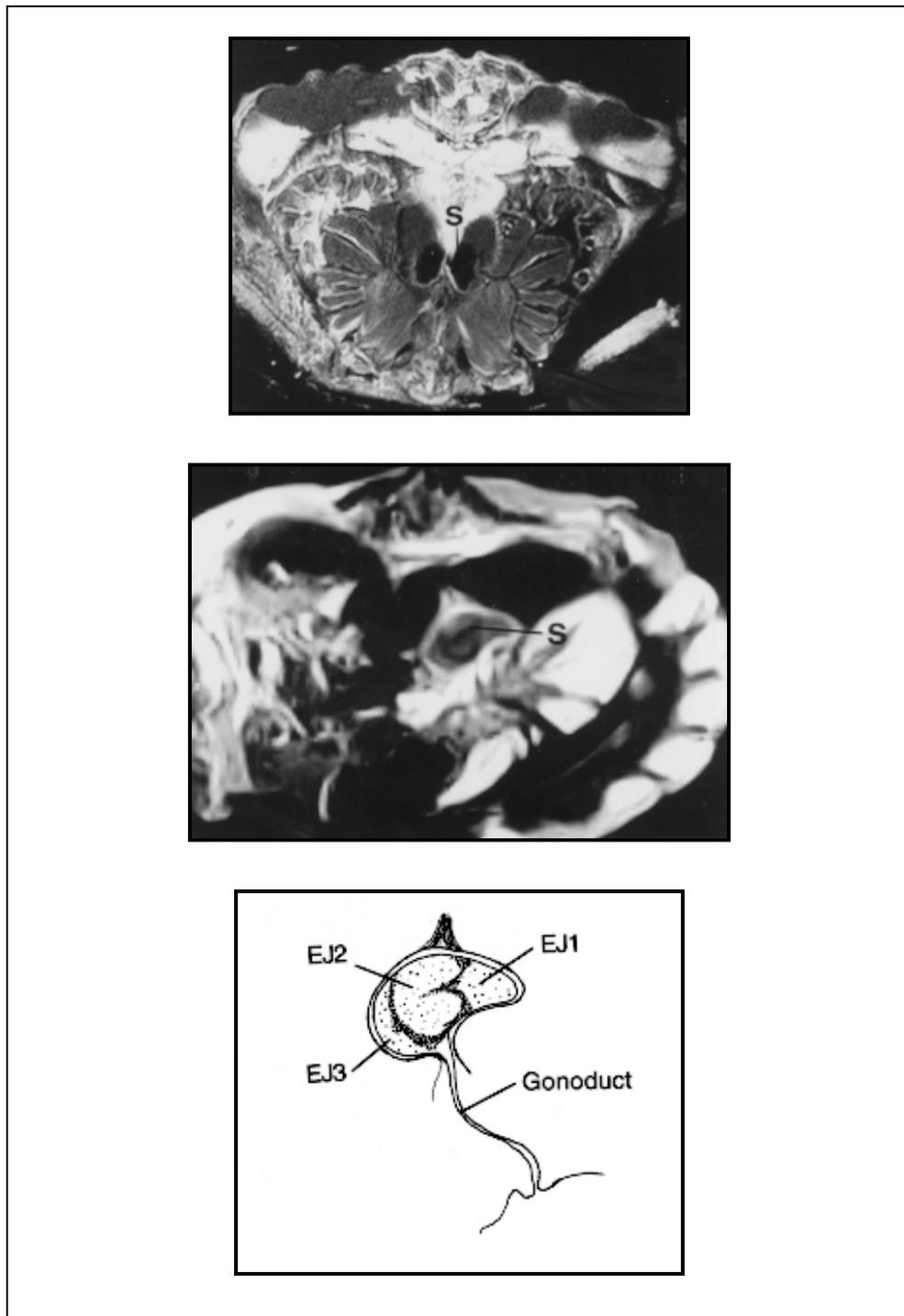


Figure 3. Dorsal (upper) and longitudinal (lower) images of a female *Pseudocarcinus gigas* produced by computerised tomography (CT) showing high resolution of spermathecae (S). Unlike MRI, CT is sensitive to calcified structures and the carapace is seen as a white band.

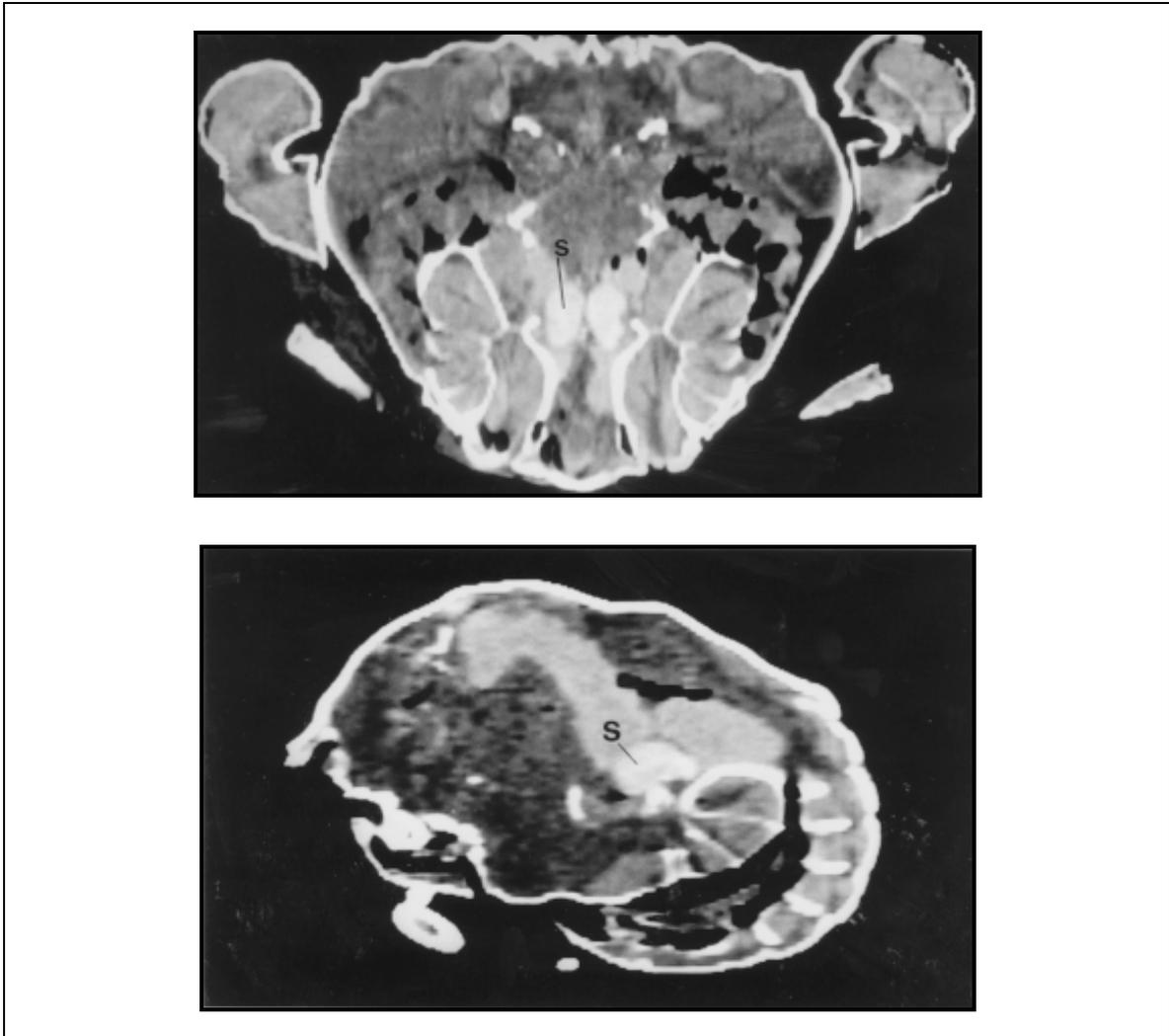
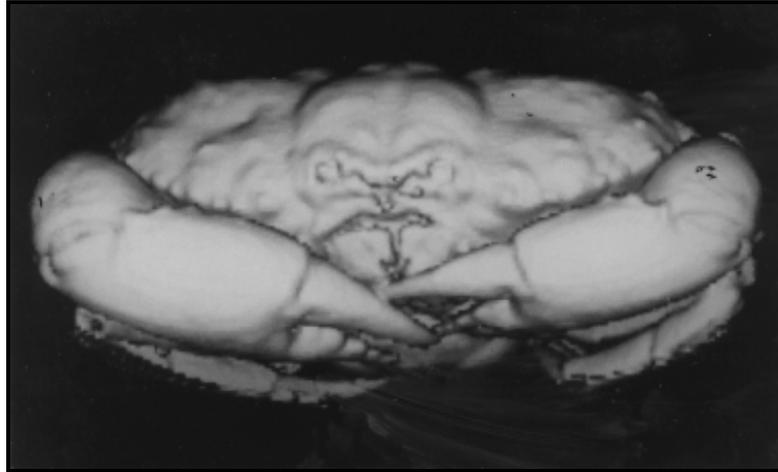


Figure 4. Three dimensional surface image of female *Pseudocarcinus gigas* reconstructed from CT scans. Both MRI and CT scans can be processed into three dimensional images and "sliced" along any plane.



Discussion

The thick exoskeleton of *P. gigas* impaired the use of both biopsy sampling and ultrasound. No entry location for the biopsy could be found other than via the gonopore, which was unsuitable, since the gonoduct tore when a biopsy tube was introduced. Ultrasound has been used in research of organisms with soft bodies, such as marine mammals and fish (Gales and Burton, 1987; Bonar et al., 1989), but it appears to be unsuited to crustaceans.

Conventional x-radiography produced relatively poor images, since the boundaries between the spermathecae and surrounding tissue were blurred. Consequently, the exact size and form of the spermathecae could not be determined. *Pseudocarcinus gigas* have particularly thick carapaces, around 3.0 mm in relatively x-ray opaque larger females. The definition of spermathecae by conventional x-radiography may be greater in species with a thinner carapace. If higher resolution can be achieved, x-radiography may have application where the objective is simply to determine whether insemination has occurred. This can be important in experiments such as those reported by Koga et al. (1993) and Sainte-Marie and Lovrich (1994), where female crabs were dissected to confirm mating. The technique may also have application in studies assessing repeated spawning, using stored sperm where it is necessary to determine whether sperm reserves have been depleted. Conventional x-

radiography is relatively inexpensive (around \$US10 per exposure and several crabs can be x-rayed on each plate), and more readily available than the other feasible techniques, CT and MRI scans.

Of the methods tested, only CT and MRI produced clear images of the spermathecae so that size could be measured and some internal structure viewed. The calcified exoskeleton did not interfere with imaging in either method. The shell was effectively transparent to MRI, since this technique relies on resonance of hydrogen nuclei (protons) which tend to be in low concentration within calcified tissues (Young, 1984). Resolution of internal structure was greater with MRI than with CT, and separate regions could be discerned which may relate to separate sperm deposits. Several authors have made observations based on separate sperm deposits in work investigating sperm storage and competition in brachyurans (e.g., Paul, 1984; Diesel, 1989; Sévigny and Sainte-Marie, 1996). The technique of MRI provides the option of measuring or viewing contents of the spermathecae before, or perhaps even during, mating. Although logistically difficult, MRI may also have application in observing changes to spermathecae during the process of extrusion and fertilisation. An additional benefit from computerised scanning methods is that 3-dimensional plots may be generated, either of the external surface structure (CT), or of the internal boundary between shell and soft tissues (MRI).

Although CT scans were of lower resolution than MRI, additional information is gained as calcified structures are detected. Where calcified structures are the subject of research, such as in the examination of skeletal growth of corals, CT scans are valuable (Logan and Anderson, 1991). In crustaceans, a benefit of imaging internal calcified structures is in the location of tissues, as calcified plates in the stomach and between limb muscles can serve as landmarks.

Practical considerations in the use of MRI and CT imagery include the costs of the procedures and the restraint of the crabs to prevent movement during imaging. Both procedures are usually available from medical imaging facilities and fees for research use vary widely. *Pseudocarcinus gigas* is a highly valued crustacean, with individuals wholesaling at around \$US100. With *P. gigas*, and other highly valued crustaceans, costs of imaging are offset by the reduced purchase of specimens, since sampling is nonlethal and the same individuals may be used repeatedly. While crabs were dissected after imaging in this study, this would not usually be necessary. Further reduction in costs can be made by scanning more than one individual simultaneously and this also increases the rate of specimen

processing. In CT-scanning undertaken subsequently to this project, groups of 3 giant crabs were scanned simultaneously to examine ovarian development, and it was possible to process over 50 crabs per hour. Crabs must be motionless during scanning so paralyzing agents such as xylazine-HCl, ketamine-HCl, procaine-HCl, or clove oil should be used (Oswald, 1977; Gardner, 1997).

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*Spermatogenesis and the Reproductive
Tract of the Male Giant Crab*
Pseudocarcinus gigas

13

Abstract

The reproductive tract of the male giant crab consists of paired elongate testes, located immediately below the hypodermis of the carapace, and convoluted vas deferens. The vas deferens consists of the anterior vas deferens (AVD; white), the mid-vas deferens (MVD; white), the posterior vas deferens (PVD; transparent), and the ejaculatory duct (ED). The ejaculatory duct exits at the ventral base of the fifth pereiopod. Sperm cells are produced in the testis and are carried by the seminiferous tubule to the AVD. Luminal secretions produced in the anterior portion of the AVD condense around clumps of sperm cells to produce a single, thin, envelope layer around the spermatophore. Spermatophores are ovoid, non-pedunculate, and contain numerous, closely packed sperm cells. A granular luminal substance is secreted in posterior portions of the AVD and this secretion, plus spermatophores, are stored in the MVD. The PVD and ED contain an agranular secretion which contains few, or no, spermatophores. This luminal substance in the PVD is likely to serve as the sperm plug in the spermathecae of females. Investigation of physiological maturity of giant crabs should focus on the presence of spermatophores in the MVD as this is the main site of spermatophore storage.

Introduction

Current management arrangements for the giant crab fishery include a minimum size limit, which is the same for both sexes, and restrictions on the taking of ovigerous females. Both these practices promote the harvest of males rather than females so there is concern that sperm limitation may occur in the residual population. In response to this concern, research has been undertaken on the sexual maturation of male giant crabs. This chapter contributes to that research and describes the anatomical and histological structure of the reproductive system.

Numerous other papers have described the histology of the male reproductive tract of various crab species. However, there is species-specific variation in the gross anatomy of the tract, form of the spermatophore, luminal secretions, and storage location of

spermatophores within the vas deferens (Uma and Subramoniam, 1984). This information is required to assess accurately the onset of physiological maturity, as determined by the production of spermatophores (Paul, 1992).

Materials and methods

Male giant crabs *Pseudocarcinus gigas* were obtained in March and July 1996 from commercial fishers who had captured the crabs at approximately 350 m depth off eastern Tasmania. Fifty-eight specimens were examined and these ranged in size from 81 to 208 mm carapace length. Crabs were killed in a bath of clove oil in seawater at 0.125 ml/l (Gardner, 1997). The reproductive tracts were carefully removed and examined for their gross anatomy and morphology. Tissue was preserved in 10% neutral buffered formalin for at least a week before processing and sectioning by standard paraffin histology. Sections were cut at 5 μ m and stained with haematoxylin and eosin. As luminal secretions in the vas deferens tended to wash off during staining, slides were coated with albumin.

Results

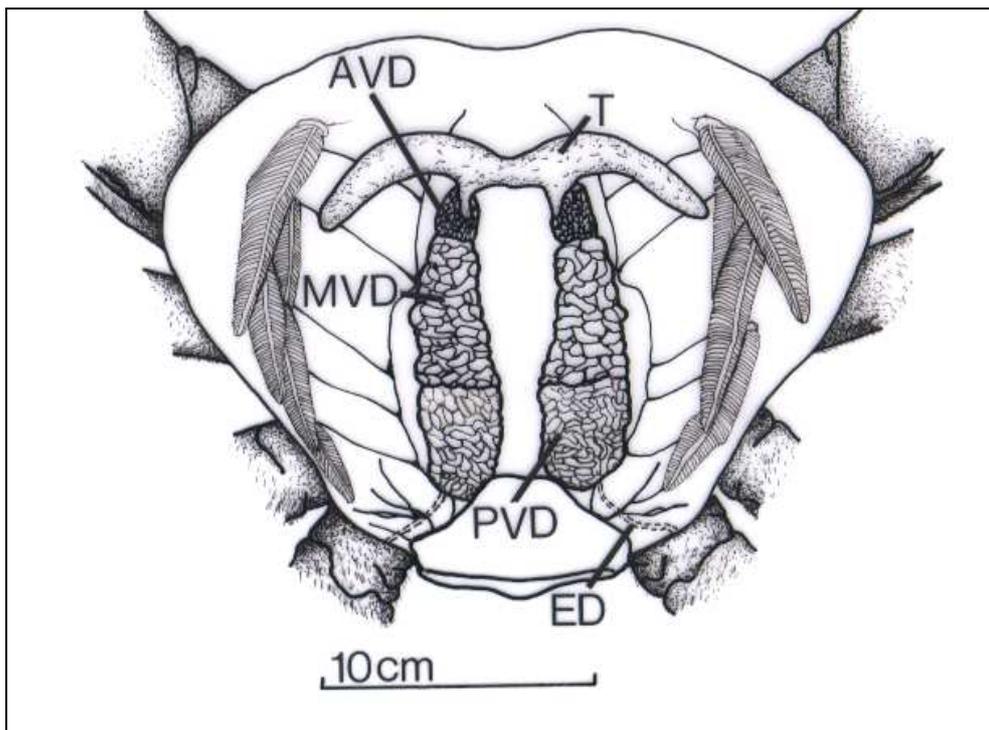
General morphology

The male reproductive tract consists of paired testes and elongate, convoluted vas deferens (Figure 1). The paired testes are white and are interconnected medially by a commissure. They lie dorsal to the hepatopancreas and immediately below the hypodermis of the carapace, extending forwards from the middle of the antero-lateral border of the carapace, lateral to the stomach, and ending medially to the anterior vas deferens. The vas deferens is divided into 4 regions: the anterior vas deferens (AVD); mid vas deferens (MVD); posterior vas deferens (PVD); and ejaculatory duct (ED).

The AVD is highly coiled into a roughly spherical mass and is an opaque dull white. It is approximately 1.5-2 mm in diameter. The MVD is more loosely coiled and of greater

diameter (approximately 5 mm) than the AVD. This part is white and is the largest portion of the system, being marginally larger than the PVD. The PVD is transparent rather than white, clearly differentiating it from the MVD. It is swollen, highly convoluted, and around 3 mm in diameter. It is easily ruptured and extends directly to the ejaculatory duct through a crescent shaped aperture in the internal calcified plates of the fifth pereiopod. The ejaculatory duct passes through the musculature of the coxa to the penis which is located on the ventro-medial border of the coxopodite of the fifth pereiopod. The ejaculatory duct is a simple uncoiled tube and the diameter varies from 2 mm in the proximal region to 1 mm distally.

Figure 1. Gross anatomy of the *Pseudocarcinus gigas* male reproductive tract, viewed dorsally. T - testis; AVD - anterior vas deferens; MVD - mid vas deferens; PVD - posterior vas deferens; ED - ejaculatory duct. The testis is dorsal to the hepatopancreas and stomach which have been removed to expose the vas deferens.



Histology of the reproductive tract

Testis

The testis consists of blind lobes, which may be divided into lobules, converging on a seminiferous tubule. Lobules have an outer layer of thin connective tissue tunica and an inner layer of squamous epithelium. The lumen of lobules contain cells in spermatogenesis; sections through distal regions of lobules are filled with spermatogonia while sections through more proximal regions are partially filled with sperm cells (Figure 2, upper). Sperm cells are not generally located within the centre of the lumen, rather, they tend to collect along one wall forming a lens-shaped area in transverse cross-section. The seminiferous duct has a cuboidal epithelium and the lumen is entirely filled with sperm cells (Figure 2, lower).

Anterior vas deferens

The histological structure of the AVD changes along its length although it is composed of three basic layers: an outer covering of connective tissue, a middle muscular layer, and an inner epithelial lining. These layers are also present in the MVD and the PVD. In the most anterior segment of the AVD, the epithelial lining is cuboidal with secretory globules in the cytoplasm. The lumen is filled with sperm mass, which is not always fully divided into spermatophore packets, and a luminal substance. The luminal substance appears to encapsulate the sperm masses by condensation (Figure 3, upper).

In the mid-AVD, the epithelium becomes multinucleate columnar (25-30 μm high) with considerable glandular activity and sperm masses are more dispersed due to greater proportion of luminal substance. All sperm cells are massed into ovoid spermatophores with a distinctive envelope layer (110-230 μm on longest axis) (Figure 3, lower). The posterior AVD is similar with a columnar epithelium although this is more elongated (30-40 μm), also with glandular activity. Luminal secretions in the mid- and posterior AVD are granular and more eosinophilic than secretions in the anterior AVD.

Figure 2. Histology of the testis. Upper figure shows meiotic spermatogonia and spermatids (bar=35 μm). Lower figure shows a lobule with a seminiferous duct along one margin (bar=80 μm).

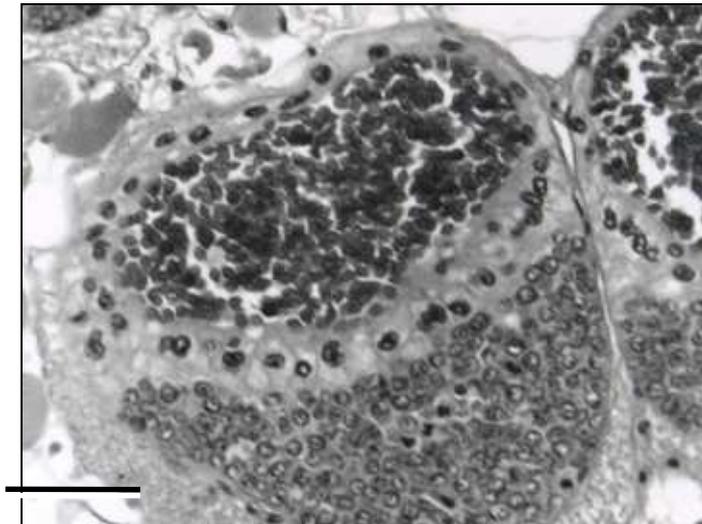
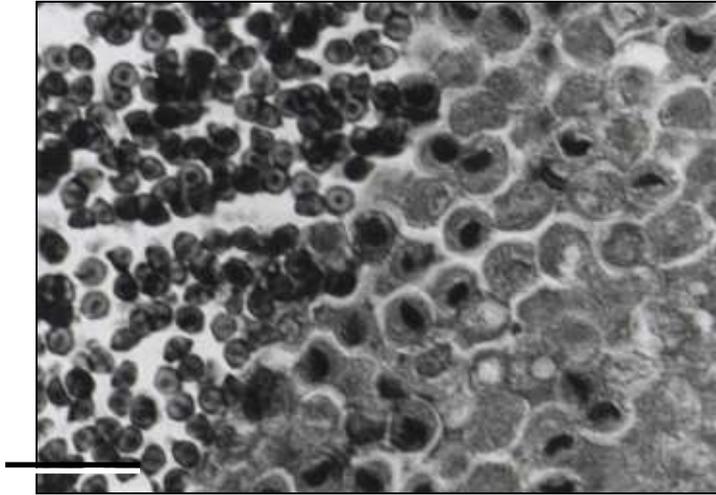
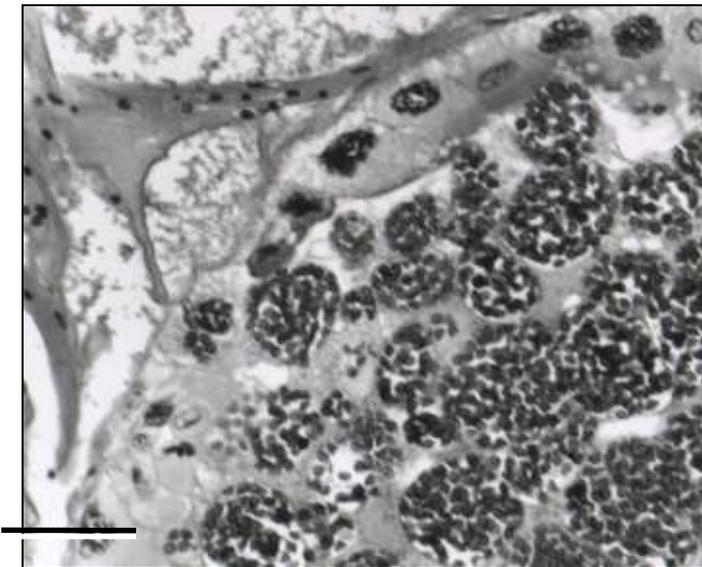
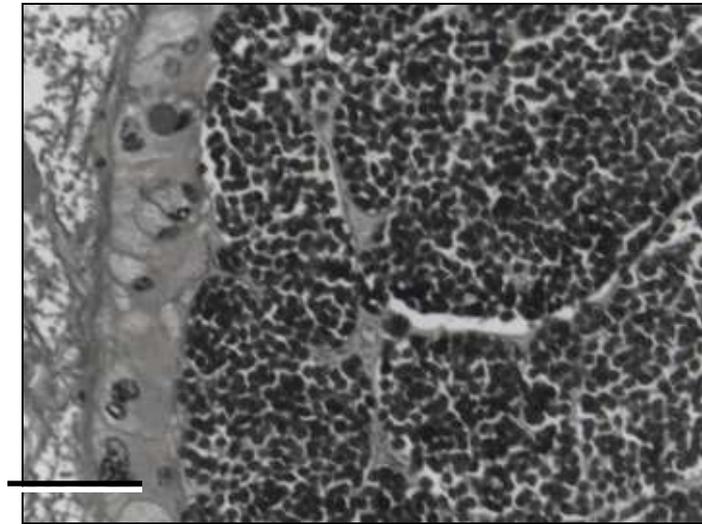


Figure 3. Histology of the anterior vas deferens (AVD). Upper - anterior segment of AVD with lumen filled with spermatids which are not yet formed into spermatophores. Luminal secretions have begun to encapsulate sperm masses (bar=80 μm). Lower - Mid section of the AVD. Sperm cells are massed into spermatophores although these remain closely packed (bar=80 μm).



Mid-vas deferens

The anterior region of the MVD is initially similar to the AVD although the epithelium gradually becomes less elongated (from 30-40 μm to 25 μm high) so that it approaches a cuboidal type epithelium (Figure 4, upper). There appears to be little glandular activity in the mid-region of the MVD. The muscular layer is also thinner while the lumen is larger than in the AVD. The lumen is filled with a granular luminal substance and scattered spermatophores. Granules range in size from 2 to 100 μm although most are 10 μm (Figure 4, lower).

Posterior vas deferens

The connective tissue layer is thicker than in the MVD (20 μm). In the anterior third, the epithelial layer is low columnar with rounded infoldings into the lumen and appears to be secretory (Figure 5, upper). The epithelium gradually changes to a cuboidal epithelium with no secretory activity. Basophilic, simple branched acinar glands open into the lumen of the duct and are particularly numerous in the anterior third of the PVD. Luminal contents change along the length of the PVD with anterior portions filled with: a granular substance - apparently the same as that in the MVD; an agranular less eosinophilic substance; and occasional spermatophores. The agranular luminal substance appears to be secreted in the PVD and this displaces other luminal contents. After the first third of the PVD, spermatophores are no longer present within the lumen which is filled with the agranular substance (Figure 5, middle).

Ejaculatory duct

The ejaculatory duct (ED) is lined by a simple columnar epithelium (30-40 μm) which is produced into folds, more pronounced distally (Figure 5, lower). The connective tissue layer and muscle layer are thicker than in the PVD. The lumen contains a substance which is predominantly agranular and similar to that in the PVD.

Figure 4. Histology of the mid vas deferens (MVD). Upper- Spermatophores in the anterior MVD are well formed with a distinct envelope layer and are surrounded by luminal secretions (bar=80 μm). Lower - the lumen of the MVD is filled with granular secretions and spermatophores are now more widely separated than in the posterior region of the AVD (bar=80 μm).

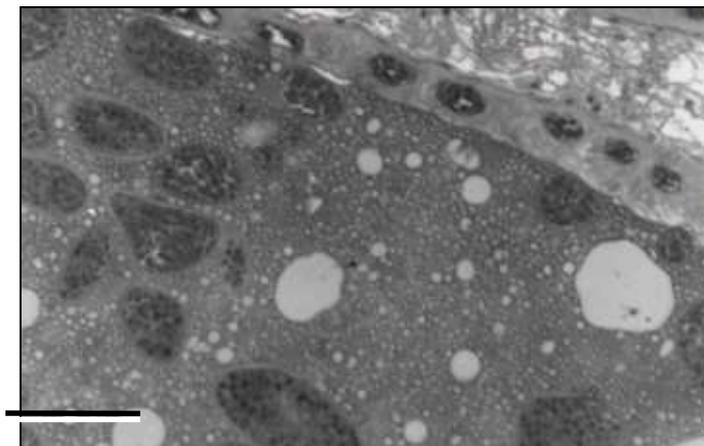
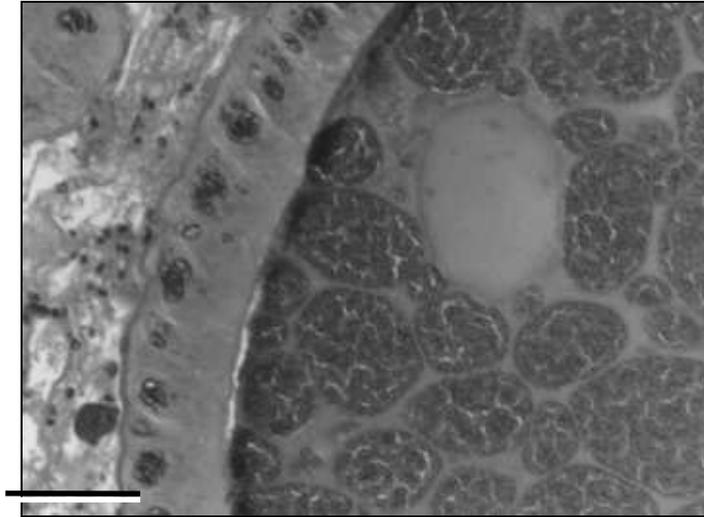
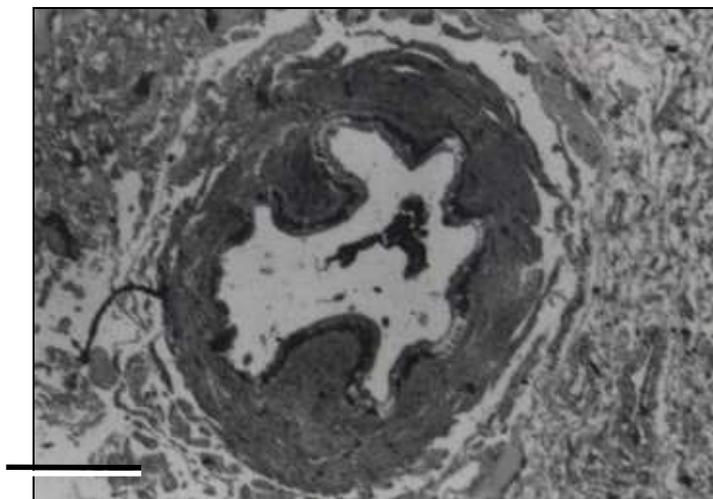
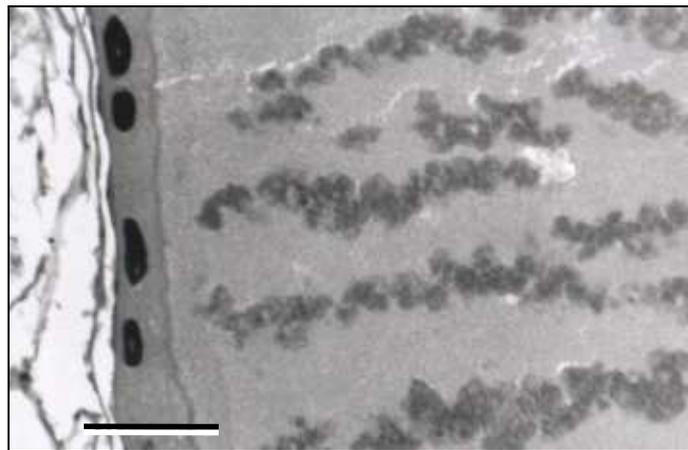
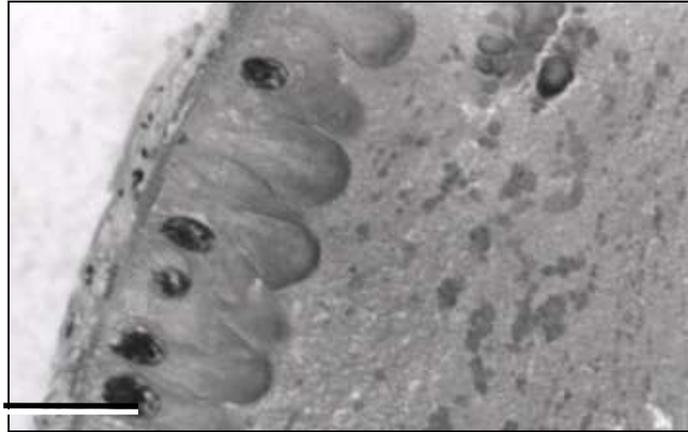


Figure 5. Histology of the posterior vas deferens (PVD) and ejaculatory duct (ED). Upper - section through the anterior PVD. Note that spermatophores are seldom seen in the lumen and that the epithelial cells are rounded with infoldings protruding into the lumen. The lumen is mainly filled with an agranular substance although there are occasional granules, similar to those in the MVD (bar=35 μm). Middle - mid PVD showing change in the epithelium to a low cuboidal epithelium (bar=35 μm). Lower - ED with the lumen produced into folds and surrounded by a thick muscular layer (bar=450 μm).



Discussion

The spermatophores of *Pseudocarcinus gigas* are typical of brachyuran crabs as they are non-pedunculate and vesicular, unlike anomuran spermatophores (Uma and Subramoniam, 1979; Subramoniam, 1993). A distinctive, single, envelope layer was present around the spermatophore which appeared to be deposited in the initial section of the anterior vas deferens. This envelope layer has been reported in *Portunus sanguinolentus* (Ryan, 1967), *Geryon fenneri* (Hinsch, 1988), *Scylla serrata* (Subramoniam, 1993), and *Chionoecetes opilio* (Beninger et al., 1988). An aggregation of sperm cells without an envelope layer has been reported in several species including the spanner crab, *Ranina ranina* (Ryan, 1984). There is also variation within the Brachyura in the number and density of sperm held in the spermatophore; *P. gigas* has a spermatophore similar to *Ovalipes ocellatus*, where numerous sperm cells are closely packed with little fluid between sperm (Hinsch, 1986).

Luminal secretions of *P. gigas* appear to be similar to those of *Scylla serrata*, in both function and site of production. Uma and Subramoniam (1984) classified luminal secretions of *S. serrata* into four groups: A,B,C and D. These were produced in the most anterior portion of the AVD, the posterior portion of the AVD and the MVD, the PVD, and the ED respectively. The spermatophore envelope of *P. gigas* was secreted in the anterior portion of the AVD and appears to correspond to luminal substance A. The granular substance secreted in the posterior portion of the AVD appears to be luminal substance B, and this is considered to have a nutritive function (Jeyalectumie and Subramoniam, 1991). The PVD of *P. gigas* is filled with an agranular substance, which appears to be the luminal substance C described by Uma and Subramoniam (1984). Given the absence of spermatophores in luminal substance C, and the proximity to the ED, it is probable that this substance forms the sperm plug in the spermatheca of the female (Diesel, 1989). Uma and Subramoniam (1984) identified a fourth luminal secretion in the ED, which they labelled "D". In *P. gigas*, the luminal substance in the ED appeared to be the same as that in PVD ("C") and there appeared to be no justification to classify them separately based on histology alone.

Observations on the presence or absence of spermatophores in the male reproductive tract are useful for fisheries management in establishing the onset of physiological maturity. For instance, Comeau and Conan (1986 and 1992) examined the vas deferens of tanner crabs

Chionoecetes opilio to determine if spermatophores were produced, although they also proposed that the presence of spermatophores may not necessarily mean that the crab is functionally able to mate. Paul (1992) asserted that it was improbable that a crab would expend energy producing sperm unless there was some chance of using it. This discussion over the use of gonad maturity to determine the potential of males to mate is fuelled by observed differences in physiological and morphological maturity. That is, male crabs tend to produce spermatophores before they fully develop morphological secondary sexual characteristics such as enlarged chelae.

There is potential for this debate to be obscured by the false classification of a crab as physiologically mature when only low numbers of spermatophores are produced, especially if the anterior vas deferens is examined rather than the main storage region. For instance, Van Engel (1990) determined physiological maturity of *Callinectes sapidus* by the presence of spermatophores in the AVD although seminal products are stored in the MVD in this species (Johnson, 1980). Although most brachyurans store spermatophores in the MVD, there is interspecific variation with some species storing spermatophores in the PVD (e.g. *Libinia emarginata* Hinsch and Walker, 1974; and *Uca lacteus* Uma, 1978). Thus, it is clearly important to establish the site of spermatophore storage, before conducting sampling to determine physiological maturity. In *P. gigas*, spermatophores are formed in the AVD and stored in the MVD as evidenced by the high density of numerous, well formed spermatophores in this large region of the tract.

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*Maturation of Male Giant Crab
Pseudocarcinus gigas and the
Potential for Sperm Limitation in the
Tasmanian Fishery*

14

Abstract

Onset of sexual maturity of the male giant crab (*Pseudocarcinus gigas*) from Tasmania was assessed to determine whether protection is provided by the current minimum legal size of 150 mm carapace length (CL). Maturity was assessed by morphometric analysis of chela development, change in vaso-somatic index (VSI) with size, and production of spermatophores. Males were observed to develop through three morphological groups based on development of the molariform chela relative to CL: morphologically immature, morphologically adolescent, and morphologically adult. Onset of morphological adolescence occurs at 134 mm CL and this was not influenced by sample site (east and west Tasmania). Males produce spermatophores while they are morphologically immature and the mid vas deferens of all animals sampled greater than 90 mm CL contained numerous well-formed spermatophores. VSI increases with CL for morphologically immature and adolescent crabs. Male crabs appear to suffer higher incidence of cheliped loss than females, which may be due to competition for mates. Tank trials demonstrated that female giant crabs carry broods in successive years without moulting and are able to fertilise eggs using sperm stored for at least four years. Information on functional maturity of males, from observations of mating pairs, is required to fully assess the potential for sperm limitation in the fishery. Nonetheless, there are factors that reduce the risk of sperm limitation: both females and males are harvested; females do not moult every year thereby raising the operational sex ratio; and females can store sperm for extended periods.

Introduction

Crab fisheries are frequently managed by restricting catch to males as this was traditionally considered to have relatively little impact on the reproductive output of the remaining stock. More recently, concern for the effects of reduction of males has prompted research on male maturity in several established fisheries including those for *Chionoecetes bairdi* (Brown and Powell, 1972), *C. opilio* (Conan and Comeau, 1986; Sainte-Marie et al., 1995), *Scylla serrata* (Knuckey, 1996), *Callinectes sapidus* (Wenner, 1989; Van Engel, 1990), and

Paralithodes camtschaticus (Paul and Paul, 1990). Indications of high-exploitation of male giant crab *Pseudocarcinus gigas* (Lamarck, 1818) has prompted similar research for this fishery.

A critical step in estimating the risk of sperm depletion due to fishing is to determine the size at which male crabs are able to reproduce. Several methods have been used and the most direct is the observation of mating pairs, either in the field (Ennis et al., 1988; Oransanz et al., 1995), in tank based trials (Adams and Paul, 1983; Paul and Paul, 1996a), or from the presence of mating scars (Knuckey, 1996). These approaches are impractical with *P. gigas* for several reasons: they are seldom found in water shallower than 100 m; commercial fishers do not catch mating pairs in traps; no mating has been observed with males held in tanks with females for periods of 12 months; and no evidence of mating scars have been detected on males.

Alternative methods for estimating size at which males are able to reproduce (i.e. functionally mature) were summarised by Paul (1992). They include examining the vas deferens for the presence of spermatophores (physiological maturity) and morphometric techniques such as ratios of reproductive tract weights and chela size to body size (morphometric maturity). Due to difficulty in determining functional maturity of *P. gigas* males, these alternative approaches were applied to assess the onset of maturity, and also to investigate regional differences between the two main areas of the Tasmanian fishery.

Aspects of the female reproductive biology, such as sperm storage in the spermatheca, also influence the potential for sperm limitation (Wenner, 1989; Paul and Paul, 1992; Sainte-Marie and Carriere, 1995). An additional objective of this study was to provide information on the viability of stored sperm in *P. gigas* and the cycles of egg extrusion and moulting.

Materials and methods

All crabs were collected by commercial fishers fishing for giant crabs in 200–350 m depth, along the rim of the continental shelf. Crabs were captured in traps from two sites (Fig. 1) off eastern and western Tasmania. Populations of adult *P. gigas* from these regions are relatively separate as few crabs are found in Bass Strait or around the southern region of Tasmania.

Figure 1. Sampling locations in eastern and western Tasmania, Australia. Relatively few crabs are found in Bass Strait or around southern Tasmania indicating these populations are not continuous.

Morphological maturation

Preliminary analyses were conducted on measurements of 296 male giant crabs to determine suitable parameters for assessing morphometric maturity. Parameters measured were: abdomen length; width of abdominal segment 1; width of abdominal segment 5; chela propodus width; chela propodus height; chela propodus length; and length of merus of the 5th pereopod. The most suitable parameter for assessing morphometric development appeared to be chela propodus length (CPL) as other parameters either developed in a simple linear relationship with carapace length (CL), or showed the same pattern as CPL but with greater scatter (i.e., lower correlation with CL). CPL was measured on the molariform chela from the proximal edge of the lower articulation knob to the distal tip of the immovable finger.

Sampling of CPL was then extended to a total of 533 crabs from eastern (N=186) and western (N=347) Tasmania ranging in size from 46 to 235 mm CL. All morphological sampling was conducted from March 1996 to June 1997. In addition to morphological measurements, missing pereopods or left handed molariform chelae were recorded. Missing limbs without a blackened plaque over the wound were not recorded as these had occurred recently, probably during handling after capture. Female crabs were also sampled from the same location for limb loss and incidence of left handed molariform chelae (N=114 and 319 respectively).

Physiological maturation

To determine the weight and maturity of gonads, 61 male crabs were obtained from eastern Tasmania in March and July 1996 ranging in size from 81 to 208 mm CL. Based on the analysis of morphological groups described below, this sample included 22 morphologically immature, 29 morphologically adolescent, and 10 morphologically mature crabs. Crabs were killed in a bath of clove oil in seawater at 0.125 ml l⁻¹ (Chapter 11; Gardner, 1997) and immediately dissected to remove the reproductive tract. The paired testis and vas deferens were gently blotted and weighed separately to the nearest 0.1 g. Vas deferens weight was divided by CL to provide an index of vas deferens development: vaso-somatic index (VSI).

Carapace length (CL), rather than whole weight, was used to scale the index of vas deferens weight as it is independent of development of the molariform chela.

One vas deferens and one testis were preserved in 10% phosphate buffered saline for histological processing while smears from the other mid-vas deferens were examined microscopically for the presence of spermatophores, after staining with 5% neutral red. Specimens preserved in formalin were processed, sectioned, and stained by standard haematoxylin-and-eosin paraffin histology then examined for spermatogenesis. Males were classed as physiologically mature when the mid-vas deferens, the site of spermatophore storage in *P. gigas*, contained numerous well formed spermatophores, i.e. ovoid spermatophores with a complete, single envelope layer enclosing densely packed sperm cells, 110-230 μm on the longest axis (see Uma and Subramoniam (1984) for similar histology). The two methods used to prepare specimens for determining physiological maturity (smears and histology) provided confirmation of classification and there was no disagreement between readings with the two techniques.

Sperm storage by females

To examine the viability of sperm stored for extended periods, 31 ovigerous females were captured in traps from depths in the range of 300-380 m off the east coast of Tasmania (41°15'S;148°40'E) in May 1994. Ovigerous females had only just begun to be observed by fishers so these egg masses were regarded as recently extruded. Females ranged from 2.2-3.5 kg and were maintained in two 4 m³ tanks with flow through water supply and fed twice weekly. Substrate was placed in tanks during ovipositioning to ensure adherence of eggs to pleopods (Shields, 1991). Females were individually tagged (see Levings et al., 1996) and monitored until September 1997 to record the viability of broods and patterns in reproductive biology. It was found that broods were produced annually and were held from around May to November; consequently, females were monitored for four reproductive cycles. Females were held without males for the duration of the experiment.

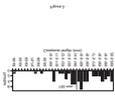
To examine changes in the weight of spermathecae in relation to the size of the female, 90 females were obtained from eastern Tasmania between June 1994 and August 1995 (samples of around ten individuals per month except during seasonal closures in the fishery: September to December). These females were killed as described for males and dissected to remove the paired spermathecae which were blotted and weighed separately;

the mean weight of the two spermathecae was used for analyses. A small proportion of female giant crabs do not produce eggs each year so females were classed as reproductively active or inactive based on the presence of eggs and the development of the gonad. This classification could only be done during months where crabs were ovigerous or in advanced gonadogenesis (i.e. excluding January and February when gonadogenesis was commencing).

Analysis of data

Morphological groups based on chela allometry were defined by iterative K-means cluster analysis (Everitt, 1974; Massart and Kaufman, 1983). Chela allometry of these groups was then defined by linear regression of CPL on CL (Sokal and Rohlf, 1981). The effect of site on morphometric development of chela was assessed by classic linear regression (Myers, 1990). Chela morphometric data have been log transformed in similar analyses with other brachyuran species (Comeau and Conan, 1992; Sainte-Marie et al., 1995). However, analysis of normal quantile plots indicated that regressions were best conducted with untransformed data in *P. gigas*. Slopes of regressions were compared by analysis of variance (ANOVA; Sokal and Rohlf, 1981) and the abscissa value for the point of intersection (I) between regression lines was calculated as $I = (b_2 - b_1)(a_1 - a_2)^{-1}$, where a_1 and a_2 are the slopes and b_1 and b_2 are the Y-intercept values of the two regressions (Sainte-Marie et al., 1995). This procedure was also applied to VSI data, which were log transformed.

The significance of differences between sexes in limb loss and incidence of left handed molariform chela were assessed by contingency table analysis using G-tests (Sokal and Rohlf, 1981).



Results

Maturity in males and chela allometry

Large numbers of spermatophores were present in the mid-vas deferens of all males greater than 90 mm carapace length (CL; N=59) and no spermatophores were detected in either animal sampled below 82 mm CL (N=2). The sample size of crabs around this CL is insufficient to pinpoint the size at onset of gametogenesis. However, onset of gametogenesis clearly occurs at a size considerably less than the minimum legal size of 150 mm CL (Fig. 2) and probably less than 110 mm CL for most males.

Figure 2. Sampling to determine onset of physiological maturity (spermatophore production) in male giant crabs Pseudocarcinus gigas. Minimum legal size is 150 mm (dashed line) carapace length. Solid columns represent physiologically mature crabs, and shaded columns represent physiologically immature crabs.

Cluster analysis of points in the scattergram of chela propodus length (CPL) on CL defined three clear clusters which are termed morphologically immature, morphologically adolescent, and morphologically adult (Fig. 3). Site appeared to have no significant effect ($P>0.70$) on size of onset of adolescent chela morphology so data were combined for subsequent analyses. Slopes of morphologically adolescent and morphologically immature crabs were significantly different ($P<0.0001$) and intersected at 133.5 mm CL. There was considerable overlap of morphologically adolescent and adult males with the largest morphologically adolescent male 207 mm CL and the smallest morphologically adult crab 174 mm CL. Mean CL of morphologically adolescent and adult crabs were 172 mm and 204 mm respectively; that is, CL of morphologically adult crabs were around 19% larger than adolescents.

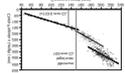


Figure 3. Scattergram of chela propodus length (CPL) on carapace length (CL) for samples of morphologically immature, adolescent, and adult male *Pseudocarcinus gigas* taken from May 1995 to June 1997. Data for crabs collected on eastern and western coasts of Tasmania are combined. Males were classified into morphological maturity groups by cluster analysis as described in text. Equations of regressions are: $CPL = 1.245CL - 22.895$ for immature males (lower scatter points, $N = 32$, $r^2 = 0.97$, $P < 0.0001$); $CPL = 2.318CL - 166.026$ for adolescent males (middle scatter points, $N = 291$, $r^2 = 0.62$, $P < 0.0001$); and $CPL = 2.285CL - 86.762$ for adult males (upper scatter points, $N = 210$, $r^2 = 0.544$, $P < 0.0001$). "I" represents the abscissa value at which the regressions of morphologically immature and adolescent males intersect.

VSI increased with male size for both morphologically immature and adolescent crabs ($P < 0.001$; Fig 4). No effect of carapace size on VSI was detected for morphologically adult crabs although most of these crabs were of similar size and sample size was small. The slopes of regressions of log vaso-somatic index (VSI) against log CL were significantly different for morphologically immature and adolescent crabs ($P < 0.01$) and they intersected at 140 mm CL (Fig. 4). Note that this abscissa value of 140 mm CL should be interpreted cautiously as it is largely an artefact of the morphological grouping variable which had an abscissa value of 133.5 mm CL.

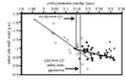


Figure 4. Relationship between log vaso-somatic index (VSI; wet weight of the vas deferens / CL) and log carapace length (CL) of morphologically immature (E), adolescent (J), and adult (H) male giant crabs *Pseudocarcinus gigas*. Note that weight of vas deferens was scaled against CL, rather than body weight, to reduce confounding effects from chela development. Equations of regressions are: $\log(VSI) = 3.672\log CL - 8.858$ ($N = 20$, $r^2 = 0.69$, $P < 0.0001$) for morphologically immature males; and $\log(VSI) = 1.213\log CL - 3.578$ ($N = 29$, $r^2 = 0.23$, $P < 0.01$) for morphologically adolescent males. No significant effect of CL on VSI was detected for morphologically mature males ($N=10$; $P > 0.50$). "I" represents the abscissa value at which the two regressions intersect.

Limb loss and incidence of left handed molariform chelae

Male crabs had significantly higher incidence of left-handed molariform chelae than females ($P < 0.05$; Fig. 5). Although there appeared to be a trend of greater limb loss in male crabs, this was not significant ($P > 0.05$).

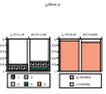


Figure 5. Proportion of male and female giant crabs *Pseudocarcinus gigas* with missing pereiopods (left) and with left handed, molariform chela (right).

Sperm storage by females and annual reproductive patterns

Females held in tanks had an annual reproductive cycle with most females producing broods in successive years (Fig. 6). Around one quarter of females did not produce broods in the three seasons after capture and these individuals went on to moult in 1995/96. However, this pattern did not occur in 1996/97 when females that had not produced broods in 1996 were able to produce broods in 1997 without moulting. Sixteen of the seventeen crabs remaining alive at the end of the trial in September 1997 were able to produce fertile broods although there had been no contact with males since capture in May 1994. No attempt was made to measure fecundity as nutrition affects fecundity in crustaceans (Harrison, 1990) and the supplied diet of mackerel (*Trachurus* sp.) was unlike the natural diet which is primarily echinoderms, anomurans, and gastropods (Heeren and Mitchell, 1997). Nonetheless, in the final season, many females produced large broods that appeared normal. No females survived moulting; the spermathecae of these females remained full although it was clearly not possible to assess the viability of stored sperm retained through a moult.

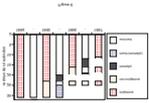
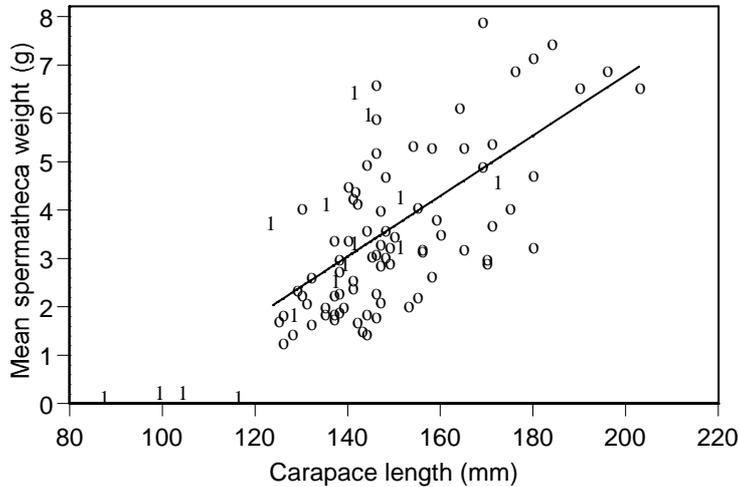


Figure 6. Reproductive history of 31 female giant crabs *Pseudocarcinus gigas* held in tanks without males from May 1994 to September 1997. Females were captured with eggs. Moulting occurred in January and February and all moulting individuals died. Embryogenesis was observed in all egg masses from females classed as ovigerous.

Several of the dissected females appeared to be skipping a reproductive season as they did not have developing ovaries or broods during the brooding season (12.8% of females with sperm deposits; Fig. 7). Levings et al. (1996) stated that the size of onset of maturity of female crabs in Tasmania is around 115 mm CL and most of these reproductively inactive females were considerably larger. All females greater than 124 mm CL had deposits in their spermathecae. Weight of spermathecae increased with female size ($P < 0.0001$) indicating that larger females have mated more often than smaller females and that they continue to accrue sperm reserves (Fig. 7).

Figure 7. Scattergram of average wet weight of spermathecae (SW) on carapace length (CL) of female giant crabs *Pseudocarcinus gigas*. Females with eggs or developing ovaries are represented by open circles (E) and reproductively inactive females are represented by crosses (I). The regression excludes the four females without sperm deposits and the equation is: $SW = -5.712 + 0.0624 CL$ ($N = 90$, $r^2 = 0.41$, $P < 0.0001$).



Discussion

Maturation of males

Male *Pseudocarcinus gigas* pass through three distinct morphological stages based on allometry of the chela which have been termed morphologically immature, morphologically adolescent, and morphologically adult. While the use of morphological indices for assessing maturity is controversial (see Paul, 1992; Comeau and Conan, 1992; Sainte-Marie et al., 1995), it is a useful method for determining whether there are regional differences in size of onset of maturity. Also, in the absence of information on functional maturity, morphological indices can provide the best estimate of size of maturity in some species (Somerton and Macintosh, 1983). There did not appear to be any regional effect (between east and west Tasmania) on size of onset of morphological adolescence in *P. gigas*. This suggests the information on physiological maturity of *P. gigas* obtained from specimens collected on the east coast may be equally applicable to west coast populations.

To use morphological information for fisheries management, it is necessary to relate changes to moult increments and these relationships are not necessarily simple (Paul and

Paul, 1995a). The mean CL of morphologically adult *P. gigas* was 19% larger than the mean CL of adolescent crabs. This is around the same size as the moult increment of male crabs reported by Levings et al. (22%; 1996) although it should be noted their estimate was drawn from only four individuals between 115 and 140 mm CL. Nonetheless, this indicates that the distinct stages of chela development correspond to different instars and similar patterns have been shown in numerous brachyurans, including other xanthids (Finney and Abele, 1981). The data presented indicate that the morphologically adult stage of *P. gigas* may be terminal as with *Chionoecetes opilio* (Sainte-Marie et al., 1995) although it is possible that additional moults may occur as with *Chionoecetes bairdi* (Paul and Paul, 1995b). These could result in morphological stages beyond the upper size limit sampled which is limited at around 225 mm CL by the legal maximum neck diameter of pots.

Unlike *Chionoecetes* spp., the morphological stages of *P. gigas* do not correspond to distinct stages in the onset of gametogenesis as crabs of all morphological stages had spermatophores in the mid vas deferens and possessed well developed gonads (Brown and Powell, 1972). Several authors have regarded the presence of spermatophores as indication of functional maturity (Hartnoll, 1969; Paul, 1992) although this relationship is debated in other species where morphological development seems to be an important determinant of functional maturity (Van Engel, 1990; Comeau and Conan, 1992; Sainte-Marie et al., 1995; Knuckey, 1996). In regards to *P. gigas*, the morphological stages of chela development represent three possibilities for fisheries management. First, physiologically mature males of immature morphology may be functionally mature. These animals are generally protected by the current minimum size (150 mm) so impacts of fishing will be slight. Secondly, males may not become functionally mature until morphological adolescence, despite the presence of spermatophores at much smaller sizes. Male *P. gigas* begin to exhibit morphological adolescence at around 135 mm CL with most morphological adolescents being larger than legal size, so little protection would be provided by current legislation. The third alternative is that only morphologically adult crabs are functionally mature. Current minimum size limits would be ineffective for protecting breeding male crabs in this alternative and most could be harvested as morphological adolescents well before reaching functional maturity.

Without direct measures of mating, it is not possible to ascribe definitively the maturation of *P. gigas* to any of the options listed above, however, there are clues to their mating behaviour. Species with larger males relative to females, as in *P. gigas*, tend to have a

polygamous, female-centred competition system (Martin and May, 1981; Christy, 1987). The large molariform chela of morphologically adult male *P. gigas* is of limited use for feeding (Heeren and Mitchell, 1997) and is used aggressively in tanks so that opposing males are killed although females have never been observed to damage each other (similarly with *C. bairdi*; Paul and Paul, 1996b). Regenerated molariform chela often regrow as incisiform chela so the greater incidence of "left-handed" molariform chela in male *P. gigas* may be a result of greater limb loss due to inter-male aggression (Cheung, 1976; Smith, 1990; Simonson and Hochberg, 1992; Abello et al., 1994). On the basis of these indirect observations on behaviour, it is probable that the morphological stages of development of the chelae have functional significance in mating and that only morphologically adolescent or adult crabs are important for fertilisation. Production of spermatophores before males are functionally mature appears to be common in brachyurans (Oransanz et al., 1995; Knuckey, 1996) so this is unlikely to indicate functional maturity in morphologically immature *P. gigas*. Spermatophore production at this stage may simply indicate the onset of gonadogenesis in preparation for mating at subsequent stages, as VSI increases sharply in relation to CL for morphologically immature crabs (note VSI was calculated using CL as the somatic scale so the relationship is not merely volumetric).

The implications of removing large males from the population have caused concern in some crab fisheries as they are more successful in restraining females during the precopulatory embrace (Comeau and Conan, 1992; Hankin et al., 1996). The increase in *P. gigas* spermatheca weight with CL indicates that females mate on several occasions as they grow (Fig. 7) and spermathecae of large females contain separate regions of sperm deposit that appear to correspond to separate ejaculates (Gardner et al., 1998). Consequently, there is potential for large females to avoid mating if large males are removed by the fishery and if male size influences mating success (Christy, 1987).

Removal of large males by fishing may also lead directly to sperm limitation where small males replace larger males after competition for possession of females is reduced (Ennis et al., 1988). For instance, males in exploited populations of *C. opilio* have reduced sperm reserves, presumably due to more frequent mating (Sainte-Marie et al., 1995). More frequent mating is of limited concern where small males are able to inseminate several females (Sainte-Marie and Lovrich, 1994), however, this is not the case for all species. Paul (1992) noted that large male *Paralithodes camtschaticus* are capable of inseminating several females while smaller males may be limited to a single mating. The implications of the

removal of large males from breeding populations of *P. gigas* are unknown so future monitoring of VSI as the fishery develops is advisable.

Sperm storage

Irregular moulting of female crabs and associated reproductive asynchrony have the potential to inflate the operational sex ratio and to increase competition for females (Wenner, 1989; Orensanz, et al., 1995). Inflation of the operational sex ratio occurs in *Cancer gracilis* where sperm can be retained through moults and is used to fertilise several broods (Orensanz, et al., 1995). *P. gigas* are also able to retain sperm through moults and stored sperm remains viable for extended periods, so that at least four annual broods can be fertilised. This prolonged viability of stored sperm in *P. gigas*, combined with the production of successive broods without moulting suggests that the operational sex ratio is inflated. This acts to reduce the risk of sperm limitation.

Summary

The onset of morphological adolescence occurred at around 135 mm CL in male crabs from both east and west Tasmania, which is slightly below the legal minimum size limit (150 mm CL). Although spermatophores are produced by morphologically immature *P. gigas*, it is unlikely that these crabs are functionally mature as VSI is still increasing at this stage. Also, spermatophore production by functionally immature males appears to be common among the Brachyura. Morphological adolescents and morphological adults are likely to be distinct moult stages with most animals at this stage larger than the legal minimum size. Aspects of the reproductive biology of females which reduce the risk of sperm limitation are: 1) successive annual broods are produced without moulting; and 2) sperm can be stored in the paired spermathecae, retained through moults, and remains viable for at least four years.

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*Ovarian Development and Female
Reproductive Biology*



Abstract

The gross anatomy of the female reproductive tract of the giant crab *Pseudocarcinus gigas* is typical of brachyurans. The mating system was investigated and although copulation was never observed, indirect evidence from the anatomy of the vagina and apparent mating scars indicates that mating occurs while the female is soft-shelled, which is consistent with other members of the family Eriphiidae. Females produce broods at more than one instar and retain ejaculates through moults. Ejaculates are stored in paired ventral-type spermathecae. Separate regions of sperm cells were observed in the spermathecae and these were presumed to be separate ejaculates. The number of these presumptive ejaculates increased with female size with up to three regions identified in some individuals. Internal sperm plugs were apparent although external sperm plugs were never observed. However, this may have been an artefact of sampling as all stored spermatophores had dehisced (released spermatids), indicating that mating had not occurred recently. Patterns of ovarian development and observations of crabs held in tanks demonstrated that the reproductive cycle is annual, although females may fail to produce a brood each reproductive season. Hepatosomatic index (weight of hepatopancreas relative to total weight) was reduced in response to ovarian development although there was no evidence of depletion of reserves in the hepatopancreas during gonad development. Moisture content of the hepatopancreas declined as gonad development proceeded and protein concentration (dry weight) remained stable. Carotenoid concentration in the hepatopancreas showed strong seasonal trends although these appeared unrelated to vitellogenesis as trends were similar between reproductively active and inactive crabs. Ovarian carotenoid concentration increased during vitellogenesis and peaked immediately prior to extrusion.

Introduction

Information on reproductive biology is essential for management of exploited crab species as it forms the basis of biologically rational controls on fishing effort, especially for input controls such as closed seasons and size limits. The only published information on female

reproduction in *Pseudocarcinus gigas* (Lamarck, 1818) is by Levings et al. (1996) who reported seasonal patterns of capture of ovigerous females, determined a regression of egg mass weight against carapace length, and noted that not all females become ovigerous during the incubation period (stated as June/July to October/November). They also stated that females migrate from 270 m depth to 210-140 m to release larvae, and that they mate while soft-shelled in June and July, although no data were presented in support.

The genus *Menippe* is in the same family as *P. gigas* and they are the most taxonomically similar exploited crab species (Family Eriphiidae⁷). A considerable amount of information has been published on *Menippe* species⁸ which serves as a useful guide to probable reproductive patterns in *P. gigas*. However, while there is similarity in reproductive biology within taxa, aspects such as mating behaviour may vary within brachyuran families (Norman, 1996) and even within genera (Orensanz et al., 1995).

Given the limited biological information on reproduction in female *P. gigas*, it was considered useful to investigate fundamental aspects, as well as issues relating directly to fisheries management. The anatomy of the reproductive tract is described with emphasis on the spermathecae as the structure of this organ reflects aspects of the mating system (Jensen et al., 1996). Additional research on the mating system was conducted by maintaining crabs in tanks for several years in an attempt to observe mating.

Information on the mating system is also useful for managing the harvest of male crabs as males tend to suffer higher exploitation and there is concern that sperm limitation may result (Chapter 14). The operational sex ratio can be lowered, and thus the risk of sperm limitation increased, by factors such as mating while the female is soft-shelled (during short seasonal periods) and by prolonged mate guarding behaviours (Christy, 1987).

Seasonal patterns of development were investigated by holding animals for prolonged periods in tanks and from changes in the size, composition and histology of dissected ovaries. Seasonal changes in the hepatopancreas were also monitored as this organ is considered to be an important organ for storage of nutrients prior to gonadogenesis, and

⁷ Eriphiidae is synonymous with Oziidae and Menippidae (Holthuis 1993).

⁸ *Menippe adina* was separated from *Menippe mercenaria* in 1986 so earlier studies referring to *M. mercenaria* may have been based on *M. adina*, or on hybrid material (Williams and Felder, 1986; Wilber, 1989a).

its composition tends to follow opposite trends to the ovary (Kulkarni and Nagabhushanam, 1979; Castille and Lawrence, 1989; Dy-Penaflorida and Millamena, 1990).

Materials and methods

Collection of specimens for dissection

A total of 101 female crabs were captured in traps set at 150-300 m by a commercial fisher. All samples were collected from the east coast of Tasmania (41°10'-42°00'S; 148°30'-149°00'E) between May 1994 and August 1995. It was intended to collect at least 10 crabs monthly although this was not always possible due to poor weather or closed seasons (Table 1). Specimens ranged from 83 to 203 mm carapace length (CL).

Commercial fishers reported that not all crabs became ovigerous during the winter months. To improve clarity of results in gonad analyses, females were classed as reproductively active or inactive, on the basis of gonad development. As this classification discriminated on the basis of gonad development, it was theoretically possible for a female crab to be ovigerous, yet classed as having inactive gonads - for the next spawning season. This distinction was not clear in samples collected in June and August as gonadogenesis was not advanced. Consequently, no crabs sampled in these periods were classed as inactive. Overall, 15 of the total 101 female crabs were classed as inactive.

Table 1. Number of crabs sampled in each month from May 1994 to August 1995.

	1994							1995								
	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
Active	3	9	9			8			12	12	3	9	4	12		9
Inactive									1	2	2	1	5			
Total	3	9*	9*			8 (6*)			13	14	5	10	9	12		9*

* number of ovigerous crabs in sample

Assessing maturity

Crabs were killed in a bath of clove oil in seawater at 0.125 ml l^{-1} (Chapter 11; Gardner, 1997a), measured (carapace length), weighed, and then dissected to remove the hepatopancreas, ovary, and spermathecae. Where crabs were ovigerous, the egg mass was removed before the female was weighed to obtain whole weight. The hepatopancreas and ovary were then weighed and subsamples were either preserved in 10% phosphate buffered formalin for histology, or frozen (-60°C) for biochemical analyses. Indices of gonad (GSI) and hepatopancreas (HSI) size were calculated as: (wet weight of organ / total body weight) $\times 100$.

Ovarian tissue and bisected spermathecae were processed by standard haematoxylin and eosin, paraffin histology and sections were cut at $7 \mu\text{m}$. The development of oocytes was quantified from histological sections by measuring the area of 50 large oocytes within the ovary by image analysis using NIH-Image™ 1.6 software. As the diameter of oocytes was often greater than the thickness of sections, it was inevitable that many oocytes would be not be sectioned through their medial plane. This problem was partially overcome by only selecting large oocytes for measurement.

Spermathecae were preserved in Davidson's fixative. Distinct regions of sperm deposits were apparent in histological sections of the spermathecae and these may have been separate ejaculates although biochemical techniques, such as electrophoretic analysis of enzymes, is required to confirm this (Sévigny and Sainte-Marie, 1996). The number of these presumptive ejaculates was counted for each female.

Composition of ovary and hepatopancreas

All analyses were duplicated and averaged. Where the duplicate analyses differed by more than 10% the analysis was repeated. To determine water content, samples were dried at 80°C for 24 h, with a final 1 h vacuum period before weighing. Samples of around 2 g were ashed at 450°C for 2 h. Samples for protein and carotenoid analyses were homogenised with a mortar and pestle. Protein was assayed by a modified Lowry procedure (Peterson 1977; Sigma Diagnostics™ #5656; Appendix 10).

Carotenoids were extracted from tissue with acetone (Appendix 10). The acetone extract was partitioned with diethyl ether which was then washed with 20 volumes of 10% NaCl to remove residual acetone. Six samples of carotenoid extract from well developed ovaries collected in May 1994 and 1995 were chromatographed by thin layer chromatography (TLC) on C8 octyl silica plates (Merck™) using a solvent mixture of 95 : 5 petroleum ether : methanol. Tissue extracts were run alongside saponified extracts (5% ethanolic KOH for 24 h at room temperature) and astaxanthin standard (Roche Pharmaceuticals™). Carotenoids present in extracts were identified as predominantly astaxanthin or astaxanthin esters. Consequently, total carotenoids were estimated from the extracts as astaxanthin by measuring their absorption in diethyl ether at 472 nm assuming an $E_{1\text{ cm}}^{1\%}$ of 2099 (Clarke, 1977). Although lipids were to be analysed, this was not possible due to the destruction of specimens during a freezer malfunction.

Reproductive behaviour

Three large, morphometrically mature (see Chapter 14) male crabs were placed with three female crabs in a 4 m³ tank from January 1995 to August 1995 and monitored daily for signs of precopulatory mate guarding. In addition, males were placed into 4 m³ tanks with females undergoing ecdysis in February 1995 and their behaviour monitored.

To permit cycles of female reproduction to be observed in tanks, 31 ovigerous females were captured in traps from depths in the range of 300 – 380 m off the east coast of Tasmania (41°15'S;148°40'E) in May 1994 by a commercial fisher. These females ranged from 2.2 – 3.5 kg and were maintained in two 4 m³ tanks with flow through water supply and fed twice weekly. Females were individually tagged. These crabs were maintained in isolation from males until September 1997 (3 years, 4 months).

Statistical analysis

The effect of female size on oocyte area (assumed to infer timing of extrusion) was assessed by standard least squares regression for two separate samples: February and April, 1995. These were also tested for normality with a Shapiro Wilk w-test (Tietjen, 1986). Non-normal distribution would provide evidence of individuals at separate development

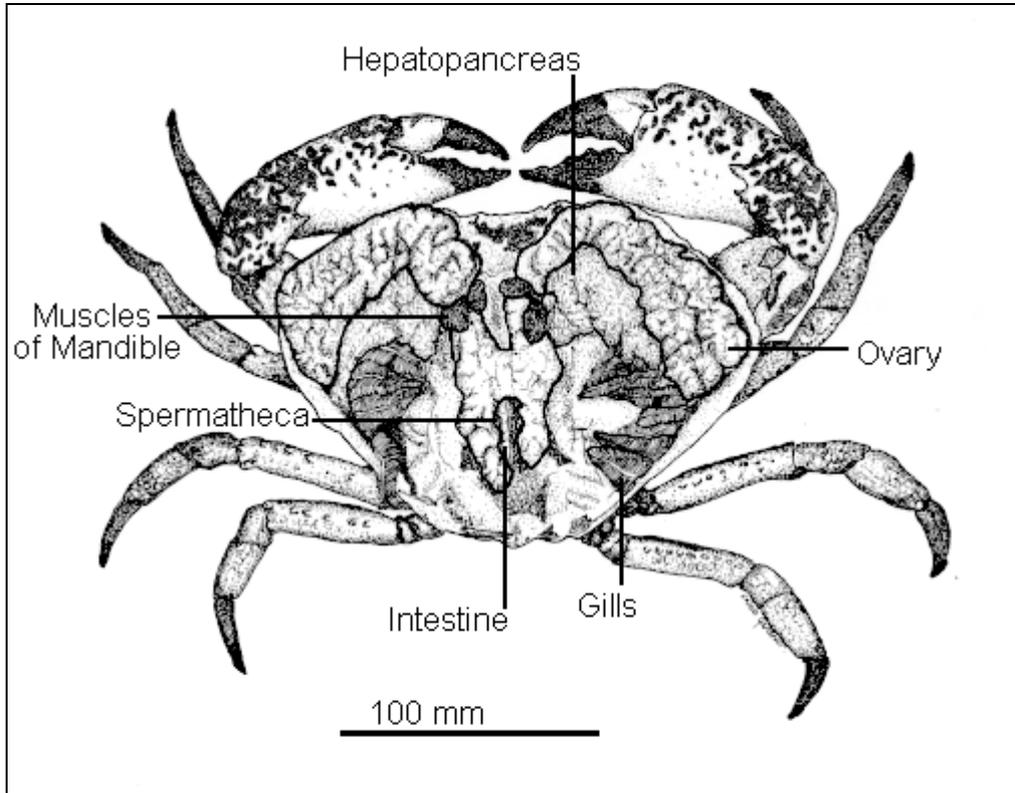
stages, possibly due to a biannual cycle. ANOVA was used to test if female size differed between crabs with 1, 2 or 3 presumptive ejaculates.

Results

General morphology of the female reproductive tract

The reproductive tract of female *Pseudocarcinus gigas* consists of the ovary, paired oviducts, spermathecae, vaginas and gonopores (Fig. 1). The ovary is surrounded by fibrous connective tissue and is located beneath the endocuticle, and dorsal to the hepatopancreas. The anterior lobes of the ovary extend to both lateral sides of the hepatic regions of the carapace. The posterior lobes extend laterally past the cardiac region to the intestinal region where they lie immediately lateral to the intestine. There is a medial commissure anteroventral to the heart. The ovary extends ventrally to the spermathecae so that the oviduct forms only a short tube of less than 10 mm passing along one side of the spermathecae. Spermathecae were ovoid, and were 41 mm on the longest axis in the largest specimen (203 mm CL). The oviducts join the spermathecae ventrally, thus the spermathecae are of the ventral type. The cuticular vaginas extend from the ventral surface of the spermathecae, through muscular tissue to the gonopores which are located on the sternal plastron of the 2nd walking leg. An external sperm plug was never observed in the vaginas of any specimen dissected here, or occluding the vulva in later sampling of 162 ovigerous female crabs (Gardner, 1997b).

Figure 1. Drawing of the gross anatomy of the female giant crab *Pseudocarcinus gigas*. The carapace, heart, and pericardiac sinuses were removed to expose the ovary. The spermathecae are largely obscured by the ovary but are located below the posterior lobes of the ovary, and lateral to the intestine.



Histology of the spermathecae

The spermathecae of all females greater than 124 mm CL contained sperm cells indicating that females had copulated at least once. Only 5 females were sampled below this size so it is not possible to assess the size at first mating. In all mated specimens, spermatophores had already dehisced and sperm cells were observed as a closely packed mass (Fig. 2). This suggests that either the spermatophores dehisce soon after copulation, or that none of the females sampled had mated recently. Masses of sperm cells were divided by basophilic proteinaceous matrix and separate sperm deposits appeared to be present, possibly as a result of separate copulations (Fig. 3). The number of these presumptive ejaculates ranged from 1 to 3 with larger females tending to have more presumptive ejaculates ($P=0.062$; Fig. 4). Females which moulted in isolation from males were dissected and found to have retained the contents of the spermathecae.

Figure 2. Sperm cells within the spermathecae. Note that the sperm cells are not bound into spermatophores.

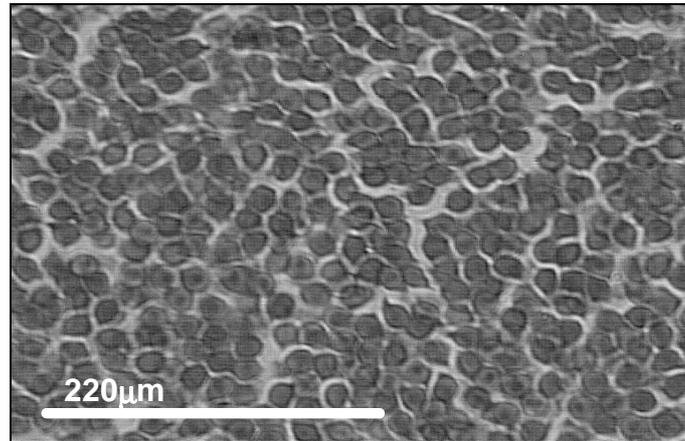


Figure 3. Spermathecae of giant crab *Pseudocarcinus gigas* bisected along the longitudinal axis. Note that three distinct deposits are present within the spermathecae, these are termed presumptive ejaculates.

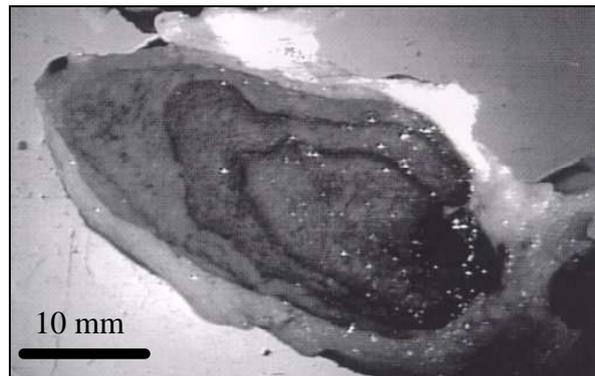
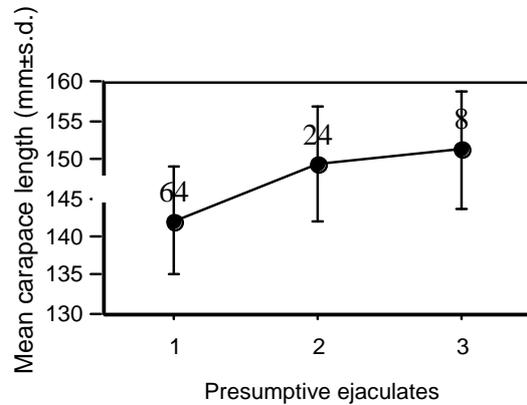


Figure 4. Relationship between size of female (carapace length) and the number of distinct regions of sperm deposit within the spermathecae. Distinct sperm deposits may be separate ejaculates. Number of females was 64, 24 and 8 for groups 1,2 and 3 respectively.



Ovarian maturation

The proportion of reproductively inactive crabs included in samples is influenced by feeding behaviour as ovigerous giant crabs are less likely to enter traps, as with many other species (Howard, 1982; Heasman et al., 1985; Schultz et al., 1996). Consequently, the ratio of reproductively inactive to reproductively active giant crabs is likely to be biased and is of limited value. Nonetheless, it is noteworthy that reproductively inactive crabs spanned a range of sizes which suggests that some of these females were not simply immature, but were “skipping” a reproductive season (Fig. 5). Results from these females were kept separate in subsequent analyses (Figs. 7 and 8).

Patterns in the gonadosomatic index (GSI) and oocyte area indicated that ovarian maturation is seasonal with increase in ovary size commencing shortly after extrusion (Fig. 7). Histological examination of the ovary indicated that oocytes separated from the germinal layer developed synchronously which indicates that only a single clutch is produced each spawning cycle (Fig. 6). For both years sampled, female giant crabs extruded eggs in late Autumn, during the months May to June. All females captured in May had highly developed ovaries while females captured in June were ovigerous. The hepatosomatic index (HSI: (wet weight of hepatopancreas/whole weight)×100) increased during spring (September to November) then slowly declined in summer (December to February). This decline in HSI occurred concurrently with a sharp increase in the gonadosomatic index (GSI) in summer (Fig. 7). The HSI of reproductively inactive

females also declined at this time, but tended to be larger than the HSI of reproductively active females, presumably due to greater space available within the carapace in the absence of developed gonads.

Figure 5. Weight of females in relation to carapace length (CL). Hollow symbols represent crabs with developing ovaries (n=86); solid symbols represent crabs classed as reproductively inactive (n=15). Whole weights of ovigerous crabs are with eggs removed. The regression is for both groups combined.

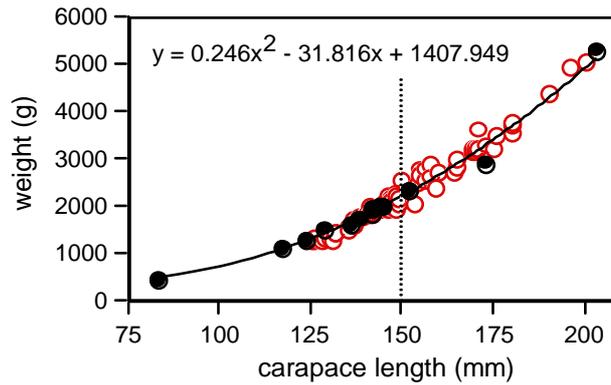


Figure 6. Oocytes from ovary in advanced development (April 1995). All oocytes are at a similar stage of development indicating that only a single clutch is produced each spawning cycle.

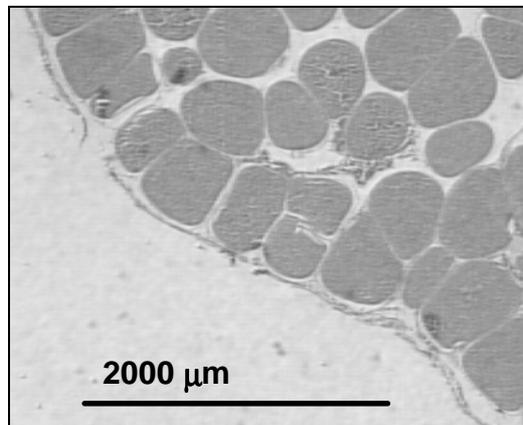
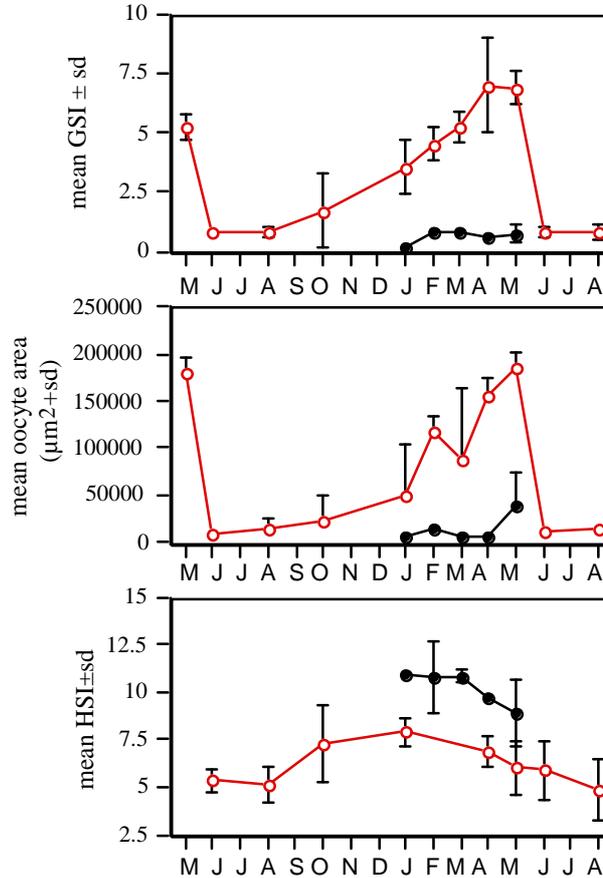


Figure 7. Seasonal changes in gonado-somatic index (GSI), oocyte size (area in cross section), and hepato-somatic index (HSI) of female giant crabs *Pseudocarcinus gigas*. Hollow symbols represent crabs with developing ovaries or brooding egg masses; solid symbols represent crabs classed as reproductively inactive. N of each sample was variable and is listed in Table 1.

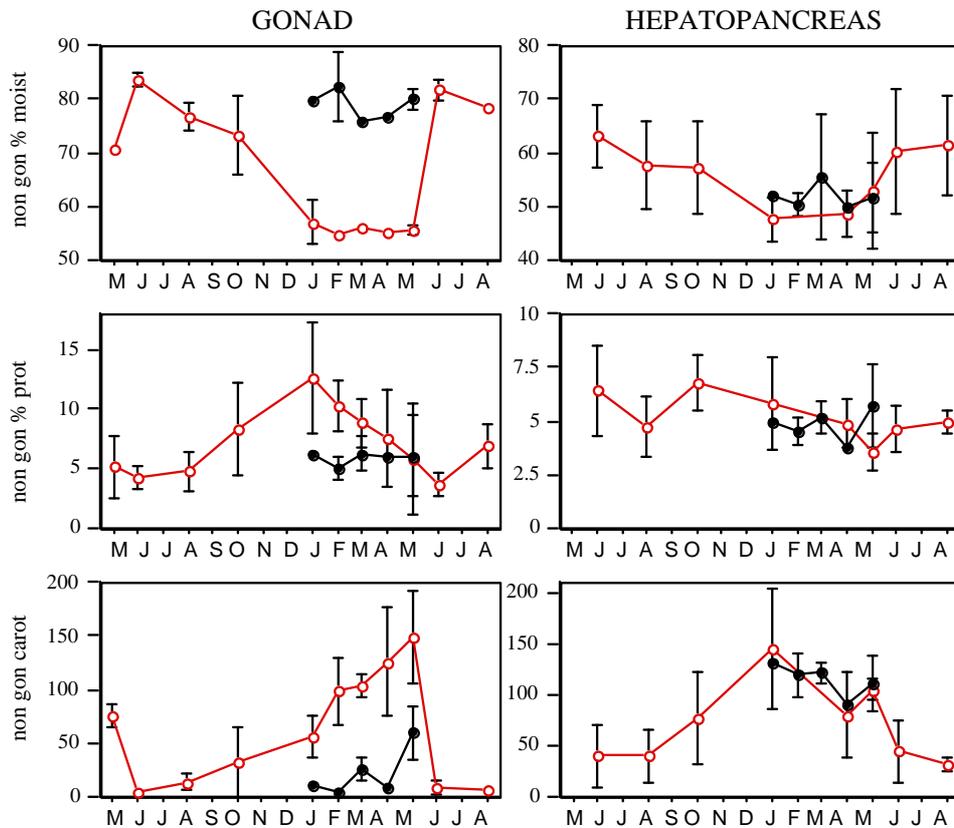


There was no evidence of an effect of female size on timing of the reproductive cycle (assessed by oocyte area) for either February (n=12) or April (n=9) samples ($P>0.4$). However, it should be noted that sampling was not specifically designed to assess the effect of female size on ovarian development, so the sample sizes were small and the results are not definitive.

Increase in ovary size, due to vitellogenesis, initially resulted in a decline in the moisture content of the ovaries and an increase in protein content (Fig. 8). Protein content peaked in December and then declined until ovaries were fully ripe in May. Total carotenoid content of the ovaries increased throughout gametogenesis. The hepatopancreas composition of reproductively active and inactive females were similar which suggests

composition was not affected by vitellogenesis. Moisture content of the hepatopancreas was lowest during summer (December-February; Fig. 8) when HSI was relatively high. Total carotenoid composition of the hepatopancreas rose in spring (September-November; Fig. 8) in a similar pattern to the seasonal trend in HSI (Fig. 7).

Figure 8. Seasonal changes in composition of giant crab *Pseudocarcinus gigas* ovaries and hepatopancreas. Protein and carotenoid composition are expressed as ash free dry weight. Hollow symbols represent crabs with developing ovaries or brooding egg masses; solid symbols represent crabs classed as reproductively inactive. N of each sample was variable and is listed in Table 1.

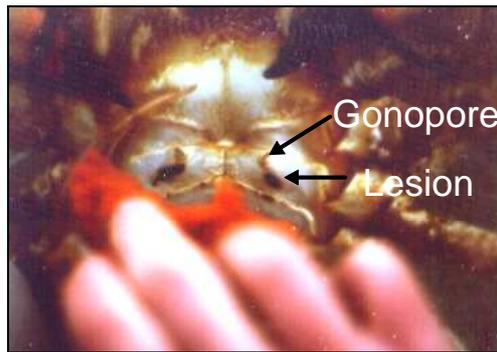


Reproductive behaviour

Neither mating nor mate-guarding were observed in tank trials, and males did not appear to respond to females undergoing moult. Consequently, it was not possible to ascribe their mating behaviour to being either soft- or hard-shelled. However, in additional research of fecundity of giant crabs (Gardner, 1997b), females were observed with melanised wounds around the gonopore (Fig. 9). These appear to be due to damage from the male's pleopods as melanised lesions in the muscle beneath these wounds had histopathology consistent

with spermatiferous granulomas, as occur in female sheep (Pers. Comm., Barry Munday, University of Tasmania). The sternum of females in intermoult is extremely thick so damage to the female sternum from the male's pleopods could only occur when the female was soft-shelled (although this does not exclude the potential for hard-shelled mating).

Figure 9. Apparent mating scars near the gonopores of a female giant crab *Pseudocarcinus gigas*. The abdomen has been pulled downwards (4 fingers are out of focus at the base of the figure) to expose the sternum. Two black lesions are visible immediately below the gonopores.



Tank observations of females and evidence for annual reproductive cycle

Detailed reproductive history of individual *P. gigas* females held for extended periods without males was presented previously in Chapter 14. In brief, most of the females held in tanks were observed to produce clutches of eggs annually, although females occasionally skipped a season. Skipping a reproductive season appeared to be associated with moulting as 6 of the 31 crabs moulted in January/February (late summer) after failing to produce a brood in the previous winter. General pattern of egg extrusion and hatching the same as observed in wild stocks, that is, eggs were extruded in May/June and hatched in October/November. Twelve of the 31 crabs produced 4 viable broods in successive years without moulting.

The distribution of oocyte area data in samples from February and April 1995 provided additional evidence for an annual cycle of ovarian development. These data were distributed normally (after exclusion of reproductively inactive females), which would not occur if there was a bimodal distribution due to a biennial reproductive cycle. The small error bars for mean oocyte area (Figure 7) for most months are also consistent with a unimodal distribution. Also, all females with broods dissected in October 1994 (late Spring; n=8) had well developed ovaries indicating extrusion would occur the following

Autumn. These results confirmed that females can produce broods annually, although they will occasionally skip a reproductive season.

Discussion

Anatomical features

The gross anatomy of the reproductive tract of female *Pseudocarcinus gigas* conforms to that of other brachyurans (Ryan, 1967; Diesel, 1989; Beninger et al., 1993; Jensen et al., 1996; Nagao et al., 1996). The ovary is bilobed and these lobes are connected by a central commissure, with the posterior lobes connecting to paired spermathecae which are linked to the gonopore by a vagina. The vagina is simple and without a bursa or other accessory structures.

Mating system

Due to the absence of direct observations of mating in *P. gigas*, it is not possible to ascribe conclusively the mating system to either hard or soft-shelled female (i.e. during inter-moult or at post-moult; Hartnoll, 1969). However, indirect evidence indicates that *P. gigas* may copulate while the female is at the soft post-moult stage: the vagina is of the simple type (Hartnoll, 1969); and the scars observed on the sternum of female *P. gigas* were consistent with soft-shelled mating. The apparent lack of a response by males to moulting females does not invalidate soft-shelled mating in *P. gigas* as the males were disturbed by moving them from a different tank, and the females had already commenced moulting before the male was introduced. Also, tank conditions in behavioural trials were far from natural as *P. gigas* inhabits deep water with most of the fishery based between 150 and 200 m.

Although there are exceptions (e.g. hard shelled mating of the portunid crab, *Thalamita sima*; Norman, 1996), the mating system of crabs is generally consistent across family taxa so the system of *P. gigas* is likely to be the same as that of *Eriphia smithii* and *Menippe* species, the only crabs of the family Eriphiidae where the mating behaviour has been reported. *Eriphia smithii* and *Menippe* species (*M. adina*, *M. mercenaria* and hybrids) mate exclusively

during the female's soft post-moult phase (Binford, 1913; Porter, 1960; Savage, 1971; Tomikawa and Watanabe, 1992) and these species are not only in the same family as *P. gigas*, but also the same subfamily (Oziinae). Phylogenetic similarity between *P. gigas* and *M. adina*, *M. nodifrons*, *M. rumphii* and *M. mercenaria* was evident from studies of larval development (Chapter 3; Gardner and Quintana, 1998).

Menippe species have mating behaviour typical of crabs which mate while the female is soft-shelled, such as protracted pre- and post-copulatory mate guarding, and lack of elaborate courtship behaviour (Hartnoll, 1969; Wilber, 1989a). The duration of precopulatory mate guarding appears to be scaled to size within the Cancridae (Orensanz et al., 1995) which suggests mate guarding may be protracted in the massive *P. gigas*. If male *P. gigas* guard females prior to copulation, the duration of this attendance is likely to be longer than for *Menippe* spp. (15 days; Wilber, 1989b; Wilber 1992), and possibly up to 21 days or more as this has been reported for the largest *Cancer* species, *Cancer pagurus* which inhabits similar open habitat in temperate regions (Edwards, 1966).

While it appears likely that *P. gigas* mate while the female is soft-shelled, electrical stimulation of the gonopore of intermoult females causes the gonopore to dilate (unpublished research by Rudolf Diesel and the author). This demonstrates that control of the gonopore is muscular and that hard shelled mating is theoretically possible, although this has also been observed with species which mate exclusively while the female is soft-shelled (R. Diesel, Pers. Comm.).

Sperm competition and the functional anatomy of the spermathecae

Diesel (1991) divided the spermathecae of higher brachyuran crabs into two groups, ventral and dorsal, based on the position of the attachment of the ovary and vagina. Both types of spermathecae are found in the super family Xanthoidea, which includes *P. gigas*, with dorsal spermathecae in the Pilumnidae (Diesel, 1991) and ventral type spermathecae in Eriphiidae (e.g. *Menippe mercenaria*; Wilber, 1989b). The presence of ventral type spermathecae in both *P. gigas* and *M. mercenaria* is consistent with their phylogenetic similarity indicated by larval development as mentioned previously (Chapter 3; Gardner and Quintana, 1998).

Female xanthoid crabs typically have several post-pubertal instars (Tomikawa and Watanabe, 1992) and this occurs in *P. gigas* as moulting was observed of females which had previously produced broods. The progression through several post-pubertal moults by

female *P. gigas* was also indicated by the broad size range of individuals with developing ovaries. Females which moulted were found to have retained the spermathecal contents as reported in *M. mercenaria* (Cheung, 1968). The trend towards an increase in number of presumptive ejaculates with female size may result from additional inseminations at each moult and the position of these presumptive ejaculates in the spermathecae indicates that sperm is displaced dorsally, which would result in last male sperm precedence.

Diesel (1991) described two kinds of sperm plug: internal plugs which seal off previous ejaculates within the spermathecae; and external sperm plugs which extend up the vagina and out the gonopore. These plugs are generally considered to function in preventing paternity by other males (Jensen et al., 1996) although it has also been proposed that the plug is produced by the female (Bawab and El-Sherief, 1989) and that its role is to reduce damage to the female by repeated mating (Diesel, 1991).

A basophilic, proteinaceous matrix without sperm cells or spermatophores was observed within the spermathecae of *P. gigas* females and this appeared to act as an internal sperm plug, sealing off separate ejaculates. However, no external sperm plug was observed despite the presence of an external sperm plug in other crabs in the family Eriphiidae (Tomikawa and Watanabe, 1990). External sperm plugs are short lived and function only within a single receptive period (Orensanz et al., 1995) so it is possible that they are present in *P. gigas*, despite the fact that none were observed in this research. As with *Menippe* species, *P. gigas* may produce 4 broods between moults fertilised by stored sperm so recently mated females may be encountered only occasionally (Porter, 1960; Chapter 14). This is consistent with unpublished analyses of tag recapture data of mature size female *P. gigas* which indicate that moulting may occur only every 4 years or more (R. McGarvey, Pers. Comm.). Consequently, if an external sperm plug is produced during mating, it may only be present in a small proportion of the population for a brief period each year.

It is also noteworthy that no spermatophores were observed within the spermathecae, that is, they had already dehisced prior to sampling. This may be evidence of a prolonged delay between mating and sampling as spermatophores remain intact a considerable time after mating in *Chionoecetes opilio* (Beninger et al., 1988; Beninger et al., 1993), and until after dissolution of the sperm plug in *Portunus pelagicus* (Ryan, 1967).

Female reproductive cycle

Ovigerous giant crabs are found from May/June to October/November (winter and spring) but around 50% of the females captured by commercial fishers during this period are non-ovigerous (Levings et al., 1996). Until 1995, this was interpreted as evidence for a biennial reproductive cycle until females maintained in tanks for the present study produced eggs in successive years, and ovigerous females were found to have developing ovaries (as cited by Levings et al., 1996). Additional evidence of an annual cycle is presented here with distinct annual trends in oocyte diameter and GSI, and no bimodal distribution in oocyte size (as occurs in biennial species; Jensen and Armstrong, 1989). The presence of non-ovigerous *P. gigas* females has been reported in populations of other species with strongly seasonal, synchronised reproduction (e.g. *Cancer magister*, Hankin et al., 1989) and in *P. gigas* it appears to be associated with moulting (Chapter 14). The proportion of non-ovigerous females in the population is likely to be less than the 50% determined by trapping surveys as ovigerous females tend to avoid traps (Howard, 1982).

Seasonal synchronisation of reproduction patterns is typical of temperate decapods (less so in deep water; Haefner, 1978) although there is considerable variation in the duration of the reproductive cycle (Sastry, 1983). Smaller temperate crabs often produce several broods within a seasonally restricted period (Griffin, 1971) while larger crabs may have biannual cycles (e.g. *Chionoecetes opilio*, Sainte Marie, 1993; and *Paralithodes platypus*, Jensen and Armstrong, 1989), and even triannual cycles (*Erimacrus isenbeckii*, Nagao et al., 1996). An annual cycle, as in *P. gigas*, is typical of larger crabs found at similar latitudes (e.g. *Ovalipes catharus*, Armstrong, 1988).

The timing of the female reproductive cycle may also vary with female size so that timing of larval release varies (Attard and Hudon, 1987; Bakir and Healy, 1995). From the analyses of oocyte size conducted in this study, there was no evidence that timing of oviposition in *P. gigas* was affected by female size (as in *Scylla serrata*; Heasman et al., 1985). However, this result is only a general indication as the sample size was small, and the size of extruded eggs is known to vary with female size which could confound analyses (Chapter 16; Gardner, 1997b).

Ovary and hepatopancreas interactions

Both HSI and GSI began to increase in spring, possibly in response to increased foraging behaviour and food supply associated with warmer water. This is supported by the observed decline in moisture content of the hepatopancreas during this period.

Dichotomous trends in moisture content of the hepatopancreas and gonad due to transfer of nutrients during vitellogenesis have been observed in *Scylla serrata*, a tropical species (Nagabhushanam and Farooqui, 1982), but the trends in moisture content of the two organs are similar in *Crangon crangon* and *P. gigas* which are both temperate species (Haefner and Spaargaren, 1993). The effect of gonad development on moisture content of the hepatopancreas may be overshadowed in temperate species by seasonal change in food availability and consumption rate.

HSI peaked in late Summer (February 1995 sample) and then declined while GSI continued to increase. It is tempting to attribute the decline in HSI to the transfer of nutrient reserves from the hepatopancreas to the gonad, and physical displacement by the enlarging gonad, but HSI of reproductively inactive crabs followed the same trend. Overall, HSI was lower in reproductively active crabs than in reproductively inactive crabs, which is expected given the limited volume available within the body of a crab for ovarian development.

Despite large fluctuations in the protein composition of the gonad, there was no evidence of transfer of protein from the hepatopancreas. Protein in the hepatopancreas remained relatively stable, which has been reported in other crabs, and there appeared to be no difference between reproductively active and inactive females (Heath and Barnes, 1970; Nagabhushanam and Farooqui, 1982; Mourente et al., 1994). Percentage protein content of the gonad began to decline several months before extrusion which has been reported in other decapods during late ovarian maturation (Dy-Penaflorida and Millamena, 1990). This relative decline in protein may be due to the deposition of lipid-rich lipovitellin in oocytes (Fyffe and O'Connor, 1974; Clarke, 1979; Castille and Lawrence, 1989).

The absence of lipid analysis in the present study makes comment on the stages of vitellogenesis in *P. gigas* difficult, although the pattern of carotenoid and lipid concentration in the ovary may be linked. This is because carotenoids in decapod ovaries are usually incorporated into vitellogenin molecules and increase in concentration during ovarian development in proportion to the deposition of vitellogenin (Wallace et al., 1967; Fyffe and

O'Connor, 1974; Komatsu and Ando, 1992; Dall et al., 1995). This deposition of carotenoid during vitellogenesis causes the ovary to change in colour which has been used as a guide to ovarian development by numerous authors (e.g. Meredith, 1952; Dayakar and Rao, 1992), although there is no consistent pattern in some species (eg. *Scylla serrata*: Heasman et al., 1985).

The concentration of carotenoids in the gonad of *P. gigas* females increased rapidly from January until oviposition, presumably due to secondary vitellogenesis. Secondary vitellogenesis did not appear to result in depletion of carotenoids in the hepatopancreas, as concentration in reproductively active females was similar to that of reproductively inactive females. This indicates that carotenoid was not depleted from the hepatopancreas during vitellogenesis as has been reported in other decapods (Dall et al., 1995).

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Effect of Size on Reproductive Output of Female Giant Crabs



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Abstract

Fecundity and egg size of giant crabs (*Pseudocarcinus gigas*) were determined from egg masses of 162 crabs sampled from three sites in south-eastern Australia: western Victoria; western Tasmania; and eastern Tasmania. Crabs ranged in carapace length from 126 to 220 mm and egg number ranged from 830,000 to 2,500,000. Egg number and egg size increased with size of female. There appeared to be a decline in number of eggs and size of eggs with successive broods (inferred from carapace-condition) produced between moults. Sampling locality appeared to have little effect on reproductive output. Comparison of regressions of oocyte number in ovaries and number of eggs beneath the abdomen indicated there was no detectable loss of eggs during oviposition. Regression of an allometric model of log egg number to log crab size had a slope of 1.76 which was significantly less than 3.0. This indicates there is not a simple volumetric relationship between the variables, which would tend to occur if increasing fecundity with female size was a simple function of increased body space available for ovarian development. This pattern appeared to be a function of decreasing egg number and size with successive broods, and the trend of increasing egg size with female size.

Ovigerous giant crab *Pseudocarcinus gigas* (photo by Karen Gowlett-Holmes)



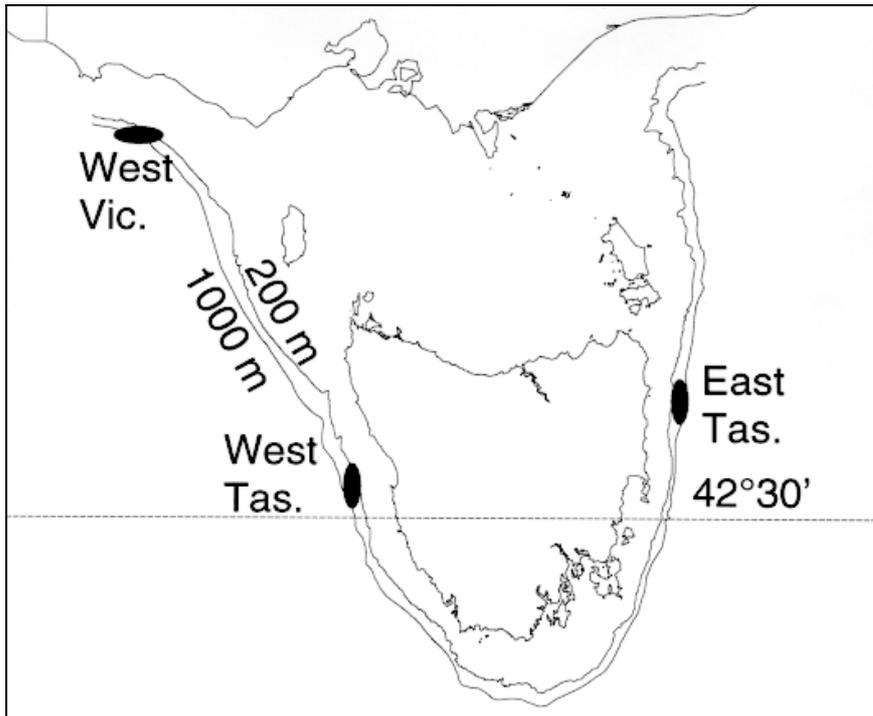
Introduction

Female giant crabs produce broods annually (see previous chapter) and fishery restrictions currently exclude them from harvest while ovigerous. However, unlike many crab fisheries⁹, females may be harvested when they are not carrying broods. As most fishing effort for giant crabs is in February and March, prior to oviposition, exploitation rate of females may be high in some areas. High exploitation of females can affect recruitment so information on fecundity is critical for management.

Information on the fecundity-size relationship can be combined with growth information, size frequency, onset of maturity, and the proportion mature over the size range to develop fundamental management models such as egg per recruit and relative population fecundity (Annala and Bycroft, 1987). The objectives of this study were to assess the effect of the following factors on fecundity in giant crabs: (1) size of the female, (2) regional variation between three widespread sites around south-east Australia (Fig. 1) representative of the major fishing areas, and (3) successive broods produced between moults (moult frequency remains poorly understood). In addition, the effect of these same variables on egg size was examined.

⁹ e.g. *Cancer magister* in USA (Hankin et al., 1996); *Lithodes santolla* and *Paralomis granulosa* in Chile and Argentina (Vinuesa et al., 1996); *Chionoecetes japonicus* in Japan (Kon, 1996); and *Chionoecetes opilio* in Canada (Sainte-Marie et al., 1996).

Figure 1. Location of sampling sites in western Victoria, western Tasmania, and eastern Tasmania. Latitude 42°30'S approximates the southern limit of the fishery for *P. gigas*.



Materials and methods

Sampling

Ovigerous female crabs (n=166) were collected by commercial fishers using traps during the period 10 August to 15 September 1995 from three regions: eastern Tasmania (n=30), western Tasmania (n=121), and western Victoria (n=11) (Fig. 1). As Howard (1982) noted with *Cancer pagurus*, female *P. gigas* feed less when ovigerous and are less likely to enter pots. This behaviour appears to have affected sampling from western Victoria where only 11 crabs were collected despite considerable effort.

In addition to sampling of ovigerous females, 13 females in late ovarian development but before extrusion, were collected for oocyte counts, in April and May 1995, from north-east Tasmania.

Preliminary research in 1994 investigated changes in eggs in relation to development which was categorised by the scheme of Subramoniam (1991). This preliminary study showed that although the size and mass of individual *P. gigas* eggs changed with development, relatively little change occurred in the first 2 stages of embryonic development in: dry weight, 3.4% compared with 11.0% throughout development; diameter, 1.8% compared with 7.5% throughout development; and composition, e.g. moisture, 2.2 % compared with 8.9 % throughout development. Consequently, sampling was restricted to eggs up to and including development stage 2 (Subramoniam, 1991).

The following measures of size were recorded for all specimens: whole weight (with egg mass removed), carapace length (CL, mm), abdomen width, chela length, and chela height. The sample included 21 crabs missing 1 leg and 2 crabs missing 2 legs; the whole weight (W, g) of these individuals was adjusted by correcting for missing limbs with a regression formula, $W = -4076.3 + 37.35[CL] + 0.0325[CL]^2$ (n=138, $r^2=0.95$). Female *P. gigas* appear to produce clutches annually, and several clutches of eggs may be produced between moults (see chapters on sperm limitation and female reproduction). It was considered that fecundity may be altered in these successive clutches so a measure of shell wear was used to quantify time since the previous moult. Shell wear, or “carapace-condition”, was recorded using the following scheme:

Carapace-condition 1	clean bright shell; little to no fouling. If gooseneck barnacles present, then < 5 mm across longest axis, little to no wear apparent on the dactylus of pereopods.
Carapace-condition 2	bright shell; fouling often heavy but composed almost entirely of gooseneck barnacles; wear apparent on the dactylus of pereopods with the bristles completely removed in places.
Carapace-condition 3	shell is often faded; fouling is heavy and is composed of many organisms besides gooseneck barnacles, especially colonial ascidians and bryozoans; the dactylus of pereopods is heavily worn with abrasion on the shell surface.

It is important to note that the carapace-condition grade does not provide a measure of the actual number of clutches produced by a female since moulting. For instance, it is assumed that a carapace-condition 3 (heavy wear) female is likely to have had more clutches of eggs

since moulting than a carapace-condition 1 female (clean shell), but it does not imply that the crab has produced 3 broods.

Derivation of egg counts

Half of each egg mass was removed from the crabs by severing four of the eight pleopods at their base in an alternate fashion, to remove possible bias from uneven distribution of eggs between pleopods. Eggs were then removed from the pleopods by severing setae at the point of junction with the pleopods and pooled.

Mean individual egg dry weight was calculated based on the weight of two sub-samples of at least 250 eggs, counted and then weighed collectively ($\pm 10\mu\text{g}$). Samples were dried at 80°C for 24 h, with a final 1 h vacuum period. Dry weight of the whole egg mass was estimated by weighing the blotted egg mass and then calculating mean moisture content from two sub-samples of 1.5 g. Total number of eggs per female was then estimated from the values of mean individual egg dry weight and the dry weight of the whole egg mass.

Derivation of oocyte counts

The number of oocytes was compared with the number of eggs in abdominal clutches (after correcting for female size) to assess realised reproduction. That is, what proportion of the oocytes were lost during attachment to pleopod setae.

Whole ovaries were removed by dissection and weighed to obtain whole wet weight. Number of oocytes in ovary was estimated for each sample as described for abdominal egg counts except that portions of the ovary were first fixed in Davidson's fixative. This hardened the yolk so that oocytes could be teased apart and counted. Fixation resulted in leaching of some components from the oocytes, so it was necessary to calculate a correction factor by weighing the sub-sample before and after fixation.

Egg diameter

Sub-samples were taken from each abdominal egg mass and teased apart in sea water. The diameter of 50 eggs were then recorded by image analysis using NIH-Image™ 1.60

software. Only those eggs which were round and appeared normal were measured. Egg volume was calculated from egg diameter.

Statistical analyses

Four crabs, from western Tasmania, were excluded from analyses as they had few eggs and were not representative of the population. Three had a single small chela which appeared to be partially regenerated; chelae loss before extrusion has been shown to affect brood size so these individuals were excluded (Norman and Jones, 1993). The fourth crab had eggs attached to only a small proportion of available pleopod setae which suggested that extrusion may have taken place after capture and without a natural substrate present (Shields, 1991).

The relationship between egg number and size was assessed with an allometric model of log fecundity (abdominal egg number) and log size (carapace length) as recommended by Somers (1991). Somers (1991) noted that the theoretical slope of linear regressions of log fecundity–log crab size should equal 3.0 as the relationship should be simply volumetric. Analysis of factors affecting the slope can provide additional information on the reproductive biology and this technique was applied to *P. gigas*. Analysis of factors affecting the slope of this model was by *t*-tests.

In attempt to explain patterns of fecundity for fisheries management, the log fecundity–log size model was compared with models which included additional effects of carapace-condition, site, and interactions, using *F*-tests on the residual sum of squares (Draper and Smith, 1981). All additions to the model were tested using the residual mean square from a model incorporating all possible terms as the scale factor. This stepwise multiple regression is the same as that used for developing fecundity models currently in use for managing the Southern Rock Lobster *Jasus edwardsii* fishery (R. Kennedy, Tasmanian Department of Primary Industry and Fisheries, Pers. Comm., 1997).

The effect of female size, site, carapace-condition, and interactions on individual egg dry weight and diameter were also assessed by stepwise multiple regression. As with egg number, data were log transformed to account for allometric relationships between reproductive traits. Additional curvature in regressions, after transformation, was tested by including polynomial functions in stepwise multiple regression (Sokal and Rohlf, 1981).

Where the categorical effects of site and carapace-condition were found to reduce error significantly ($P < 0.05$), comparisons within the effects were made by correcting for size effects with analysis of covariance (ANCOVA). Comparisons could then be made between separate sites or carapace-condition by contrasts.

Effect of size of crabs on carapace-condition was assessed by one way analysis of variance (ANOVA); where crab size had a significant effect the individual means were compared by Tukey-Kramer HSD. Where appropriate, power details (as β , PC type II error) are supplied for non-significant results (Searcy-Bernal, 1994).

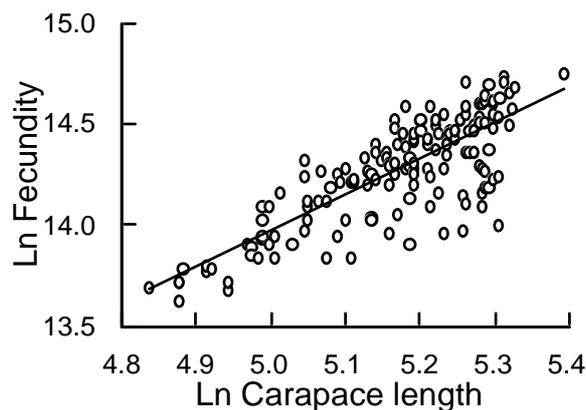
Comparison between oocyte number and egg number were made by comparing regression elevation differences with ANCOVA to correct for size of female (Sokal and Rohlf, 1981). All analyses were performed with JMP™ 3.1 software.

Results

Fecundity model

Egg number increased with adult size for all size measures used: whole weight, carapace length, abdomen width, chela length, and chela height (Appendix 11). As variation was similar for each measure of size, carapace length was selected for use in subsequent analyses as this measure of size is already used in the fishery for describing size limits (Fig. 2).

Figure 2. Effect of body size of female giant crabs, measured as carapace length (mm), on fecundity (number of eggs). Trends were similar for all other measures of crab size: weight, abdomen width, chela length, and chela height.



The number of eggs produced by each female ranged from 706,000 (carapace length = 131 mm) to 2,545,000 (carapace length = 203 mm) with a mean of 1,575,000. Sample data from the different sites are summarised in Table 1. The hypothesis that increase in size leads to no increase in egg number was tested and rejected ($P < 0.001$). Additional terms were then added to the model to attempt to explain variation. Inclusion of the effect of site in the model did not significantly improve model fit ($P > 0.1$, $\beta = 0.79$) so site was not included in subsequent analyses. Inclusion of carapace-condition in the model significantly reduced the unexplained error compared to the model with carapace length only ($P < 0.001$). This was due to a trend of decreasing fecundity with deteriorating carapace-

condition (Table 2; Fig. 3). The effect of including a term for extra curvature ($(\ln \text{carapace length})^2$) was tested but did not significantly improve model fit ($P > 0.3$, $\beta = 0.13$).

Table 1. Characteristics of female giant crabs from three sites in southern Australia. Data (mean \pm s.d.) for three grades of carapace condition pooled. Significantly different sites denoted by superscripts in ascending order of intercept on regression ($P < 0.05$). Significance of difference in female size between sites not tested; female size used only as covariant to establish the effect of site on reproductive output. NS, not significant; β , power.

	Site			P
	East Tasmania (n=30)	West Tasmania (n=117)	West Victoria (n=11)	
Carapace length, mean \pm SD (mm)	156 \pm 13.7	183 \pm 17.0	147 \pm 16.6	-
Weight, mean \pm SD (g)	2488 \pm 617.8	3859 \pm 915.4	2072 \pm 644.4	-
Egg number, mean \pm SD (millions)	1.35 \pm 0.294	1.67 \pm 0.435	1.07 \pm 0.407	NS $\beta = 0.26$
Egg number, coefficient of variation	31.94	26.93	34.20	-
Egg mass dry weight, mean \pm SD (g)	76.5 \pm 26.2	99.1 \pm 26.2	69.6 \pm 28.7	NS $\beta = 0.45$
Individual egg dry weight, mean \pm SD (μ g)	56.37 \pm 5.03	58.17 \pm 4.55	56.64 \pm 5.88	NS $\beta = 0.06$
Egg diameter, mean \pm SD (μ m)	562.6 \pm 22.5 ^a	600.0 \pm 26.2 ^b	579.2 \pm 23.1 ^b	<0.001

β is power

Table 2. Summary of characteristics of females of carapace-condition grades 1, 2 and 3 Data (mean \pm s.d.) for three sites pooled. For qualitative carapace-condition grades, see text. Significantly different carapace conditions denoted by superscripts in ascending order ($P < 0.05$). Analysis of the effect of carapace-condition on egg measures by ANCOVA to correct for the significantly different carapace length of females. For egg mass dry weight, the pattern of ascending superscripts relates to intercepts of the model, rather than to mean values, because this corrects for carapace length.

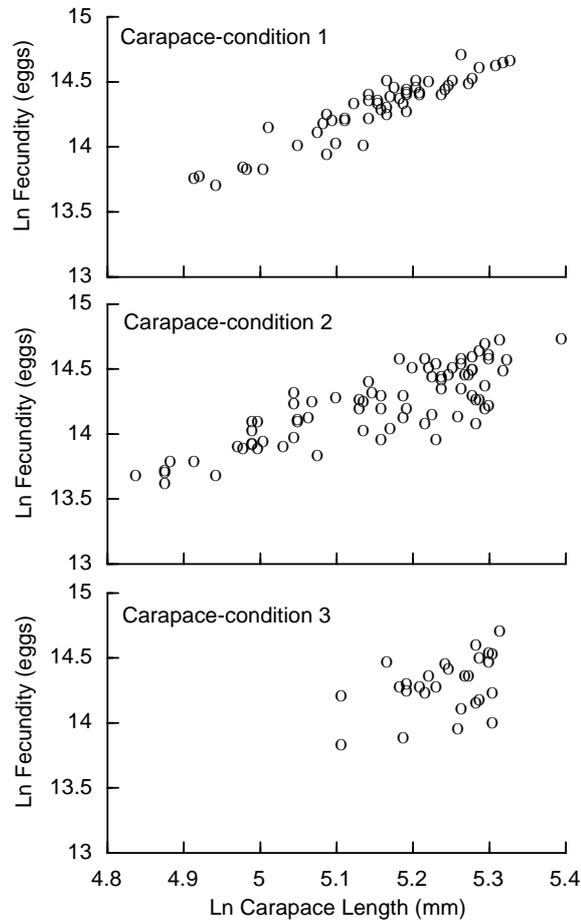
	Carapace-condition			P
	1 (n=53)	2 (n=78)	3 (n=27)	
Carapace length, mean \pm SD (mm)	174 \pm 17 ^a	173 \pm 22 ^a	190 \pm 10 ^b	<0.001
Weight, mean \pm SD (g)	3462 \pm 982 ^a	3422 \pm 1110 ^a	4194 \pm 609 ^b	<0.01
Egg number, mean \pm SD (millions)	1.64 \pm 0.40 ^b	1.53 \pm 0.52 ^b	1.57 \pm 0.43 ^a	<0.0001
Egg number, coefficient of variation	24.42	33.71	27.15	-
Egg mass dry weight, mean \pm SD (g)	99.7 \pm 26.6 ^c	89.1 \pm 29.6 ^b	92.4 \pm 26.3 ^a	<0.001
Individual egg dry weight, mean \pm SD (μ g)	59.89 \pm 3.56 ^c	56.72 \pm 4.69 ^b	56.61 \pm 5.74 ^a	<0.0001
Egg diameter, mean \pm SD (μ m)	597.2 \pm 21.49	586.8 \pm 33.78	594.0 \pm 26.32	NS $\beta = 0.66$

β is power.

Table 3. Parameter estimates of fecundity models for female giant crabs. $M_{y,x} = \beta_0 + [CC]_i + \beta_1 \ln [CL]$, where CC is carapace condition, CL is carapace length and i is 1, 2, 3 using \ln transformed fecundity. Correction factor for converting the results of the model back to the original scale of measurement is 1.0045819 for the full model, and 1.0119281 for the model with length alone, i.e. predicted values (as numbers of eggs) are ($e^{M_{y,x}}$) 1.0045819.

Parameter	Full model		ln length	
	Estimated value	SE	Estimated value	SE
β_0 (intercept)	4.598535	0.56149	5.202213	0.56429
β_1 (slope)	1.868524	0.10831	1.754983	0.10915
Carapace-cond. offset: grade 1;	0.070893	0.01841		
grade 2;	0.017130	0.01713		
grade 3	0			

Figure 3. Relationship between Ln fecundity and Ln carapace length for each carapace-condition in female giant crabs.

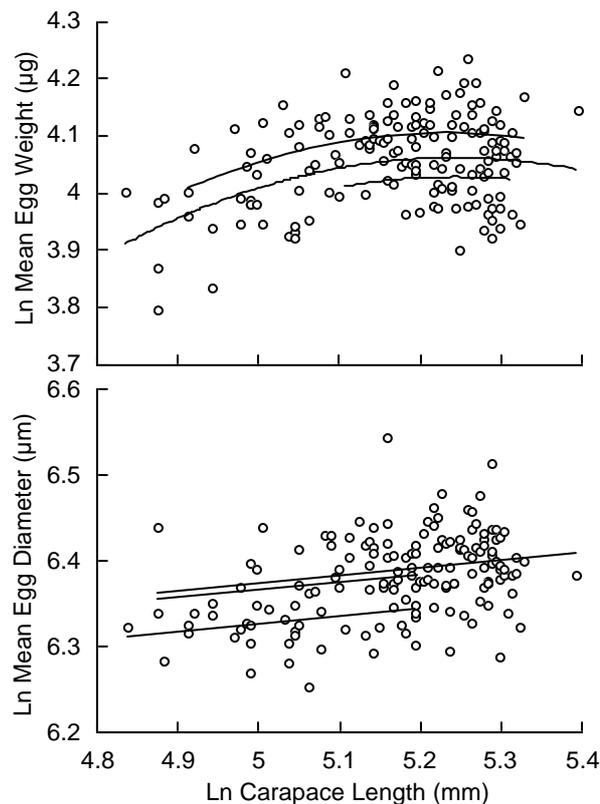


In summary, the most accurate model for describing the egg number-size relationship incorporates the effects of log carapace length and carapace-condition (Table 3). The slope of this model was significantly less than 3.0, based on the associated 95% confidence limits (Table 4). Most of the model error was explained by inclusion of size with only a relatively slight improvement in fit achieved by incorporating carapace-condition (4%). As improvement in fit with carapace-condition was relatively small, albeit significant, model parameters are also presented for the model with carapace length alone (Table 3). A summary of the effect of carapace-condition on reproductive output is outlined in Table 2.

Effect of female size on egg size

Although there was a great deal of variation, the dry weight of individual eggs was significantly affected by female size ($P < 0.0001$; Fig. 4). Incorporation of carapace-condition into the model for mean egg weight significantly improved fit ($P < 0.0001$) while site did not ($P > 0.05$, $b = 0.12$). All grades of carapace-condition were significantly different and there was a trend of declining egg size with a deterioration in carapace-condition (Table 2). Including a term for extra curvature ($(\ln \text{ carapace length})^2$) significantly improved fit ($P < 0.05$), suggesting the increase in egg size with female size levels out as the females become larger.

Figure 4. Effect of size of female giant crab (carapace length) on egg size. (a) Effect of maternal size on egg dry weight: upper regression, carapace-condition 1 ($r^2 = 0.17$), mid regression, carapace-condition 2 ($r^2 = 0.18$), lower regression, carapace-condition 3 ($r^2 = 0.10$). (b) Effect of maternal size on mean egg diameter: upper regression, western Tasmania ($r^2 = 0.09$), middle regression, western Victoria ($r^2 = 0.85$), lower regression, eastern Tasmania ($r^2 = 0.08$).



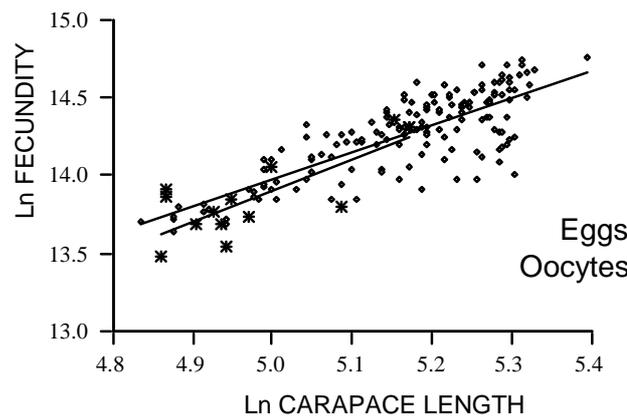
Mean egg diameter increased significantly with size of the female ($P < 0.05$; Fig. 4), and terms for extra curvature ($\beta = 0.29$) or carapace-condition ($\beta = 0.56$) did not significantly

improve fit ($P > 0.05$). The model was significantly improved by including site ($P < 0.001$). This resulted from significantly smaller diameter eggs in the eastern Tasmania samples while eggs from western Victoria and western Tasmania were not significantly different ($P > 0.05$).

Realised reproduction

There was no significant difference between the number of oocytes in the ovary and the number of eggs held under the abdomen which suggests that there is no egg loss during oviposition (Fig. 5; $P > 0.6$, $\beta = 0.16$).

Figure 5. Comparison between fecundity estimated by the number of mature oocytes in the ovary, and the number of eggs held under the tail after oviposition. Linear regressions against \ln carapace length are shown: the lower regression is for oocytes.



Analysis of factors affecting slope

The slope of the allometric model of log carapace length–log fecundity, was 1.76 and significantly less ($P < 0.05$) than the theoretical value of 3.0 (Somers, 1991). This appeared to be due to the observed decline in egg number with deterioration in carapace-condition and the observed increase in egg size with female size (Table 4). Both of these factors were reassessed by including carapace-condition and total egg volume (egg number by egg volume) in the model as independent and dependent variables respectively. Although inclusion of these factors produced a slope of 2.63, this was still significantly less ($P < 0.05$) than the theoretical value of 3.0.

Table 4. Regression statistics of reproductive output to evaluate whether the relationship between carapace length (CL) and fecundity (F) is volumetric with a slope of 3.0.

Model 1a: simple allometric model of F predicted by CL; model 1b predicts total egg-mass dry weight (W) from CL. Models 2a and 2b: as 1a and 1b, but with the addition of carapace-condition (CC) as an independent variable. Model 3 predicts volume of the whole egg-mass as the product of individual egg volume (V_e) and F from CL and CC. All continuous variables were log transformed. All models were tested against the null hypothesis (H_0) that the slope (β_1) was not different from 3.0.

Model	Slope (β_1)	95% Conf. Limits on slope (L1-L2)	$H_0; \beta_1=3.0$ (t-test)	r^2 value
1a) $y = \ln F; x = \ln CL$	1.76	1.55-1.97	Reject	0.635
1b) $y = \ln W; x = \ln CL$	2.25	1.95-2.56	Reject	0.581
2a) $y = \ln F; x = \ln CL \ \& \ CC$	1.87	1.66-2.08	Reject	0.675
2b) $y = \ln W; x = \ln CL \ \& \ CC$	2.40	2.10-2.69	Reject	0.638
3) $y = \ln (V_e \times F); x = \ln CL \ \& \ CC$	2.63	2.31-2.95	Reject	0.647

Discussion

Site

The absence of a site effect on number of eggs and egg dry weight suggest that the models listed (Table 3) are appropriate for stocks throughout Tasmania and through to western Victoria. Fishing grounds for *P. gigas* in Tasmania are roughly split into western and eastern regions with few animals captured in Bass Strait or below 42°30'S on the east coast of Tasmania. Consequently, sample sites were from the extreme ranges of the fishery for these States. Site had a significant effect on egg diameter ($P < 0.001$) with females from eastern Tasmania ($n=30$) producing smaller eggs than those from the other two sites ($n=132$). This observed effect of site on egg diameter is surprising as individual dry egg weight did not appear to be affected by site. It is important to note that the effect of site on

diameter was very weak, although significant, so there is unlikely to be any biological implication.

Effect of carapace condition on reproductive output

The significant improvement in model fit of log length-log fecundity by incorporating carapace-condition indicates that there is a decline in fecundity with successive broods within an instar.

The effect of successive broods between moults on fecundity has been examined previously in snow crabs, *Chionoecetes opilio* (Majidae) which terminally moult into maturity and produce only two broods. Sainte-Marie (1993) observed an increase with consecutive clutches (opposed to a decline in *P. gigas*) in the order of 20% per brood. The relationship in *P. gigas* is more complex as there does not appear to be terminal moult into maturity, so broods are produced at several instars. This pattern is similar to most *Cancer* species where viable sperm may be retained for greater than 2 years and used to fertilise successive broods without moulting. A pattern of declining fecundity for successive broods produced between moults, as in *P. gigas*, also occurs with *Cancer magister* and *Cancer anthonyi* (Hankin et al., 1989; Shields et al., 1991).

Some females in carapace-condition groups 2 and 3 appeared to have lower fecundity than those in group 1. Biological causes for this observed effect of carapace-condition on fecundity may include: damage to pleopods that may physically prevent a crab from carrying a full egg mass (Hankin et al., 1989); reduced ovarian development in response to depletion of spermatophore reserves from previous extrusions (Hankin et al., 1989); and senescence of older females. The potential for damage to pleopods to impair attachment of eggs in carapace-condition 3 crabs could not be assessed from information collected in the present study, but Hankin et al. (1989) did observe short pleopods on several non-moulted *C. magister* females. Large, carapace-condition 3, *P. gigas* females have been held in tanks to extrude their broods and no egg loss appeared to occur (Gardner, unpublished).

Heavily fouled, grade-3 *P. gigas* females were significantly larger than other grades (Table 2) and are unlikely to have natural predators except during ecdysis. Consequently, it is feasible that this group does contain senescent females. Large female European edible crabs (*Cancer pagurus*) are also often heavily fouled and termed “granny crabs” (Pearson,

1908) which has been attributed to both prolonged intermoult duration and senescence (Edwards 1979). However, as with *C. magister* and *C. anthonyi*, decline in fecundity of *P. gigas* with successive batches of eggs produced between moults confounds detection of senescence (Hankin et al., 1989; Shields, 1991).

While the decline in fecundity of grade 3 females may be an effect of senescence, this is unlikely to be the case with grade 2 females. The latter were well represented across the size range collected, but many had significantly smaller clutches than did grade 1 females ($P < 0.05$). If there is a limiting factor on reproductive output, such as remaining sperm reserves, there are advantages to a strategy of reducing clutch size to optimise future survival, rather than risking mortality during moulting to re-mate (Begon and Parker, 1986). Mating has never been observed in *P. gigas*, but there is indirect evidence that it occurs while the female is soft-shelled (see previous chapter).

The effect of carapace-condition on fecundity is important when modelling fecundity for fishery management as it suggests that changes to stocks, other than those detected by monitoring size of individuals, have the ability to affect egg production.

Egg size

Size of individual eggs did not remain constant but declined significantly, albeit slightly, with deterioration in carapace-condition and increased with carapace length ($P < 0.0001$). Change in individual egg size with deterioration in carapace condition and increase in CL has also been reported in *Chionoecetes opilio* in which older, multiparous females produced smaller eggs (Sainte-Marie, 1993). Change in egg size with female size is important if the amount of nutrient reserves available to the embryo is increased (Clarke, 1993). This has been demonstrated in the lobster *Homarus americanus* where larger females produce eggs with greater energy content (Attard and Hudon, 1987). Although significant, the effect of female size on egg size in *Pseudocarcinus gigas* was weak and there was considerable variation between individuals; consequently, female size on egg size may have little effect on nutrient reserves.

Differences in egg size may affect not only the nutrient reserves available to the larvae, but may affect timing of larval release (Wear, 1974). It appears that even small differences in egg size can have profound effects on egg development; Sainte-Marie (1993) noted that

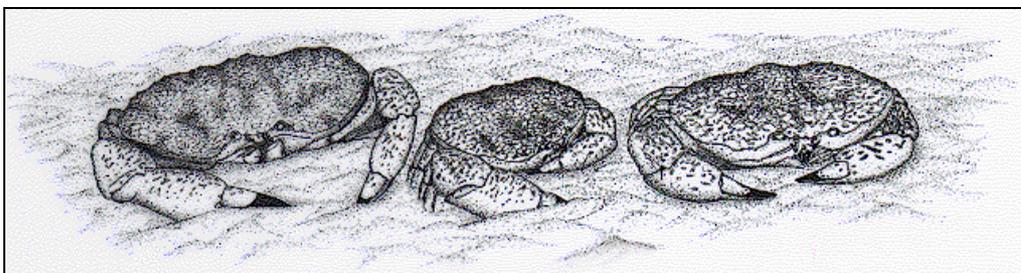
development takes around 3 months longer in primiparous *C. opilio*, with larger eggs, than in multiparous snow crab females, even though egg diameter differed by only 1.4-2.7%. This suggests that the observed effect of female size on egg size in *P. gigas* may influence larval release, although it was not possible to assess this in the current study.

Realised reproduction

Corey (1991) listed factors that may cause loss of eggs at oviposition in crustaceans: incomplete extrusion of eggs; infertile eggs; lack of proper attachment of eggs to pleopods; and the extrusion of too many eggs for attachment. Comparison between counts of oocytes and eggs held under the tail indicated that none of these factors contribute to significant egg loss in *P. gigas*. Corey (1991) speculated that the most important factor affecting attachment of eggs in the freshwater crayfish, *Orconectes* spp, was rapid water movement.

Female *P. gigas* held for long term sperm retention trials (see Chapter 14) were held in a 200 m³ tank with a 15 cm deep sandy substrate and observed during oviposition. Females buried their abdomens in substrate during oviposition and remained in this position for several weeks (Fig. 6). This behaviour has been observed in other brachyurans and is known to reduce loss of eggs during oviposition (Crothers, 1969; Wear, 1974; Edwards, 1979; Wild, 1983; Shields, 1991; Shields et al., 1991).

Figure 6. Female giant crabs *Pseudocarcinus gigas* during oviposition with their abdomens buried into pits in the substrate.



Relative reproductive expenditure with increase in female size

As would be expected, egg number increased with size of the female. With log transformed data, the slope of this relationship has a theoretical value of 3.0 as egg number is a volumetric measure while size is linear (Somers, 1991); however, in *Pseudocarcinus gigas*, the slope was 1.76 and significantly less than 3.0 ($P < 0.05$). Somers (1991) postulated that values less than 3.0 could be caused by: separate age classes; changes in the proportionate size of the ovaries relative to the female; change in egg size relative to female size; and senescence. Additional factors which may have been important in this study were the effects of site, and successive clutches between moults.

Of these hypotheses, it was possible to assess the effect of change in egg size relative to female size, site, successive clutches between moults, and to some extent the effect of separate age classes. There was no evidence that site affected fecundity as it did not improve the regression fit. Likewise the inclusion of a term for extra curvature did not improve fit, suggesting that there was no distinct trend of separate age classes as shown by Wenner et al. (1987) in *Emerita analoga*. However, by adjusting for carapace-condition (or possibly senescence as discussed above) the slope was raised (see effect of carapace-condition, Table 4). Also, there were indications that changes in the size of eggs with size of females was affecting the slope, based on comparisons between regressions for log fecundity and log total egg mass dry weight, the latter having higher slopes. This would be expected given the observed relationship between female size and egg size in *P. gigas* (Fig. 4); if a female crab is producing larger eggs then fewer could be contained within the space available to the ovary within the body cavity. This hypothesis was supported as slope of the regression for total egg volume (number of eggs x egg volume) was 2.63, and far closer to the theoretical value of 3.0, although still significantly less ($P < 0.05$).

In summary, the fecundity of female giant crabs did not follow a simple volumetric relationship; the relationship is affected by declining fecundity between successive clutches, and also by an increase in egg size with female size. Despite correcting for both these factors, the slope was still less than 3.0 which may be due to error from the coarseness of the measure of time since moulting by carapace-condition, or there may be an additional biological process involved. If moulting occurs in periods of greater than 3 years then carapace-condition may mask patterns as this was only divided into 3 groups. Also, if senescent females were present, they were likely lumped with fouled, but healthy, females

into carapace-condition 3. While there is a general assumption that clutch size should increase with female size, optimising reproductive output by optimising future growth and survival can lead to a declining clutch size with age (Charlesworth and Leon, 1976; Begon and Parker, 1986); such a biological pattern may be operating with *P. gigas* females.

Additional factors affecting fecundity

All samples for this study were collected in the early stage of embryonic development, as preliminary trials had shown that variation in individual egg weight was least during that period. This prevented analysis of possible decline in brood size through development which has been shown to be important in other crustacean species (Annala and Bycroft 1987; Kuris and Wickham 1987). Nemertean and amphipods were observed in eggs sampled from *P. gigas* so a decline in brood size during development from predation is possible.

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*Composition of Eggs in Relation to
Embryonic Development and Female
Size*



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Abstract

The size and composition of eggs from 22 giant crabs *Pseudocarcinus gigas* were monitored over 165 days to determine trends through embryogenesis. Egg composition was most stable during the early stages of embryogenesis so additional sampling (n=143) was conducted during this period to assess the effect of female size, sampling location (east and west Tasmania), and successive broods between moults, on egg composition. During embryogenesis, eggs increased in diameter and moisture content while organic dry weight declined. Total carotenoid content did not change significantly while protein declined and lipid increased (as proportion of ash-free dry weight). This indicates that protein was used preferentially to lipid which is atypical of most decapods and may be an adaptation to the deeper water habitat of *P. gigas*. Females with heavy and intermediate carapace wear were considered more likely to have produced previous clutches and they produced eggs with significantly less carotenoid. The eggs of larger females contained significantly more water, less protein, and less carotenoid while there was no effect on total lipid ($P < 0.05$). Although the effects of female size on egg composition were significant, the magnitude of the effect was small ($r^2 \leq 0.17$). Consequently, it is unlikely that larval viability is affected, or that larger females contribute more to recruitment than predicted by fecundity.

Introduction

Yolk reserves of crustacean eggs are critical to the development of the embryo and subsequent larvae. The pattern of utilisation of reserves varies between species and there is evidence that this inter-specific variation is an effect of environmental adaptation (Pandian, 1970; Pillai and Subramoniam, 1985). Ecophysiological research on yolk utilisation in decapods has been based on freshwater, inshore coastal, and terrestrial species while this chapter describes egg development and yolk utilisation in the deeper water, oceanic decapod species, *Pseudocarcinus gigas*. Given that the demands on an emerging larva in deep, oceanic water are likely to be different to those of a larva hatching in inshore areas, it was anticipated that yolk utilisation may be affected.

In commercial crustacean species, understanding factors affecting egg production is important as these may influence recruitment. Egg size has been shown to vary within species in response to a range of factors including depth (Thessalou-Legaki, 1992), region (Collart and Rabelo, 1996), female size (Attard and Hudon, 1987), and production of successive broods (Sainte-Marie, 1993). Earlier work on fecundity of giant crabs demonstrated that egg size increased with female size (previous chapter; Gardner, 1997). In addition, egg size of *P. gigas* appeared to decline with successive clutches of eggs produced between moults. It was hypothesised that these differences in egg size may influence larval viability and subsequent larval recruitment.

Although these observations on egg size of *P. gigas* indicated effects of female size and successive broods on reproductive output, they are not definitive. As noted by Clarke (1993), the assumption that larger eggs contain more nutrient is usually intuitive and is rarely tested. While egg size has been shown to reflect real differences in investment per egg by polar shrimps (Caridea; Clarke, 1993), this relationship is not present in all invertebrates and cannot be assumed (McEdward and Coulter, 1987).

The most direct measure of the “quality” of eggs, and the effect of female size or successive broods, is a measure of larval size or vitality. This can be difficult to manage, especially in larger crustaceans like the giant crab, as females must be housed separately until larval release. An indirect technique to assess egg quality was employed by Attard and Hudon (1987) in an investigation of the effect of female lobster *Homarus americanus* size on egg quality. They assessed egg composition as energy content and showed that the expenditure per egg was greater by larger females than smaller females. This suggests that larger female lobsters may contribute more to recruitment than would be predicted by fecundity alone. Similar research was conducted on giant crabs and is described in this chapter. The composition of eggs of giant crabs was analysed to test if the effects of female size and successive clutches on egg size represent real differences in parental contribution (in other words, do bigger female giant crabs produce better eggs?).

Materials and methods

Collection of samples

Two sets of samples were collected: the first was for the assessment of changes in egg composition during embryogenesis, and the second was for assessment of female size on egg composition. For the first, 22 ovigerous females were captured in traps from depths in the range of 300 – 380 m off the east coast of Tasmania (41°15'S;148°40'E) in May 1994 by a commercial fisher. Ovigerous females had only just begun to be observed by fishers so these egg masses were regarded as recently extruded. Females ranged from 2.2 – 3.5 kg and were maintained in two 4 m³ tanks with flow through water supply and fed twice weekly. Hatching occurred over a period of 2 weeks in November 1994, during which females were checked every two days to allow the date of egg sample collection to be back-calculated, relative to hatching. Females were individually tagged and samples of eggs (around 30 g) were removed at 165, 125, 75, 50, and 20 d before hatching (averaged across sample).

This first set of samples delineated a period during development when egg composition was relatively stable; sampling of females to determine the effect of female size was then conducted during this more stable period. Development of eggs was staged by a qualitative scheme (Table 1).

For the second set of samples, ovigerous female crabs (n=143) were collected during the period 10 August to 15 September 1995 from eastern Tasmania (n=30), and western Tasmania (n=113). These were the same samples used previously to determine fecundity (see Fig. 1 in Gardner, 1997). Half of the egg mass was removed from each female for analysis of composition (development of individual eggs was homogeneous within the egg mass). Four of these females were captured with more advanced eggs but all eggs retained for analysis were at development stage 2 (Table 1).

Female *Pseudocarcinus gigas* produce broods annually and appear to produce several broods between moults (Chapter 14). The time since moulting was roughly quantified by assigning a carapace condition grade ranging from grade 1, clean shelled, to grade 3, heavily worn

and fouled (Gardner, 1997). This carapace condition grade was not intended to provide a direct scale of years since the previous moult, rather, it simply assumes that a heavily fouled female is more likely to have produced previous broods than a clean shelled female.

Table 1. Classification of egg development in *Pseudocarcinus gigas*

Stage	Description
I: recently extruded	Eggs bright orange with no embryo pigmentation. The egg is translucent and contains evenly distributed yolk granules.
II: early developmental	Eggs bright orange; a clear, yolk-free streak evident at one pole; yolk granules evenly distributed throughout.
III: intermediate I	Eggs dull orange; a quarter of the yolk mass is cleared; some structural development of embryo; no black pigmentation; some faint red eye pigmentation.
IV: intermediate II	Eggs dull orange; embryo eye pigmentation visible as small, grey/black patches; the inner yolk sac appears slightly detached from the surrounding capsule.
V: late stage	Eggs brownish orange; eye pigmentation is black; embryo well formed; heart beat is obvious and scattered pigmentation is visible; some yolk remains.
VI: pre-hatch	Eggs brownish orange to burgundy; embryo fully formed; pigmentation more defined with lines on the abdomen; small amount of pale yolk; frequent movement.

Analysis of egg size and composition

For measuring diameter, sub-samples were teased apart in sea water and the diameter of 50 eggs was then recorded by image analysis using NIH-Image™ 1.60 software. *Pseudocarcinus gigas* eggs are round and only those eggs which appeared normal were measured.

All analyses were duplicated. Eggs were blot dried before weighing to obtain an initial wet weight. To determine water content, eggs were dried at 80°C for 24 h, and cooled for 1 h under vacuum before weighing. Samples of around 2 g were ashed at 450°C for 2 h. Mean individual egg dry weight for each brood was obtained by counting at least 250 eggs which were then rinsed in distilled water, dried, and weighed. Samples for biochemical analyses were stored at -60°C then thawed and ground in a mortar to a homogeneous paste.

Protein was assayed by a modified Lowry procedure (Peterson, 1977; Sigma Diagnostics™ #5656; Appendix 9). Total lipid was measured by the gravimetric method of Folch et al. (1957) using chloroform and methanol as solvents (Appendix 9).

Carotenoids were extracted from tissue with acetone (Appendix 9). The acetone extract was partitioned with diethyl ether which was washed with 20 volumes of 10% NaCl to remove residual acetone. Four samples of carotenoid extract from both early and late development stage eggs of the same females were chromatographed by thin layer chromatography (TLC) on C8 octyl silica plates (Merck™) using a solvent mixture of 95 : 5 petroleum ether : methanol. Tissue extracts were run alongside saponified extracts (5% ethanolic KOH for 24 h at room temperature) and astaxanthin standard (Roche Pharmaceuticals™). Carotenoids present in extracts were identified as predominantly astaxanthin or astaxanthin esters with low levels of a rapidly eluting red pigment, possibly β carotene, and an unidentified yellow pigment. Consequently, total carotenoids were estimated from the extracts as astaxanthin by measuring their absorption in diethyl ether at 472 nm assuming an $E_{1\%}^{1\text{cm}}$ of 2099 (Clarke, 1977).

Data analysis

The effect of embryonic development on egg size and composition was tested by repeated measures analysis as the same females were sampled throughout the trial (Mardia et al., 1979). Significance was tested by Wilk's lambda (Mardia et al., 1979).

Effect of female size on egg size and composition was initially tested by simple linear regression. Additional analyses were then conducted to assess the effects of site and carapace-condition by analysis of covariance (ANCOVA), after first establishing equality of slopes by testing for interaction with female size (Sokal and Rohlf, 1981). Where the results of ANCOVA were significant, elevations of separate carapace-condition classes were compared by t-tests. All analyses were performed with JMP™ 3.1 software (SAS Institute).

Results

Changes in egg size, mass, and composition during embryogenesis

Relatively little embryonic development occurred during the first 90 d of incubation with the majority of egg masses sampled at 75 d before hatch being at development stage 2 (Fig. 1). By 50 d prior to hatch, most egg masses had progressed to stage 3 with a quarter of the yolk cleared. Embryogenesis appeared to proceed more rapidly in the last 50 d with most yolk utilised by 20 d.

Embryogenesis resulted in a significant change in egg diameter ($P < 0.001$); individual egg dry weight ($P < 0.01$); moisture ($P < 0.001$); protein ($P < 0.001$); and lipid ($P < 0.01$; Fig. 2). Greatest rate of change in the water, protein, and lipid composition appeared to occur during the period of most rapid yolk depletion: between 50 d and hatch (Fig. 2). Mean egg dry weight declined during development while diameter increased. Total carotenoid (as ash free dry weight) did not change significantly during embryogenesis ($P = 0.22$).

Figure 1. Effect of embryogenesis on egg diameter, weight and composition (moisture, protein, lipid, and carotenoid; n=22). Moisture content is presented as percentage of wet weight while protein, lipid, and carotenoid are presented as proportion of ash-free dry weight. Days prior to hatch was averaged across each sample.

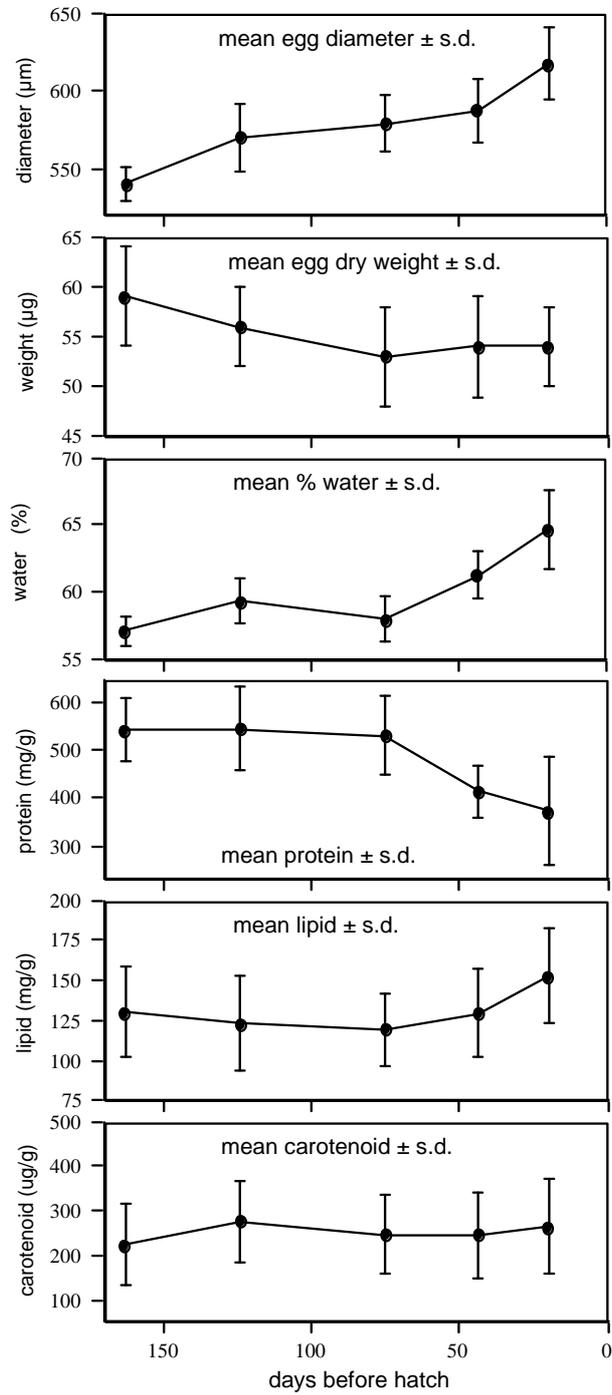
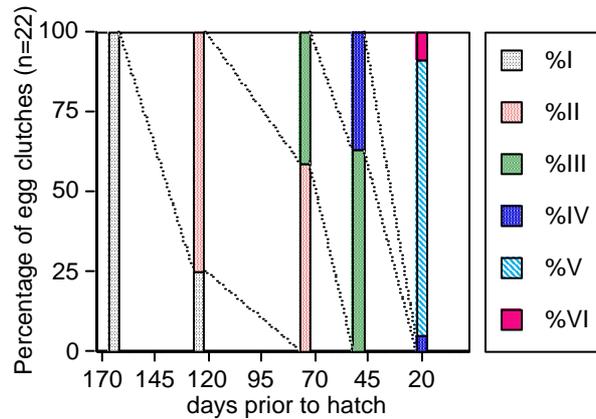


Figure 2. Developmental stages of egg masses collected at each sample period. Days prior to hatch was averaged across each sample.



Influence of female size and carapace condition on egg composition

The eggs of larger females tended to have significantly higher water content ($P < 0.01$), less egg protein ($P < 0.05$), and less total carotenoid ($P < 0.0001$; Table 2). Although significant, the effect of female size appeared to be relatively slight, especially for water and protein content ($r^2 = 0.049$). Lipid content did not appear to be influenced by female size ($P > 0.2$). Additional analyses by ANCOVA indicated site and carapace condition had no significant effect on water, protein or lipid content ($P > 0.2$; both as a proportion of ash free dry weight and as a total mass per egg). Total carotenoid did not appear to be influenced by site although it was affected by carapace condition ($P < 0.05$; Table 3). The intercepts of each carapace condition grade were compared by t-tests; these tests indicated that the significant effect of carapace condition on total carotenoid content was attributable to higher levels of carotenoid in eggs from grade 1 (clean shelled) females while grades 2 and 3 (intermediate and heavy wear) were not significantly different (Fig. 3).

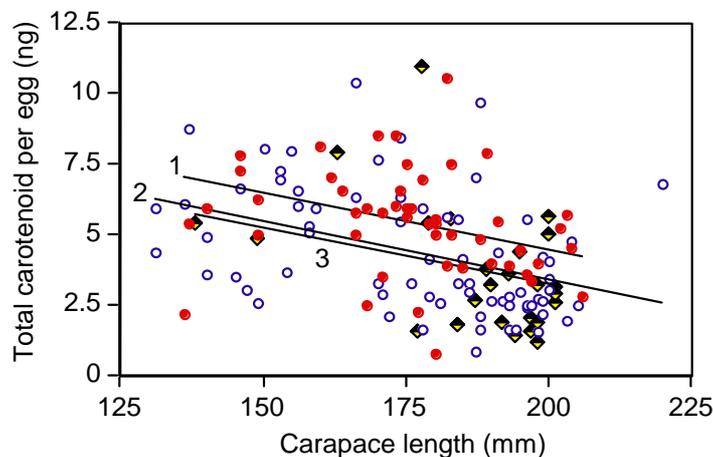
Table 2. Regressions of egg composition on female size, measured as carapace length (CL; mm). Results from 143 females were used in each analysis. Protein and carotenoid concentrations are as ash-free dry weight per gram of egg mass and per individual egg. NS, $P > 0.2$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$.

Regression equation	r ²	F-ratio
water (mg/g) = 0.673CL + 502.39	0.049	7.420**
protein (mg/g) = -1.332CL + 750.10	0.028	4.129*
protein per egg (µg) = -0.030CL + 15.14	0.037	5.564*
lipid (mg/g) = -0.190CL + 214.10	0.009	1.119 NS
lipid per egg (µg) = 0.00001CL + 1.04	<0.001	0.0001NS
carotenoid (µg/g) = -2.176CL + 603.11	0.153	25.753***
carotenoid per egg (ng) = -0.046CL + 12.75	0.170	29.216***

Table 3. Results of analyses of variance examining the effect of carapace-condition on carotenoid content. Analyses were conducted on carotenoid content as ash-free dry weight per gram of egg mass and per individual egg. Carapace length (mm) was included in the analyses to correct for the effect of female size on carotenoid content of eggs. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$.

Source of variation	Carotenoid (µg/g)			Carotenoid per egg (ng)		
	D.F.	M.S.	F-ratio	D.F.	M.S.	F-ratio
Carapace length (covariate)	1	170254	21.01***	1	86.54	22.48***
Carapace condition	2	28766	3.55*	2	19.34	5.02**
error	140	8103		140	3.85	

Figure 3. Regressions of total carotenoid per egg (as ash-free dry weight) on carapace length, for each carapace-condition class (denoted by numerals). Carapace-condition class 1 - solid dots; class 2 - hollow dots; class 3 - diamonds.



Discussion

Changes in egg size, mass, and composition during embryogenesis

General trends during embryogenesis in *Pseudocarcinus gigas* were typical of decapods; eggs took up water during development with a resultant increase in diameter while organic dry weight declined (Clarke et al., 1990; Subramoniam, 1991; Lardies and Wehrtmann, 1996; Wild, 1983). However, utilisation of protein and lipid reserves differed from the pattern of most decapods where lipid tends to decline during development with protein remaining stable or proportionally increasing (Pandian, 1970; Rao et al., 1981; Pillai and Subramoniam, 1985; Subramoniam, 1991). In *P. gigas*, protein appeared to be utilised in preference to lipid with lipid reserves appearing to decline slightly initially then increase later in embryogenesis. The apparent increase in lipid content of eggs is confounded to some extent by concurrent declines in other components but the results clearly indicate that lipid catabolism is relatively low. An increase in lipid content during embryogenesis is unusual in decapods although it has been reported previously in *Ovalipes punctatus* (Portunidae; DuPreez and McLachlan, 1984) and *Macrobrachium rosenbergii* (Palaemonidae; Clarke et al., 1990).

Enhanced lipid metabolism is a feature of cleidoic eggs (which do not exchange material other than gasses with the environment) and terrestrial crabs have been shown to produce eggs with large lipid reserves which are utilised throughout embryogenesis (Pillai and Subramoniam, 1985). Eggs of marine species have greater opportunity for release of waste products and greater reliance on protein reserves has been reported; eggs of the mole crab *Emerita asiatica* are intermediate between cleidoic and non-cleidoic as protein reserves are used continuously through development, although lipid is also depleted (Subramoniam, 1991). Eggs of *Pseudocarcinus gigas* appear to be further developed towards non-cleidoic type metabolism with greater reliance on protein reserves in a similar pattern to bony fish (Lasker, 1962).

Unlike most decapod species where the effects of embryogenesis have been investigated, *Pseudocarcinus gigas* inhabits relatively deep water of around 300 m. Habitat is known to influence yolk utilisation (Pillai and Subramoniam, 1985) and the low level of lipid

catabolism by *P. gigas* embryos may be an adaptive strategy for deeper water. Subramoniam (1991) suggested that the lower level of lipid utilisation in the shallow-water mole crab *Emerita asiatica* may be a strategy to retain higher lipid content in zoeas to increase buoyancy and provide a buffer against starvation. Buoyancy may be especially important in deeper water decapods such as *P. gigas* where newly hatched larvae appear to swim upwards in response to negative geotaxis (Gardner, 1996). Likewise, retaining lipid reserves may be of advantage in delaying starvation of zoeas in oceanic waters (19 d at 16°C in *P. gigas*; Gardner and Northam, 1997).

The predominant carotenoid in *Pseudocarcinus gigas* eggs is unesterified astaxanthin, as in *Penaeus semisulcatus* (Dall et al., 1995). Concentration of total carotenoid remained constant during embryogenesis in *Pseudocarcinus gigas* although a decline has been reported in other decapods (Dersan-Kour and Subramoniam, 1992; Dall, 1995). The apparently low utilisation rate of carotenoid reserves in *P. gigas* may be due to delay of development of carotenoid oxidation and esterification pathways until the larval stages as has been observed in *Penaeus japonicus* (Petit et al., 1991).

Effect of female size on egg composition

The composition of *Pseudocarcinus gigas* eggs was most stable during the early stages of embryogenesis, so further sampling to determine the effect of female size on egg composition was conducted during this period (egg development stage 2). Female size appeared to have a significant effect on egg composition with larger females producing eggs with more water, less protein, and less total carotenoid while there was no effect on total lipid. Although significant, the effect of female size on water and protein content was small and correlation was weak, even when analysed as mass per individual egg ($r^2=0.049$; Table 2). Based on these analyses, the effect of female size on egg size reported in Gardner (1997) is likely to have negligible effect on the viability of larvae.

In a similar study by Attard and Hudon (1987), size of female lobsters *Homarus americanus* was shown to influence energy content of eggs and it was speculated that this effect was large enough to influence larval growth and survival. Attard and Hudon (1987) concluded that the effect of female size on egg composition is likely to enhance the contribution of larger females to recruitment. In *Pseudocarcinus gigas*, it appears that the effect of female size

on egg composition is small so reproductive output of different sized females may be effectively modelled by analysis of fecundity alone.

Carotenoid content declined with female size and was also influenced by carapace condition, a qualitative measure of time since moulting. Extremely low levels of dietary carotenoids have been shown to reduce survival of crustaceans (*Penaens japonicus*; Chein and Jeng, 1992) although in *Pseudocarcinus gigas*, there was only a weak correlation between egg carotenoid content and female size ($r^2=0.17$). Consequently, the effects on larval vitality may not be important. Carotenoids cannot be synthesised *de novo* by crustaceans so the diet of females during gametogenesis will influence carotenoid content of eggs (Harrison, 1990). Levings et al. (1996) stated that *Pseudocarcinus gigas* show depth stratification of different size and carapace condition classes with most females found between 120 and 270 m. Although all females used in this study were captured at similar depth, they may have moved from different depths before capture. Consequently, the observed effect of female size and carapace condition on carotenoid composition of eggs may simply reflect different prey items encountered.

An effect of female size on composition has been recorded in other decapod species including *Chionoecetes opilio* and *Homarus americanus* (Attard and Hudon, 1987; Sainte-Marie, 1993). It has been speculated that this effect of female size may mean that larger females have greater contribution towards recruitment than would be predicted by fecundity alone. This clearly has important implications for fisheries management of *P. gigas* where minimum size limits selectively target large females for harvest. This study shows that biochemical composition of eggs was not largely affected by female size and that management by minimum size limit may be appropriate. Nonetheless, analysis of biochemical composition does not necessarily provide a measure of larval vitality which is critical in assessing the effect of female size on egg quality. Further research on the effect of female size on larval vitality is needed to fully address the issue.

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General Discussion: *Reproductive Biology of the Giant* *Crab Pseudocarcinus gigas*

18

Research conducted on the reproductive biology of the giant crab was primarily intended to provide fundamental information for fisheries management. For effective fisheries management, information is required on growth, behaviour (selectivity), and population structure and detailed information on all these factors is not available. Nonetheless, information on the reproductive biology provides a guide for basic fisheries management tools such as seasonal restrictions and size limits.

Harvest of males

Despite research on morphological and physiological maturity of male giant crabs, the available data do not clearly indicate the size of functional maturity (Chapter 14). The current minimum size limit does not protect morphologically adolescent or mature male crabs although the relationship between these morphological groupings and functional maturity is not known. Given that exploitation rate appears to be high, and that males appear to spend at least 2 years between moults (Pers. Comm., R. McGarvey, South Australian Research and Development Institute), it is probable that

very few males will live through morphological adolescence to reach morphological maturity. If morphologically adolescent crabs are not functionally mature, sperm limitation would seem inevitable.

Further research on the onset of functional maturity is important but is constrained by logistic difficulties in working with these relatively deep water crabs (as discussed in Chapter 14). A technique developed for recording mating of intertidal xanthoid crabs may be suitable. This involves immobilising crabs (Chapter 11) and attaching a trigger across the abdomen to determine if recaptured males have lifted their abdomen, and thus may have mated (Pers. Comm., P. Jivoff, Smithsonian Marine Station, Florida; Fig. 1). Male crabs are being fitted with these triggers in ongoing research but until data become available on functional maturity, management must be conservative and should aim to protect a portion of morphologically adult males. This could be achieved with a conservative quota (TAC) or by implementing a maximum size limit so that a proportion of crabs could moult directly from below the minimum size limit to above the maximum size limit.

Figure 1. Male giant crab with trigger attached to the abdomen to detect if the abdomen has been lifted since the crab was released. The trigger is mono-filament nylon and is glued to the sternum. It runs backwards to the abdomen and through a tube so that the abdomen can be lifted freely, but the nylon will slip from the tube.



Female giant crabs are harvested in Tasmania (Chapter 10) so the sex ratio is less likely to be biased by fishing than in a male-only fishery, although male giant crabs may be harvested preferentially. Males grow larger than females so less protection is offered by the minimum legal size, and restrictions on the taking of ovigerous females prevent harvest of females during 2 or 3 months of the open season. While it would be useful to monitor changes in sex ratio within the population by market measuring or by field sampling, it is a difficult goal as crabs are not distributed randomly. Giant crabs appear to live at different depths depending on sex and size (Levings et al., 1996) and commercial fishers aim to catch the smaller sized crabs which are favoured by exporters. Fisheries independent surveys conducted at fixed stations are an option for obtaining more reliable data on changes in sex ratio.

In crab fisheries where harvest of females is prohibited, research on the development of males has taken priority with the aim of limiting exploitation to sexually mature males (Hankin et al., 1996). Even with good information on the size of males in mating pairs, and thus functional maturity, there is concern about the potential for sperm limitation in several crab fisheries. Important aspects in considering the potential for sperm limitation are:

- ❑ male-only fisheries inevitably bias the operational sex ratio (Smith and Jamieson, 1991);
- ❑ the male is always larger than the female in mating pairs of many species (e.g. *Cancer magister*), so fertility of large females will be affected by intense exploitation of larger males¹⁰ (Smith and Jamieson, 1991);
- ❑ large interannual fluctuations in recruitment may lower the operational sex ratio, due to size/age dimorphism between the sexes (i.e. males need to be older than females to mate¹)(Sainte-Marie and Sevigny, 1997);
- ❑ the number of females that a male can mate with is restricted by pre- and post-copulatory mate guarding behaviour and the seasonal window of moulting by females (where mating is with soft-shelled females) (Armstrong and Jamieson, 1997); and
- ❑ the weight of ejaculate delivered to females is directly proportional to the operational sex ratio, so ejaculate reserves do not recover rapidly between copulations. This subsequently results in lowered fertilisation rate (in *Chionoecetes opilio*; Sainte-Marie and Sevigny, 1997).

Several of these issues are relevant to giant crab. Despite the lack of direct information on the mating system of *P. gigas*, indirect evidence from the anatomy of the vagina, mating scars, and taxonomic affinities (*Menippe* spp.) indicates that mating probably occurs while the female is soft-shelled (Chapter 15). Species which mate while the female is soft-shelled typically have protected pre- and post-copulatory mate guarding (Hartnoll, 1969; Wilber, 1989) which lowers the operational sex ratio and raises the risk of sperm limitation. It is also likely that males need to be larger than females for mating to be successful as indicated by their habitat (open type), dimorphism of the chelae, large differences in body size between sexes, greater limb loss in males (indirect evidence of male rivalry), and taxonomic affinity (*Menippe* spp.) (Christy, 1987; Wilber, 1992; Orensanz et al., 1995). As with *Cancer magister*, this suggests that sperm limitation

¹⁰ If growth rate of both sexes is similar until sexual maturity (typical for crabs), and if males need to be larger than their female partner, then most males involved in mating will be older than females. Consequently, they will have been subject to an additional period of natural mortality and the sex ratio will be skewed towards females (Armstrong and Jamieson, 1997).

may be a problem for larger *P. gigas* females if there is intense exploitation of males (Smith and Jamieson, 1991¹¹).

If female giant crabs can only mate when soft-shelled, then mating may occur only every 4 years or more, as preliminary analysis of recapture information from South Australia indicates that this is a typical intermoult period (Pers. Comm., R. McGarvey, South Australian Research and Development Institute). Although females were able to store sperm for at least 4 years, and produce broods with viable eggs (Chapter 14), the fecundity of heavily fouled, presumably late intermoult, females was lower than clean shelled females. If this reduced fecundity was due to depletion of sperm reserves, then fecundity of late intermoult females would be more severely reduced where the amount of sperm delivered was reduced. This has been reported in other species where the abundance of functional males has been reduced (Jivoff, 1997; Sainte Marie et al., 1997). If males need to be of a different age to females, natural variation in recruitment will alter the sex ratio and may compound the effect of fishing mortality.

Managing variation in recruitment

Classification of the life history strategies of marine organisms as *r*- or *K*-selected provided a useful guide of the potential of species to increase in population (Pianka, 1970). However, this simple continuum was limited and failed to describe some strategies, so a triangular continuum was proposed by Winemiller and Rose (1992) with three end points of opportunistic-periodic-equilibrium type strategies. As with most crabs, clawed-lobsters and spiny-lobsters, *Pseudocarcinus gigas* may be considered a periodic strategist since it is large, highly fecund and long lived. Periodic strategists are typified by fluctuating recruitment (Winemiller and Rose, 1992).

Cobb et al. (1997) considered that *Cancer* species are likely to have recruitment variability equal to or greater than that of spiny lobsters, based on their relative fecundity and larval duration. This is likely to apply to *P. gigas* also as the life history

¹¹ Note that Hankin et al. (1996) consider that there is no field evidence in support of the conclusions drawn by Smith and Jamieson (1991).

strategy is similar to that of *Cancer* species with similar fecundity (Hines, 1991; Bennett, 1995) and larval duration (Sulkin and McKeen, 1994; Bennett, 1995). Recruitment variation of spiny lobsters *Jasus edwardsii* in Tasmania can vary inter-annually by a factor of 5 (Gardner et al., 1998) so similar or greater variation may occur in *P. gigas*.

The potential for large inter-annual variation in recruitment suggested by the fecundity and larval duration of *P. gigas* may be compounded by cannibalism as fragments of giant crab exoskeleton have been found in the stomachs of giant crabs (Heeren and Mitchell, 1997). Although these shell fragments may have been from exuviae rather than live animals, cannibalism has been reported in other crabs and it is quite possible in *P. gigas* (Hines et al., 1987; Smith, 1995; Botsford and Hobbs, 1995). Cannibalism in crab populations is important to fisheries management as it can compound fluctuation in recruitment by cannibalism of smaller crabs by cohorts from high recruitment years. Several authors have considered that the inherent fluctuations in Canadian snow crab *Chionoecetes opilio* and Dungeness crab *Cancer magister* fisheries may be due to cannibalism (Comeau and Conan, 1992; Sainte-Marie et al., 1995, 1996; Botsford and Hobbs, 1995; Lovrich and Sainte-Marie, 1997).

Large fluctuations in recruitment is problematic for traditional single-species fisheries management as it becomes difficult to interpret the cause of declines in catch and catch rates (is it an effect of cyclical low recruitment or intensive exploitation?), and it removes economic stability for participants in the fishery. Understanding these patterns will only become possible in the *P. gigas* fishery by the development of pre-recruit indices (such as fishery-independent surveys of sub-legal crabs) combined with long term monitoring of at least ten years (Thresher, 1997). Managers must consider that the exploitable biomass will fluctuate between years so that fishing mortality will increase when a recruitment trough enters the fishery, and decline when a recruitment pulse enters the fishery. This is especially difficult to manage in majid crabs as they often reach legal size at the terminal moult. In periods of high recruitment, crabs will grow older with associated increase in exoskeleton wear and a decline in value (Sainte-Marie et al., 1996).

Giant crabs do not appear to have a terminal moult and their beach price is not affected by exoskeleton condition, so unlike majid crabs, it should be possible to “bank” giant crabs during periods of high recruitment into the fishery. That is, periods

of high recruitment can be exploited over several years to bridge the gap to the next period of high recruitment. Management decisions which aim to dampen variation in recruitment will be enhanced by the ability to forecast abundance trends several years in advance as has been achieved with snow crab *Chionoecetes opilio* in eastern Canada (Sainte-Marie, 1997). In the absence of this information, restrictions on effort such as total allowable catches (TAC's) should be set conservatively to permit "banking" of crabs from high recruitment years. This assumes that population cycling will continue at regular duration and that fishing mortality does not destabilise cycles.

Destabilisation of recruitment cycles through exploitation has been predicted in *Cancer magister* (Botsford, 1995) and is conceivably possible in *P. gigas* as egg production is reduced.

Egg production

Information on egg production is essential for formulating fisheries management tools, such as minimum legal size, and in modelling the effect of management strategies on egg production. Simple static models such as egg per recruit analyses incorporate fecundity, growth, and natural mortality to estimate suitable size limits to protect egg production above a certain proportion of the virgin stock. This proportion is usually set arbitrarily at 20% to 30%¹², although some species are resilient to far higher exploitation (e.g. 90% in Spanish fisheries for *Maja squinado*; Freire et al., 1997) while other crab fisheries have highly conservative management which aims to protect 100% of egg production by restricting landings to male crabs (eg in the Californian fishery for *Cancer magister*, Hankin et al., 1989).

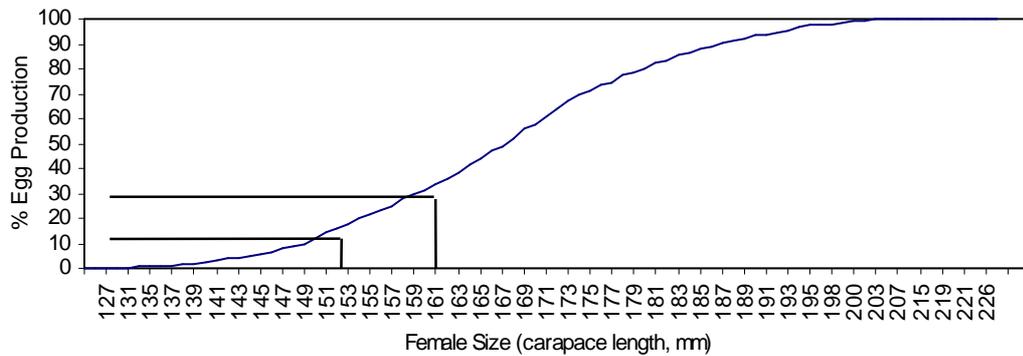
¹² These limits are generally "plucked out the air" as precise stock-recruitment-relationships (SRR) are extremely variable between species and accurate values can only be obtained through collapse of the fishery. Modeling the SRR is generally difficult and has low precision. For instance, Mace and Sissenwine (1994) conducted an extensive review of the SRR of 90 species and stocks and concluded that species such as cod would be resilient to recruitment overfishing. Unfortunately, their timing was extremely bad as cod stocks collapsed almost immediately after this work was published. Very little is known about the limits of recruitment overfishing in crab stocks. The Hawaiian spanner crab fishery collapsed but no precise data was collected (see discussions in "Workshop on Stock Recruitment Relationships in Australian Crustacean Fisheries").

Female giant crabs are harvested commercially so the egg production of fished populations will be affected. An interim size limit of 150 mm carapace length was introduced by Tasmanian fisheries management in 1994 and this was intended to protect approximately 60% of virgin egg production in North-Western Tasmania and Western Victoria. This minimum size limit was based on an analysis combining the proportion of females mature at each size interval (mm), the relationship between female size and egg mass weight, and a length-frequency distribution of female crabs obtained during months when females were ovigerous.

As an interim restriction on fishing effort, the implementation of a size limit provided an effective input control of effort until more data became available. However, there were flaws with the original analysis. Firstly, there was no measure of gear selectivity so the analysis assumed that the natural population distribution was equivalent to the distribution of catch taken by trapping. Trapping inevitably biases population surveys against smaller animals (Myers and Hoenig, 1997). Estimates of population structure were also biased by sampling when females were ovigerous, which affects their catchability (Edwards, 1978; Howard, 1982; Levings et al., 1996; Schultz et al., 1996). It is likely that this bias would also have affected estimates of the onset of sexual maturity.

To emphasise the problems with seasonal bias in population surveys of giant crabs, the analysis of egg production was repeated using data collected in Autumn 1998, which is before females become ovigerous (Fig. 2). This analysis indicates that the current size limit does little to protect egg production with only around 10% of egg production affected. However, there are problems with this analysis as there is no measure of onset of maturity (it assumes all females are mature), the population had been fished prior to the population survey (so large females will be under-represented, relative to virgin stocks), and not all females produce eggs every year (especially when they are small). All these factors cause the analysis to overestimate the egg production by females under the size limit (that is, egg production of females less than 150 mm is probably less than the 10% shown). Also, the effect of gear selectivity has not been incorporated and this is critical for estimating true population distribution.

Figure 2. Estimation of the proportion of egg production that would be protected by the minimum size limits shown on the horizontal axis. This analysis combines catch data from Tasmanian Department of Primary Industry and Fisheries research sampling (1998; n=659) with a simple fecundity model (Gardner, 1997). The resulting plot is an estimate of the cumulative contribution of different sized females to the egg production of the population. This analysis indicates that around 10% of egg production is protected by the current minimum size limit of 150 mm, and that the minimum size limit would need to be around 159 mm to protect around 30%.



Two paths can be taken to obtain useful and meaningful minimum size limits for the Tasmanian fishery. First, onset of maturity and gear selectivity effects could be estimated to improve the analysis shown in Figure 2. Onset of sexual maturity should be assessed by ovarian development to prevent bias from reduced catchability of ovigerous crabs (e.g. by CT imaging, Chapter 12). Gear selectivity could be estimated by analysis of the effect of size on recapture rates of tagged animals (provided there is only a short period between samples of 1 or 2 weeks) (Myers and Hoenig, 1997). Alternatively, minimum size limits could be formulated by an egg per recruit analysis although this requires precise growth information. A large scale tagging project is underway to obtain growth data but this is unlikely to provide enough data for a basic growth model for several years due to low recapture rates, long intermoult periods, and the narrow size range available for tagging.

Either method requires fecundity information as an input and this information is presented in Chapter 16. The models presented appear to be applicable for all Tasmanian waters as there was no effect of site, although estimates of egg production of the population would be enhanced by recording the extent of fouling on females sampled in population surveys (as carapace condition). Attard and Hudon (1987) demonstrated that the contribution of female American lobsters *Homarus americanus* towards recruitment could not be measured by fecundity alone, as egg quality was

affected by female size. Modeling the effect of size limits on giant crab egg production is more straight forward as the composition of eggs was not affected by female size in this species (Chapter 17).

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Appendix 1

Description of *Pseudocarcinus gigas* by McCoy (1889)

McCoy, F., 1889. A Prodromus of the Natural History of Victoria (Zoology). p 293.

[Zoology.]

NATURAL HISTORY OF VICTORIA.

[Crustacea.]

PLATES 179 AND 180.

PSEUDOCARCINUS GIGAS (LAM. SP.).

THE GREAT RED KING-CRAB.

[Genus PSEUDOCARCINUS (MILNE-EDWARDS). (Sub-kingdom Articulata. Class Crustacea. Section Podophthalmata. Order Decapoda. Tribe Brachyura. Family Cancridae.)

Gen. Char.-Carapace gently arched in front half, narrowed and truncated behind; wider than long, moderately depressed, the various regions and subregions elevated and embossed; front nearly horizontal, lateral anterior margins moderately curved, armed with projections or teeth; posterior lateral margins straight, converging; hind margin narrow, straight; basal joint of the external antennae very small; second joint scarcely reaching the front; third joint lodged in the orbital hiatus, but not filling it, so that the antennary fossa is not completely separated from the orbit; prelabial space not channelled; first pair of legs, especially in the male, forming very large pincers, the fingers of which are equally rounded and obtuse to the tip, unequal, and armed with very large, bluntly rounded tubercles, fewer and of greater size on the right claw,* which greatly exceeds the left in size. Hinder feet moderately long, simply pointed; abdomen of the male and female divided into seven distinct segments. Indian Ocean.]

DESCRIPTION.-Carapace slightly convex, anterior half tumid, posterior half more flattened, and bent downwards at an angle of about 145° from the anterior half; the protogastric, epibranchial, and metagastric regions tumid and bounded by broad deep furrows; the cardiac region is bounded by two furrows deeper and more angular than the rest, extending nearly to the hind margin; upper surface smooth as far as the posterior margin of epibranchial and metagastric regions, behind which the surfaces of the cardiac and mesobranchial and metabranchial regions are rough with scattered conical tubercles of very irregular size. Front between the orbits forming four projecting lobes, between which -the middle sinus is smaller than the other two; posterior superior external margin of each orbit incised by two deep parallel fissures; first joint of outer antennae very small; second joint reaching lower edge of orbit; fourth, half the length of third joint and reaching edge of front; flagellum little larger than anterior lateral portion of the carapace, with about eleven irregular, conical tubercles and divided into four lobes by small indentations on upper surface, but long narrow slits below, each lobe with two or three of the spines. Anterior legs

or chelae very large, the right much larger than the left[†]; movable finger (dactylopodite) rounded, moderately compressed, abruptly incurved at the obtuse tip; a little shorter than the fixed finger, with three elongate large, slightly compressed teeth on the basal half of the inner margin, the anterior smallest, posterior largest, a very slight angular projection at about one-fourth the length from the tip; fixed finger slightly longer than the movable one, a little broader and more compressed, but similarly abruptly incurved at the blunt apex; inner margin with three very large, rounded tubercles on basal half, the middle one largest and a slight compressed one about one-third from the tip; hand (propodite) very broad rounded externally, moderately convex on inner and outer sides; carpus (carpopodite) with two strong spines on upper inner margin, which has also three or four slight blunt tubercles near its base; next joint (meropodite) trigonal, with the upper sharp, angular margin with an irregular row of nine or ten blunt tubercles; four posterior pairs of legs, with the terminal joints

*Reversed in our plate.

† Reversed in the lithographing of our plate.

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(dactylopodites) simply pointed, villous; the next two joints (propodites and carpodites) also villous, the pile forming a close covering on upper margin, but forming a netting to bare spaces on the sides; next joints (meropodites, ischiopodites, basipodites, and coxapodites) nearly naked, but with a row of five to seven irregular, conical tubercles on upper angle, and with one smaller tubercle at middle of upper distal margin, with a few minute tubercles on each side of meropodites. *Colour*: All the underside of body, three basal joints of chelae, and underside, outerside, and most of innerside of chelae, including base of pincers, and four hind legs, yellowish cream colour; upper surface of carapace scarlet; upper surface of carpus scarlet, mottled on the sides, with the ground cream colour; upper portion and variable portion of sides of hand mottled scarlet, and the ground cream colour; velvety, close, pilose covering of last joint, and upper edge of the penultimate and antepenultimate joints and the netted pattern on their sides formed by villous lines on the four posterior pairs of legs, of rich dark brown. Two fingers of chelae rich purplish black. *Measurements of Male*: Width of carapace, 11 ins. 6 lines; length from front to posterior edge, 9 ins.; greatest depth, 5 ins.; length of abdomen, 1 in. 8 lines; greatest width at third segment, 9 lines; length of basal joint (coxa or coxapodite) of chelae, 1 in.; second joint (or basipodite), 7 lines; third joint (ischium or ischiopodite), 2 ins.; fourth joint (merus or meropodite) 4 ins. 6 lines fifth joint, carpus (carpodite), 3 ins. 9 lines long, 3 ins. 7 lines wide at distal end; sixth joint (propodos or propodite), 1 ft. 3 ins. from base to tip of fixed finger, 5 ins. 6 lines to base of movable finger, 5 ins. 4 lines wide at distal end and 3 ins. thick; seventh joint or movable finger (dactylopodite), 10 ins.; width at base, 2 ins. 3 lines; terminal joint (dactylopodite) of first pair of legs, 2 ins. 9 lines; penultimate joint (propodite), 2 ins. 6 lines; antepenultimate. (carpodite), 2 ins. 3 lines; preceding joint (meropodite), 4 ins. 6 lines; next joint (ischiopodite), 1 in.; basal joint (coxapodite), 1 in. *Female*: Much smaller than male, and with all the regions of the carapace set with irregularly sized and spaced conical tubercles about their middles, the boundaries of each region and subregion being smooth. Anterior chelae very much smaller than in the male, and more nearly equal to each other, and the fingers of the pincers much shorter and tuberculated from base nearly to apex. In addition to the spines on the carpus and other portions of the legs; as in the male, the hand has three or four large conical spines near base of upper rounded margin, and an irregularly scattered and sized series of smaller conical tubercles thence to base of movable finger (dactylopodite). About five large blunt tubercles on inner edge of movable finger, extending from base nearly to the tip, and four rather larger on corresponding intervals of fixed finger on right hand. On left, or smaller, chelae the fixed finger is much more compressed on inner edge, and the four or five tubercles still more compressed and less prominent than on the right. Inner edge of fixed finger much more compressed and with proportionately much smaller, more compressed, and less prominent tubercles than on right side. Abdomen enormously large, of seven very distinct segments. *Measurements*: Length of carapace from front to posterior margin, 8 ins. 6 lines; greatest width, 10 ins. 6 lines; length of abdomen, 8 ins.; greatest width (at sixth segment), 4 ins. 2 lines; length of right hand from carpus to tip of fixed finger, 6 ins. 6 lines; length of movable finger, 3 ins. 2 lines; greatest width of hand at base of movable finger, 3 ins.

REFERENCE. — = *Cancer gigas*, Lam., Hist. des An. sans vert, v. 5, p. 272; Milne-Ed. Hist. Nat. des Crust., v. 1, p. 409.

This gigantic and beautifully coloured crab is now figured entire and of the colours of life for the first time. It is not uncommon at the western extremity of the coast-line of the colony, particularly

about Portland, from whence examples are often brought to the fish-market. The small female with the much smaller claws seems more common than the great male with its immense powerful pincers.

EXPLANATION OF FIGURES.

PLATE 179.-Fig. 1, female, about one third natural size. Fig 1a, abdomen of female, one third the natural size. Fig.1b, antennules, or inner antennae, movable portion without great fixed base, twice the natural size. Fig 1c, antennae, or outer antennae, without the small basal joint, twice the natural size. Fig. 1g, mandible and first and second maxillipedes, natural size. Fig. 1d, third or external maxillipede, natural size. Fig. 2, abdomen of male, one-third natural size.

PLATE 180 -Fig. 1, male, about one-third natural size. (For abdomen, see pl. 179, f. 2).

FREDERICK McCOY.

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Appendix 2

Abstract: Preliminary report on the description of the larvae of *Pseudocarcinus gigas*

Quintana, R., Gardner, N.C. and Konishi, K., 1996. On the larvae of the giant crab, *Pseudocarcinus gigas* (Lamarck): a preliminary report. Abstracts for the meeting of the Japanese Society of Fisheries Science, Tokyo, April, 1996. pp. 93.

(English translation from the Japanese abstract by K. Konishi)

On the larvae of the Australian giant crab, *Pseudocarcinus gigas* (Lamarck): a preliminary report.

Quintana, R. (Aquatlas Ltd, Tasmania). Gardner, C. (University of Tasmania)
- Konishi, K. (Natl. Res. Inst. of Aquaculture)

[Introduction] - The Australian giant crab, *Pseudocarcinus gigas* (Lamarck), is well known as the largest xanthid crab. The largest record of the carapace width and whole weight is 40.6cm and 17.6kg, respectively. The distribution

ranges mainly along the southern coast of Australia and around Tasmania. The fisheries of this crab has been acutely increased in recent years, and the resources in risk of decline should be considered. Because its biological information remains poor, we tried to rear the larvae under laboratory conditions.

[Methods and Results] Thirty ovigerous females were collected from the East coast of Tasmania, and they were held in the laboratory. After 7 months, three females released the first zoeas. The larvae were cultured at 16°C and 35 ppt salinity and we got zoea 1-5 and megalopa stages by the end of experiment. Main larval characters are different from those of previously known larvae of the subfamily Menippinae as follows: 1) remarkable dorsal spine on abdominal somite 1. 2) postero-lateral spines on abdominal somites 3-4, and 3) buds of pereopods already in the first zoeal stage.

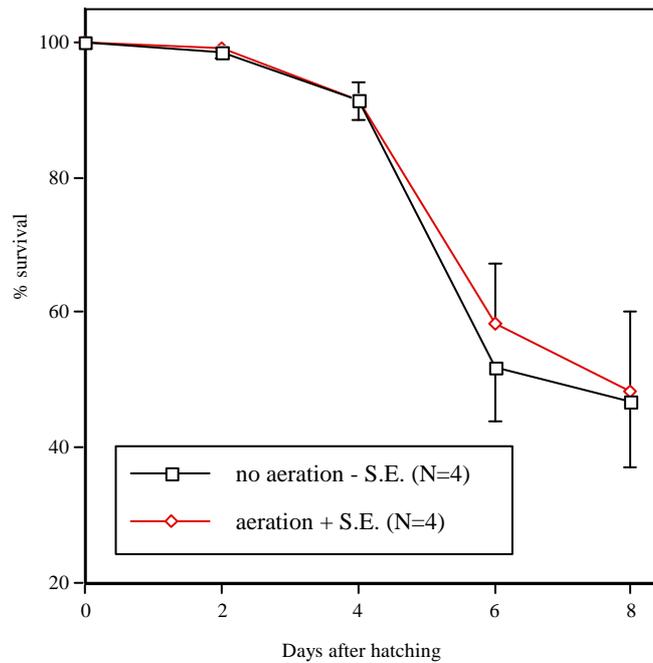
Appendix 3

Effect of aeration on larval survival in experimental culture vessels

A preliminary trial was conducted to test if larval survival was influenced by aeration. Larvae were obtained from ovigerous females and placed in 0.2 μm filtered seawater at 13°C. Instar II artemia nauplii were supplied as prey and water was changed daily.

Mean survival did not appear to be affected by lack of aeration. The flow of air was difficult to regulate to a constant level so it was considered that aeration may increase error variation between replicates. Consequently, future larval trials were conducted without aeration.

Figure 1. Percentage survival of zoea 1 giant crab larvae cultured with and without aeration in 1.8 l culture vessels. Flow rate was adjusted to approximately 8 ml per minute.



Appendix 4

Production of on-grown artemia for megalopa

Research for this appendix has been previously published as a popular article: Gardner, C. and Gardner, D. An improved method for on-growing artemia in batch culture. Austasia Aquaculture. 10(4): 42-43 & 10(5): 52-53.

Artemia are normally fed at metanauplii instar 1 or 2, but it can be useful to grow them out to larger sizes; producing these larger artemia is one of the more under-rated tasks in aquaculture as it's more difficult than producing early instars for feed. At the Marine Research Laboratories, Taroom, we've used on-grown artemia to feed large larval finfish that are not fully weaned, and also for feeding the last larval stage of giant crabs, the megalopa. Giant crab larvae were successfully reared only when enriched on-grown artemia were supplied. On-grown artemia are a valuable product in their own right and can be produced and sold to aquarium outlets as live feed.

The method for on-growing artemia described below is modified from the relatively standard method of Sorgeloos et al. (1983) with changes to two aspects: tank design and the combined use of microalgae and YM-20™ formula diet.

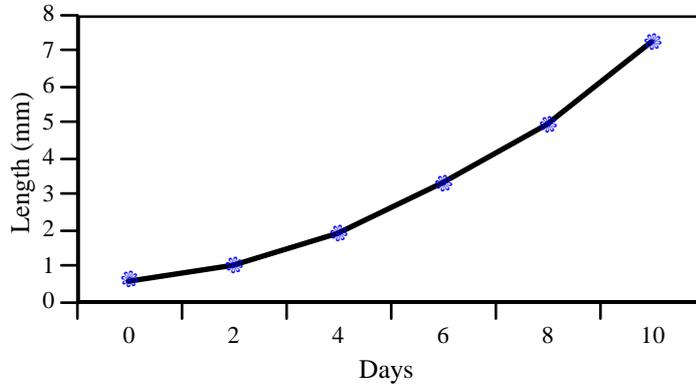
General Principles

Low density cultures of artemia can be readily on-grown to adults but the density achieved in these systems is far below that which is useful for the production of live feeds. Where large amounts of on-grown artemia are required some additional factors must be considered:

1. Artemia usually take 10-14 days to reach feeding size (around 8-10 mm, Fig 1) so if a daily supply is required, several tanks must be running simultaneously. Artemia will

be useful as feed over a fairly broad size range so cultures can be spaced a few days apart; we use 4 day intervals.

Figure 1. Standard growth rates for artemia in batch culture



2. The density in the tanks must be high for economical production. The problem of low density is compounded when several batches are running simultaneously as numerous tanks will be required.

3. Artemia only grow rapidly in warm water with a plentiful supply of a nutritious, finely particulate food in suspension. These are also perfect conditions for growing bacteria which can lead to low D.O., the build up of nitrogenous wastes, and clumping of food particles. Clumping of food particles is a problem as the particles becomes too large for artemia to ingest and anaerobic bacterial growth is enhanced by the formation of detritus.

4. For the first few days, artemia are tiny and tend to be affected by the same physical forces as the other small particles in suspension in the culture. This can be an important consideration in culture, for example; artemia are trapped by the surface tension of very fine bubbles and accumulate on the rim of the culture vessel as with protein scum; artemia collect in the detritus at the base of the tank; and artemia are hard to separate from exuviae and detritus by screening because of their similar size.

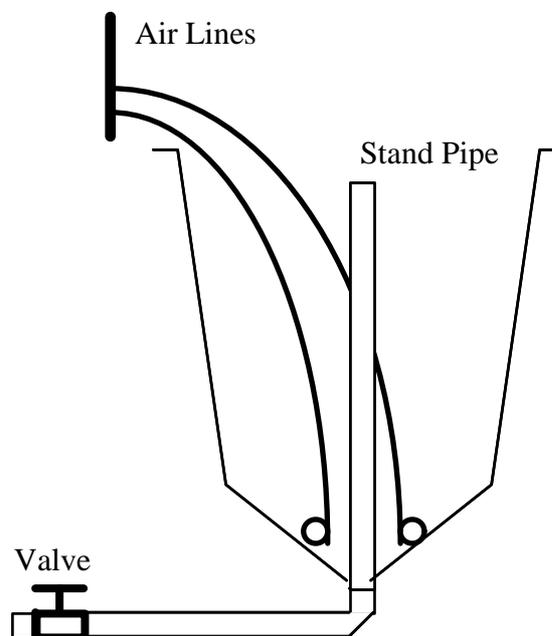
5. Bear in mind that the artemia become increasingly valuable as they grow older because more time, effort, and money has been expended. Artemia are easy to hatch so loss of 50% in a culture after 1 day is of no concern, but a similar loss at day 8 should be avoided.

Artemia have many positive attributes that can be used to make on-growing easier. Most usefully, they are relatively resilient to damage so cultures can be well aerated, and harvested or cleaned by gently pouring through a screen. Also, they will eat and grow on a large range of food types provided the particle size is correct ($< 50 \mu\text{m}$) including soy flour, micro algae, YM 20™ (a commercial diet, mainly micronised rice bran), yeast, and boiled and ground hens yolk.

Tank Design

Artemia can be on-grown in almost any vessel but it becomes considerably easier in well designed tanks. For the scale of work conducted at the Marine Research Laboratories we use large 300 l tanks (Fig. 2) and we'll describe these, but smaller tanks can be used which utilise the same principles. We've tried different tank designs but we get best results for this scale of culture with the one described below.

Figure 2. Tank system (300 l) used for on-growing *artemia* at the Tasmanian Marine Research Laboratories. Circulation is produced by upwelling from aeration which is enhanced by the narrow and deep design of the tank. The steep conical base assists in removal of detritus.

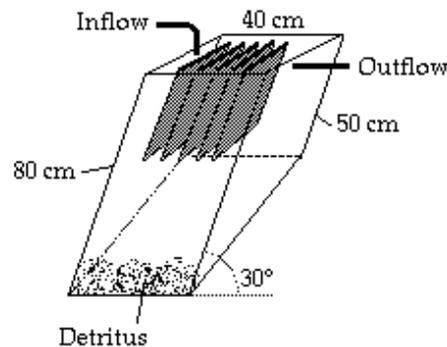


The batch culture of *artemia* described by (Sorgeloos et al., 1983) was for cultures of up to 5000 l and shallower tanks were used with circulation provided by airlifts. We found that this produced poor results as the airlifts tended to produce foam which

trapped artemia that then died around the rim of the tank. To overcome this we avoided the use of airlifts for circulation, instead, we used simple weighted air-lines in deep, narrow tanks.

1. Tanks should be relatively high and narrow with a steeply conical base. This helps ensure that all the water is kept well circulated and "dead pockets" are kept to a minimum. The steeply conical base is important in helping to collect all detritus at the mouth of the outflow, rather than letting it bank up along the base. Other systems incorporate plate separators to efficiently remove faecal wastes and exuviae (Figure 3). We utilise the tank design to collect wastes at the base of the culture tank and these are then drawn off daily. This is more laborious than using a plate separator, but the system is simpler and less time is spent in construction.

Figure 3. Plate separator used for primary water treatment. Water is circulated from the culture tank by pumping. Artemia must be prevented from entering the culture tank outflow by a filter screen. (after Sorgeloos et al., 1983).



2. Tank walls should be smooth to reduce the build-up of surface film and also to enhance water circulation.

3. We use a standpipe to prevent build up of anaerobic sludge in the drain system but this can create circulation problems in the tank if only one point of aeration is used. Wastes tend to build up on the side of the tank opposite the aeration, ie. in the dead pocket behind the standpipe. The solution is simple: distribute aeration evenly around the standpipe by using two or more air-stones or use a perforated airline ring. Air lines can be tied around weights, such as ceramic insulators, to produce large bubbles.

Feeding

Artemia will consume and grow on many food-types, but we find growth is best on YM-20, a commercial diet. Trials have been performed to compare different microalgae (*Chaetoceros calcitrans*, *Tetraselmis suecica*, *T. Isochrysis*, and *Nanochloropsis oculata*) as food sources for batch cultures of artemia and in all cases growth was poor compared to that on YM-20™ (Gardner, 1996). Artemia are obligate filter feeders so if the particle density becomes too high, the food will pass through the gut without being digested; this is especially noticeable with microalgae due to their tough cell wall.

While microalgae is a poor food source for on-growing artemia, we find that it can dramatically improve production when used in conjunction with YM-20. The reason for this improvement is unclear, algae may provide additional nutrition to the artemia, compensating for some deficiency in YM-20.

Also, cultures with microalgae present stay cleaner as the YM-20 clumps less and remains in suspension longer. This results in less fouling detritus and should also enhance food availability to the artemia. Again, it's unclear how the presence of microalgae reduces clumping of YM-20, but we suspect that bacterial proliferation may be reduced from utilisation of nitrogenous wastes by the microalgae. We've tried different algae species but find that the green algae, *Nanochloropsis oculata*, is most suitable. It seems to survive the turbulent and turbid conditions in the artemia cultures better than diatoms.

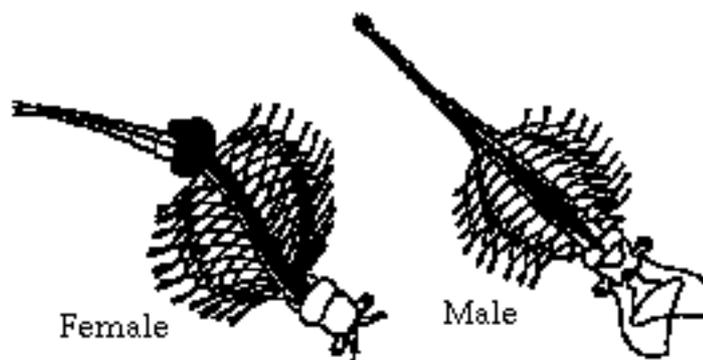
As microalgae are only added to condition the water, concentration is not critical, but we aim for about 10,000 cells / ml. Cultures should be illuminated with continuous and reasonably bright lighting (1000 lux) to promote algal growth. Artemia will strip algae from the cultures faster than it can reproduce so algae should be added daily.

YM-20 is blended in water for 1 minute then screened (50 µm) to remove lumps before being added to the cultures. The concentration of YM-20 is measured by turbidity and changed during development to suit demand. Sorgeloos et al. (1983) recommended that turbidity should be 20 cm secchi depth up to day 4, and 25 cm thereafter. We adjust this regime slightly, for ease of husbandry rather than for biological reasons. Feed density up to day 4 is reduced to 30 cm secchi depth (compared with 20 cm) to reduce the amount of uneaten feed forming detritus.

Sorgeloos et al. (1983) argued that the feed density should be reduced as the artemia become larger. This is because the artemia develop more appendages for filter feeding and so become more efficient at filtering and digesting food particles (Fig. 4). While this may be useful for producing optimum growth, we find that artemia rapidly remove particles from suspension so that they need to be fed several times each day. To reduce workload, we increase the feed density to 15 cm secchi depth past day 4 so that the artemia require feeding only twice daily.

It can be desirable to retard the growth of artemia once they've reached an appropriate size. This can be done several ways: reduction of food density; discontinue feeding YM-20 and supply microalgae only; and lowering temperature to as low as 20° C. We slow growth by only supplying *Nanochloropsis oculata* in slightly cooled water (22° C), this produces clean cultures of healthy artemia which can remain suitable for feeding for an additional 5 days.

Figure 4. Ongrown, adult artemia. Artemia develop additional feeding appendages with each moult which results in improved feeding ability. (after Planko and O'Sullivan, 1993).



General Maintenance

Stocking Density: Initial stocking density should be 12 artemia /ml. This is relatively high so that some artemia can be discarded in water changes and during cleaning. By day 6, density should be reduced to approximately 8 artemia / ml. We start harvesting

at this stage so that density is reduced daily. Density should be no more than 1.5 / ml for large artemia greater than 10 mm in length.

Temperature: Aim for 27°C in the early stages for optimal growth. This can be decreased to as low as 20°C to delay growth once the artemia have reached an appropriate size for feeding out.

Cleaning: Change the water every three days by draining artemia through a screen then wash into a new tank with preheated water.

If artemia are required for extended periods it may be worth constructing a plate separator and filter screen to automatically screen wastes (Fig. 3).

Removing Sediment: Detritus should be removed daily by swirling the tank, allowing to settle, and then draining the sediment through the bottom drain. Healthy artemia become trapped in this sludge and the loss of these can severely affect density. Cultures are initially stocked at high density to compensate for the loss of some artemia in the sediment. After day four we try to recover artemia from the detritus by washing the detritus through a 100 µm screen. If a large amount of detritus is retained on this screen then the detritus and artemia can be further separated by washing into a bucket, allowing detritus to settle, and then decanting off artemia.

Hygiene: As with all live feed production, high standards of hygiene should be maintained: wash all tanks, heaters, standpipes and airlines with chlorine (200 ppm); filter culture-water; and wash hands frequently. Wipe stand-pipes, heaters, and airlines daily.

Other Ideas

We've tried increasing the salinity in culture tanks to 45 ppt in an attempt to reduce bacterial proliferation. While the artemia survived and grew, the higher salinity appeared to kill the algae present. Euryhaline species such as *Dunaliella salina* may be better suited.

References

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Planko, D. and O'Sullivan, D., 1993. Simple Methods for small scale use of brine shrimp (*Artemia*) nauplii - decapsulation, hatching & enrichment. Aquaculture Sourcebook 4. University of Tasmania. 21pp.

Appendix 5.

Comparison of megalopas of species in the subfamily Oziinae.

Summary

The megalopas of *Pseudocarcinus gigas* were compared with megalopas of other species in the subfamily Oziinae (Xanthoidea) by features described by Martin (1988).

Pseudocarcinus appeared to lie intermediate between the genus *Ozius* (4 zoeal stages) and a cluster containing the genera *Menippe* (5 zoeal stages) and *Baptozjus* (4 zoeal stages).

Table 1. Characters examined for determining phenetic relationships of megalopa (from Martin, 1988).

1. Carapace:	Presence (yes) or absence (no) of acute anterolateral projections (yes = 0; no = 1).
2. Antennule:	Number and arrangement of setae on the distal segment of endopod (5 or more = 0; 4 or fewer = 1).
3. Mandible:	Number of spines and setae on the distal segment of palp (11 or more = 0; 10 or fewer = 1).
4. Maxilla:	Number of plumose setae fringing the scaphognathite (60 or more = 0; 59 or less = 1).
5. Maxilliped 1:	Number of long smooth setae on epipod (12 or more = 0; 11 or fewer = 1).

6. Maxilliped 2:	Number of long smooth setae on epipod (8 or more = 0; 7 or fewer = 1).
7. Maxilliped 3:	Number of long smooth setae on epipod (15 or more = 0; 14 or fewer = 1).
8. Cheliped:	Presence or absence of large recurved hook-like spine on ischium (no = 0; yes = 1).
9. Pereiopod 2-4:	Number of stout, usually serrate, spines on ventral border of dactylus (average 4 or more = 0; less than 4 = 1).
10. Pleopod 1:	Number of plumose setae on distal segment of exopod (20 or more = 0; 19 or fewer = 1).
11. Pleopod 1:	Number of hook-like setae on endopod (4 or more = 0; 3 or fewer = 1).
12. Pleopod 3:	Number of plumose setae on distal segment of exopod (18 or more = 0; 17 or less = 1).
13. Pleopod 3:	Number of hook-like setae on endopod (4 or more = 0; 3 or fewer = 1).
14. Uropods:	Number of plumose setae on distal segment of exopod (10 or more = 0; 9 or fewer = 1).

Table 2. Comparison of characters of megalopa in Oziinae.

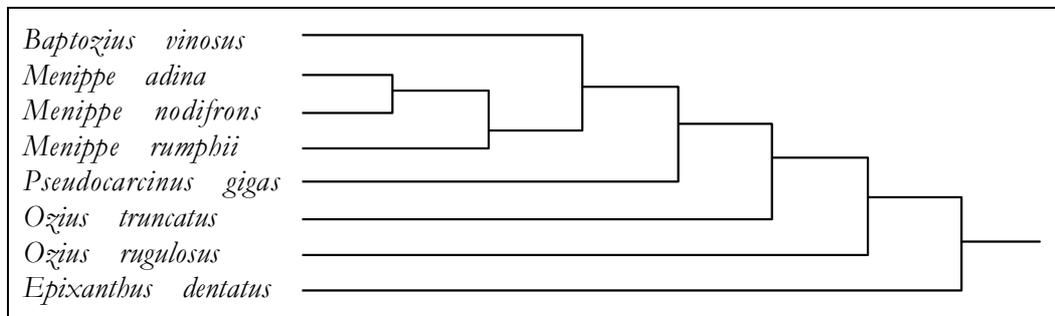
Species	Character number														References
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
<i>Baptozius vinosus</i>	no	1+4	14	90-93	11	13	29	no	3	25	5	23	5	13	Saba et al. (1978a)
<i>Epixanthus dentatus</i>	no	1+1+2	12	50	10	2	10	no	3	22	5	-	4-5	10-11	Saba et al. (1978b)
<i>Ozius rugulosus</i>	no	1+1+3	9	51	6	0	15	yes	2	19	-	19	-	10	Kakati and Nayak (1977)
<i>Ozius truncatus</i>	no	4-5	-	-	10	8	14	yes	3	22	4	22	-	12	Wear (1968)
<i>Menippe adina</i>	no	1+1+2 +3	11-14	70-78	22-23	9-10	18	no	5	21-22	3-4	-	-	12-14	Martin et al. (1988)

<i>Menippe mercenaria</i>	no	-	-	-	-	-	-	-	4-5	-	-	-	-	11-12	Kurata (unpublished) ^a
<i>Menippe nodifrons</i>	no	1+2+5	10-13	66	12-20	<10	18	no	5	20-21	3	20-21	3	11	Scotto (1979)
<i>Menippe rumpbii</i>	no	1+1+2 +3	9	65	18	8	22	no	-	20	-	18	-	12	Kakati (1977)
<i>Pseudocarcinus gigas</i>	no	1+1+4	19-23	80-88	20-26	21-22	39-53	yes	7-10	32-34	6-7	29-31	5	19-20	

^a Cited by Martin (1988)

Martin (1988) used characters 1, 4, 13, 14, 15, 16, 23, and 27 listed above to construct a phenogram and the process was repeated here with the inclusion of *Pseudocarcinus gigas*. Species were clustered by Ward's hierarchical technique (Milligan, 1980) which shows progressive dissimilarity to an initial cluster formed by the two most similar species. Martin (1988) concluded that the use of megalopa characters to determine phylogenetic relationships may be of limited value so the results of this analysis may not represent phylogeny.

Figure 1. Phenetic relationships between megalopas of the subfamily Oziinae.



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Appendix 6

Additional data from Chapter 6

Information presented in this appendix was summarised in Chapter 6.

Figure 1. Percent survival and moulting of larvae cultured at different temperatures. X axis is days.

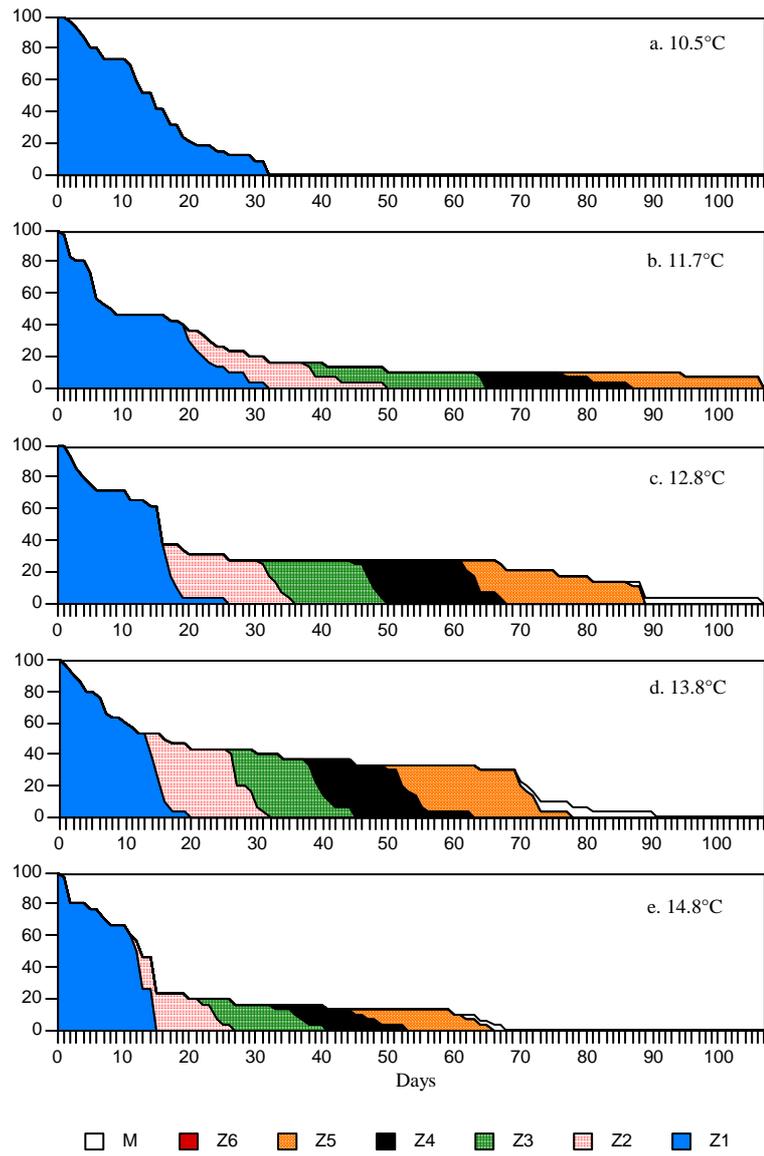
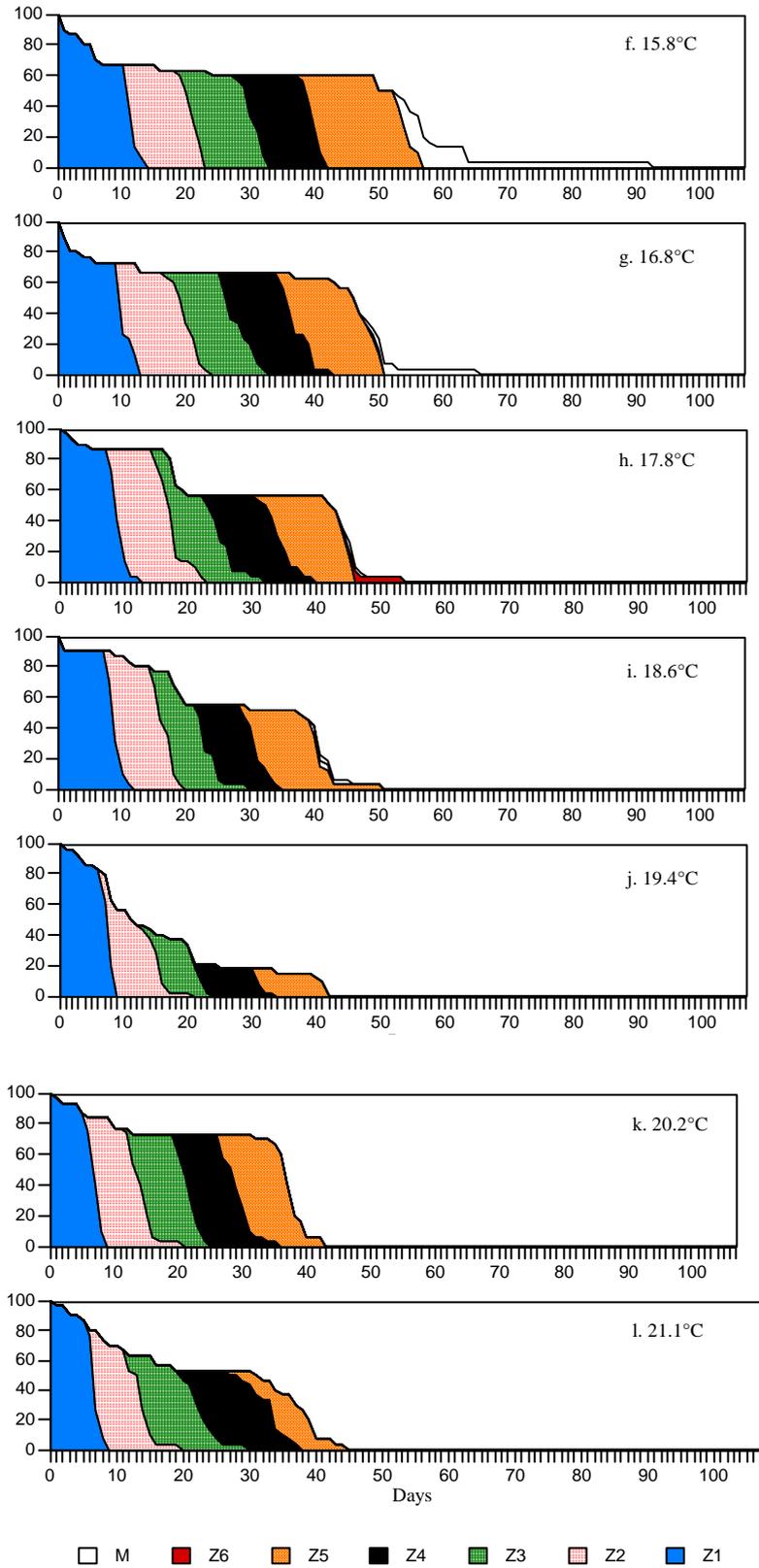


Figure 2. Percent survival and moulting of larvae cultured at different temperatures. X axis is days.



Appendix 9

Additional data from Chapter 8

Information presented in this appendix was summarised in chapter 8.

Figure 1. Percent survival and moulting of larvae in control (no chemical treatment; upper) and starvation (lower) treatments in relation to time (days).

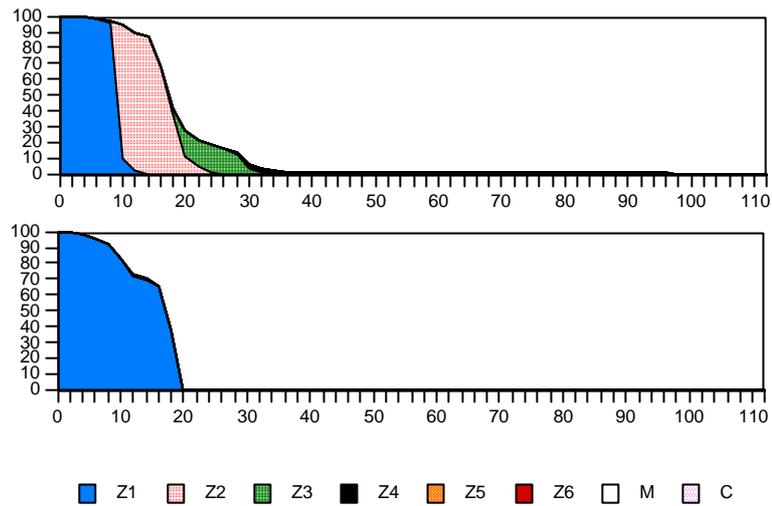


Figure 2. Percent survival and moulting of larvae in oxytetracycline treatments, in relation to time. All replicates are combined (n=4). Concentrations from upper graph to lower graph are 10, 25, 50, 100, and 200 mg⁻¹/l.

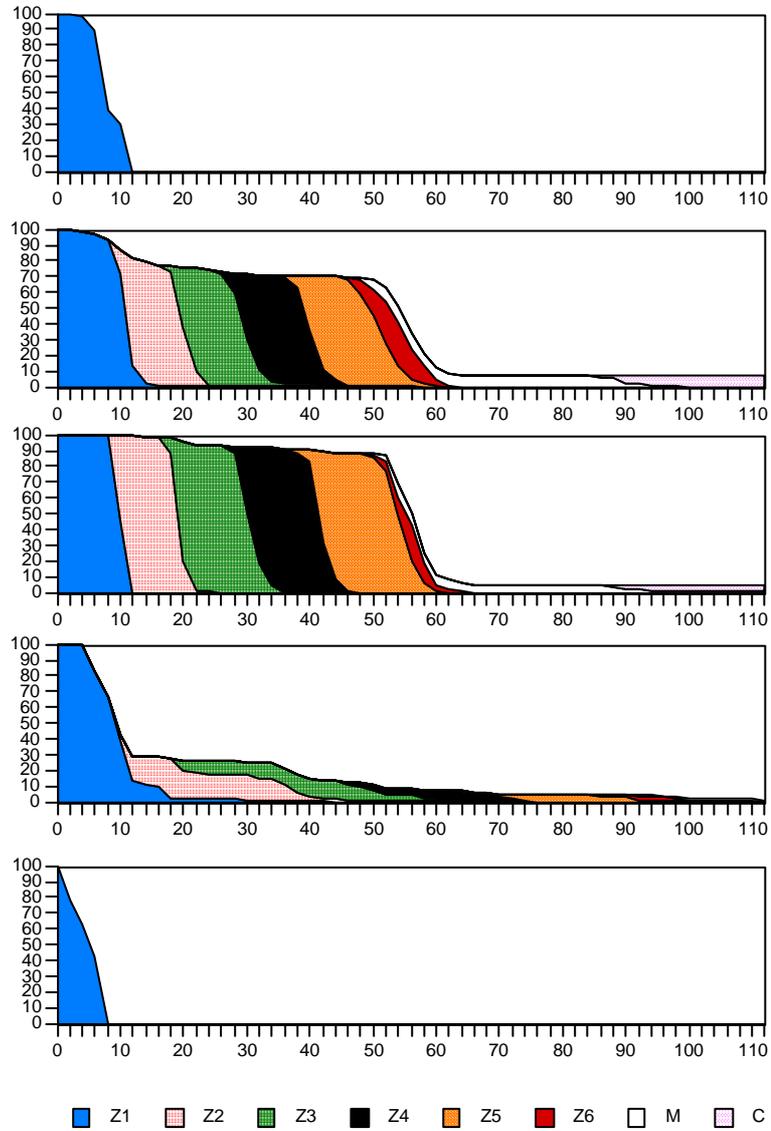


Figure 3. Percent survival and moulting of larvae in carbendazim treatments, in relation to time (days). All replicates are combined (n=4). Concentrations from upper graph to lower graph are 0.001, 0.003, 0.01, 0.03, and 0.1 mg⁻¹/l.

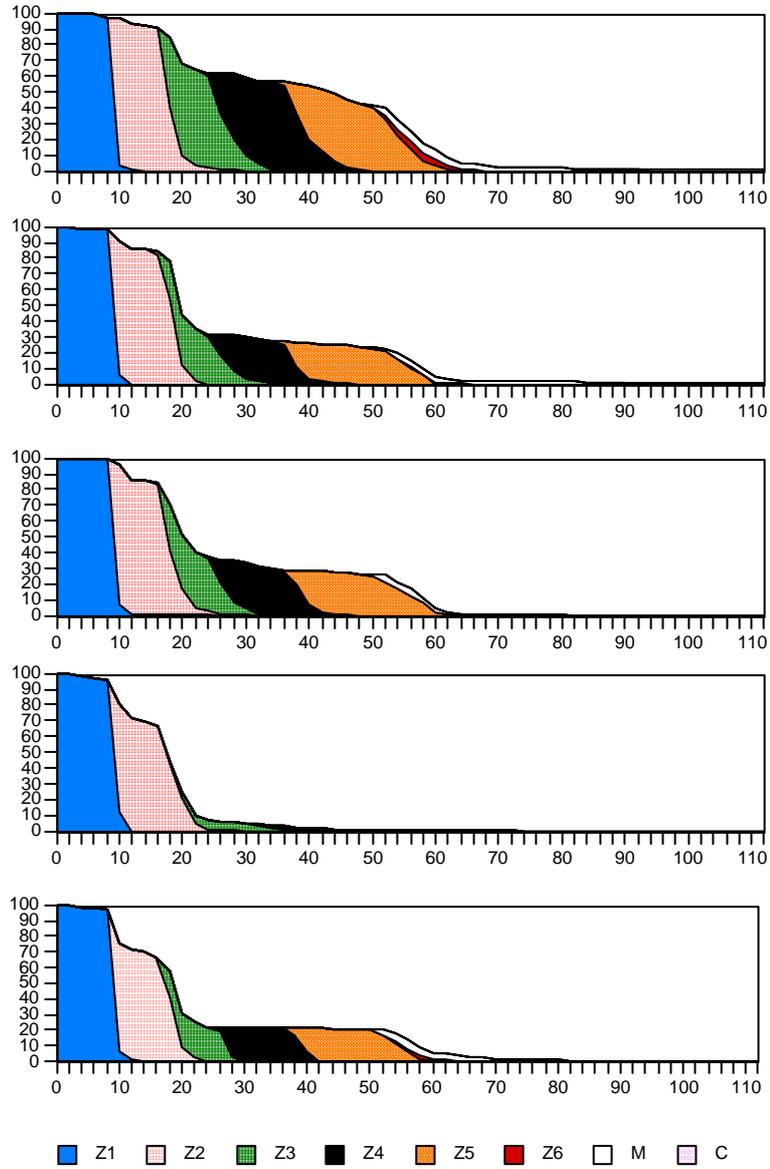


Figure 4. Percent survival and moulting of larvae in trifluralin treatments, in relation to time (days). All replicates are combined ($n=4$). Concentrations from upper graph to lower graph are 0.001, 0.003, 0.01, 0.03, and 0.1 mg^{-1}/l .

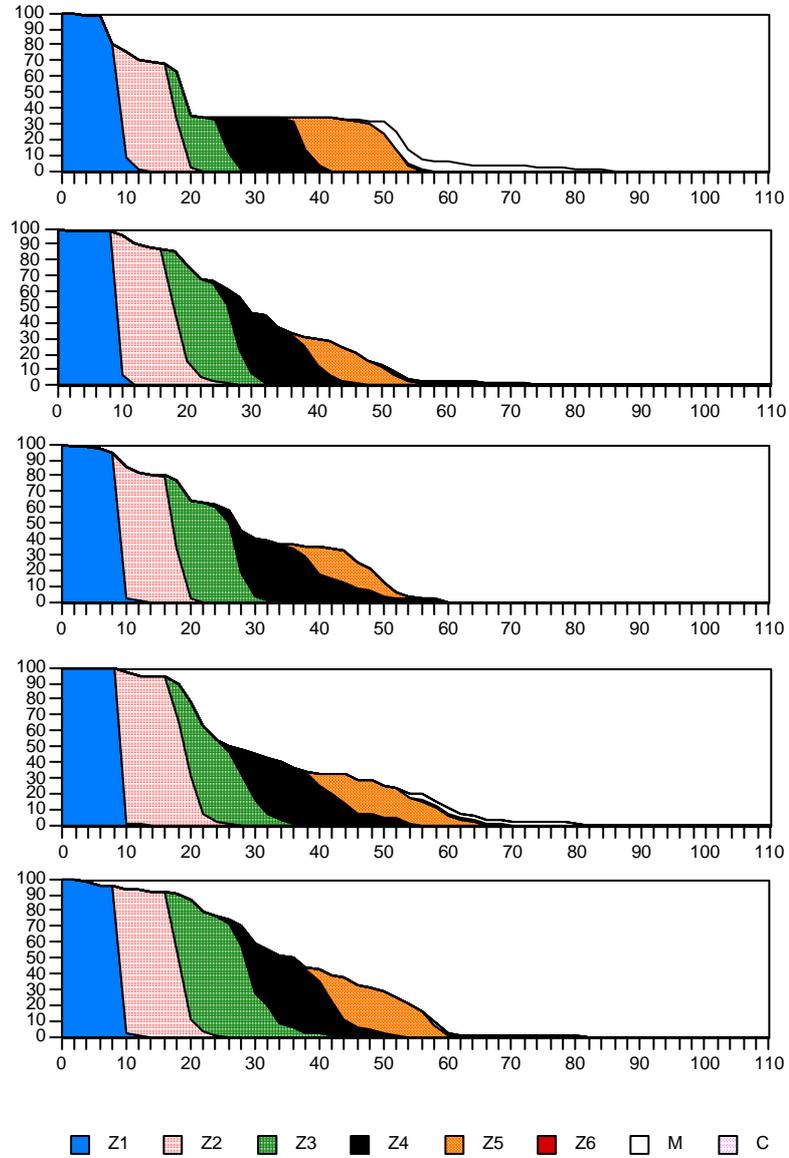


Figure 5. Percent survival and moulting of larvae in malachite green treatments, in relation to time (days). All replicates are combined (n=4). Concentrations from upper graph to lower graph are 0.001, 0.003, 0.01, 0.03, and 0.1 mg⁻¹/l.

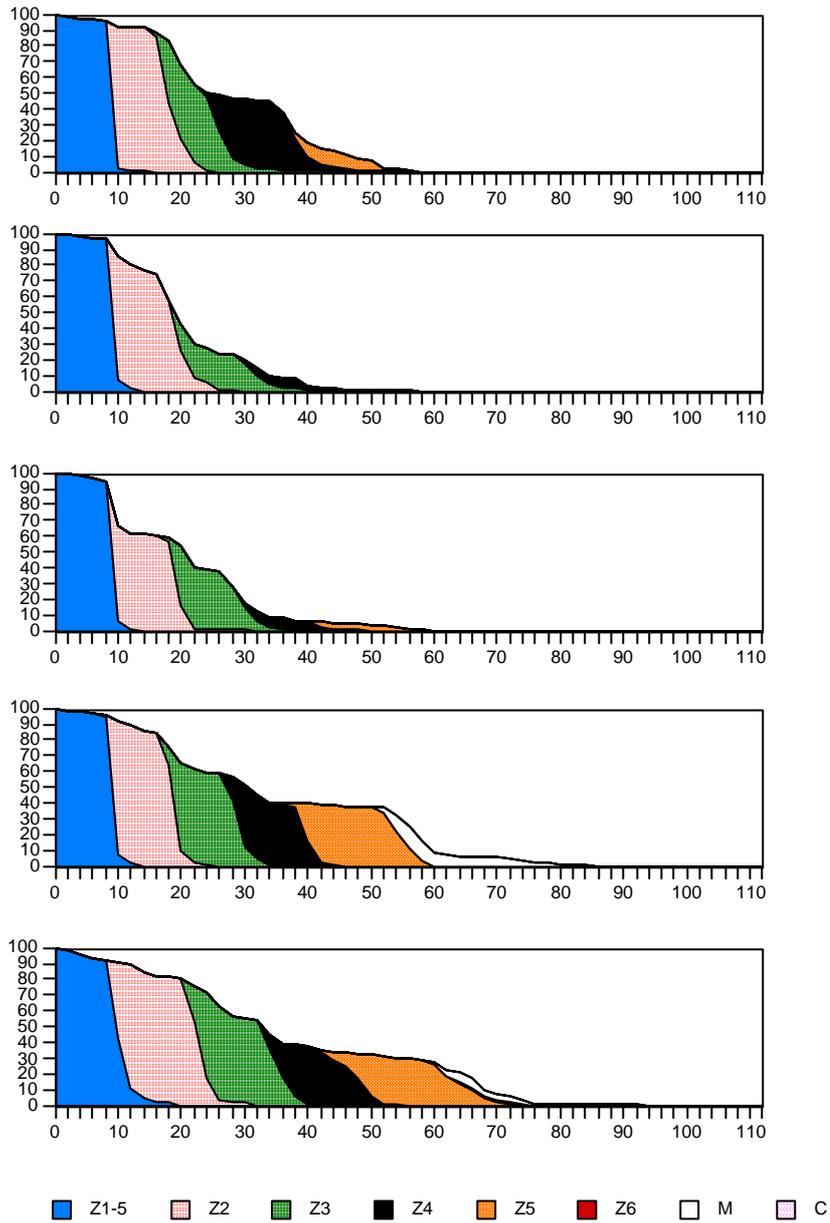


Figure 6. Percent survival and moulting of larvae in formalin treatments, in relation to time (days). All replicates are combined ($n=4$). Concentrations from upper graph to lower graph are 2.5, 5, 10, 20, and 40 mg^{-1}/l .

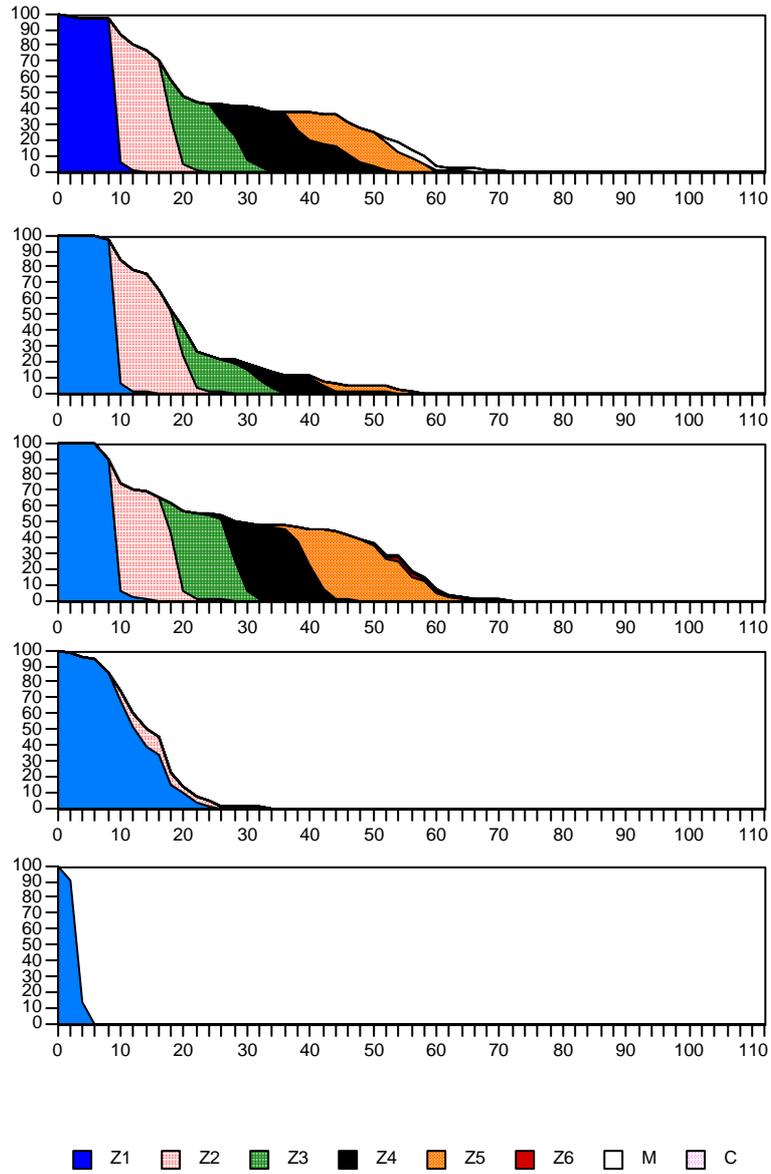


Figure 7. Percent survival and moulting of larvae in copper oxychloride treatments, in relation to time (days). All replicates are combined (n=4). Concentrations from upper graph to lower graph are 0.025, 0.05, 0.1, 0.2, and 0.4 mg⁻¹/l.

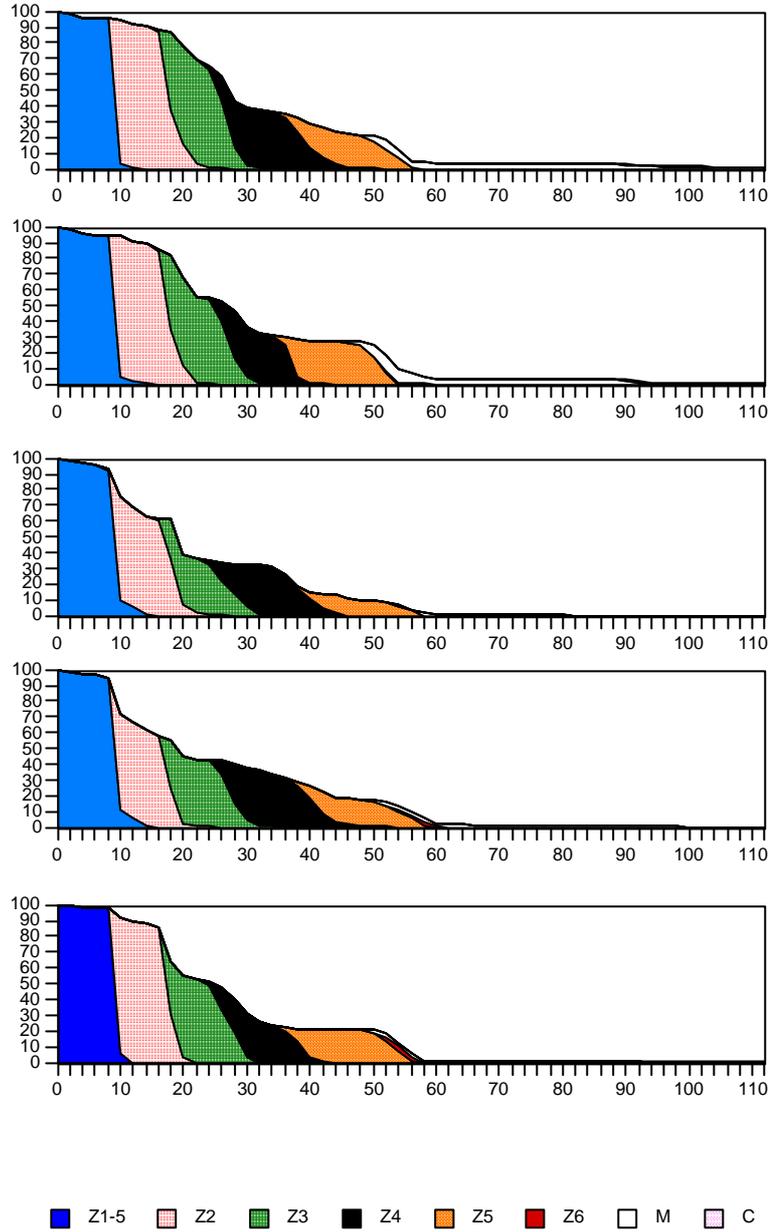


FIGURE 8. The effect of treatment concentration on the timing of moults. Lines connect means of different treatment concentrations for the moult to each successive zoeal stage (commencing with the moult from Z1 to Z2 at left). Shift towards the right suggests that the treatment has delayed the development of the larvae relative to other concentrations. Points plotted for moults stages more advanced than the moult to zoea stage 4 should be interpreted with caution as replication was frequently reduced in these later samples to less than 4. Treatment concentrations from upper row of points to lower row are: Oxytetracycline - 0.0, 25, 50, 100 mg⁻¹/l; Carbendazim - 0.0, 0.001, 0.003, 0.01, 0.03, 0.1 mg⁻¹/l; Trifluralin - 0.0, 0.001, 0.003, 0.01, 0.03, 0.1 mg⁻¹/l.

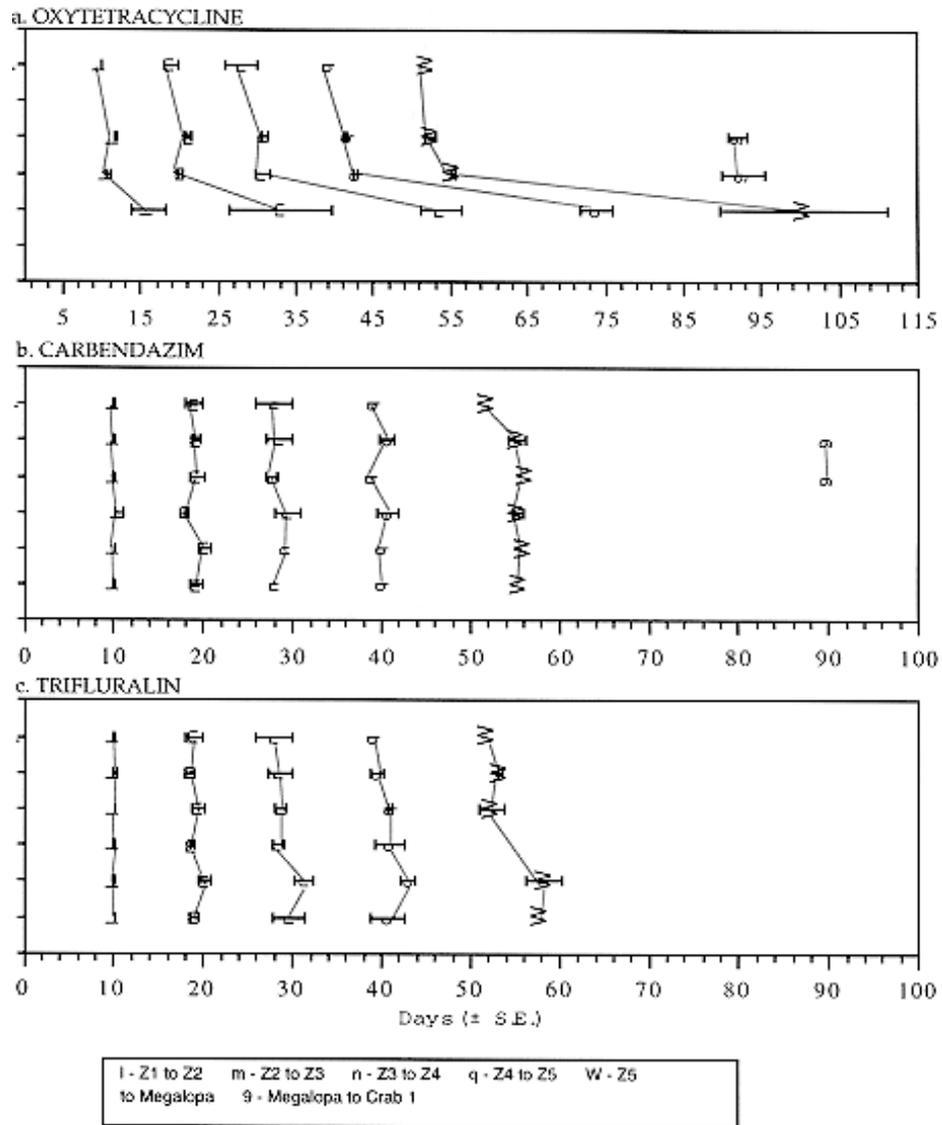


FIGURE 9. The effect of treatment concentration on the timing of moults. Lines connect means of different treatment concentrations for the moult to each successive zoeal stage (commencing with the moult from Z1 to Z2 at left). Shift towards the right suggests that the treatment has delayed the development of the larvae relative to other concentrations. Points plotted for moults stages more advanced than the moult to zoea stage 4 should be interpreted with caution as replication was frequently reduced in these later samples to less than 4. Treatment concentrations from upper row of points to lower row are: Malachite Green - 0.0, 0.001, 0.003, 0.01, 0.03, 0.1 mg⁻¹/l; Formalin - 0.0, 2.5, 5, 10, 20, 40 mg⁻¹/l; Copper Oxychloride - 0.0, 0.025, 0.05, 0.1, 0.2, 0.4 mg⁻¹/l.

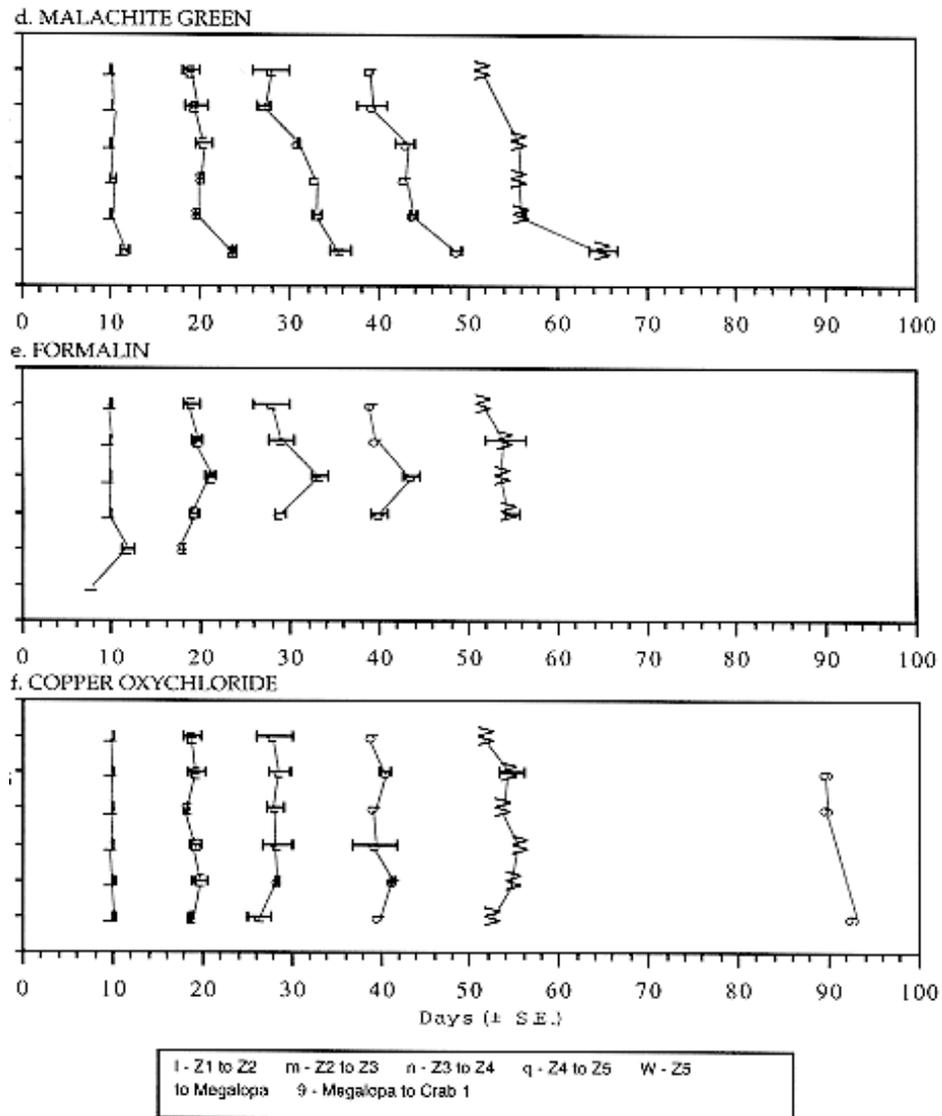


Figure 10. Effect of treatment concentration (x-axis: mg-1/l) on cause of mortality (y axis: mean % \pm SE, n=4). Where no obvious cause was apparent, larvae were scored as "normal"; larvae which died during ecdysis were scored as "moulting"; and larvae which appeared to have died as a result of fouling or deformity were scored appropriately.

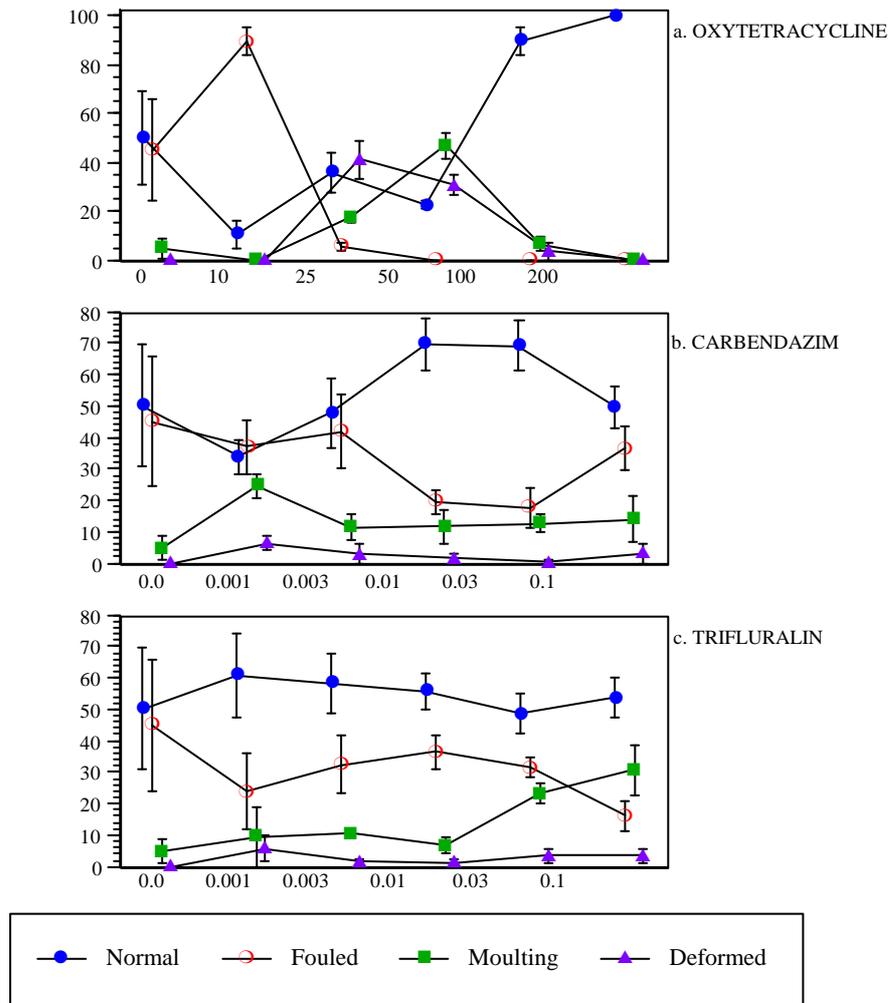


Figure 11. Effect of treatment concentration (x-axis: mg-1/l) on cause of mortality (y axis: mean % \pm SE, n=4). Where no obvious cause was apparent, larvae were scored as "normal"; larvae which died during ecdysis were scored as "moulting"; and larvae which appeared to have died as a result of fouling or deformity were scored appropriately.

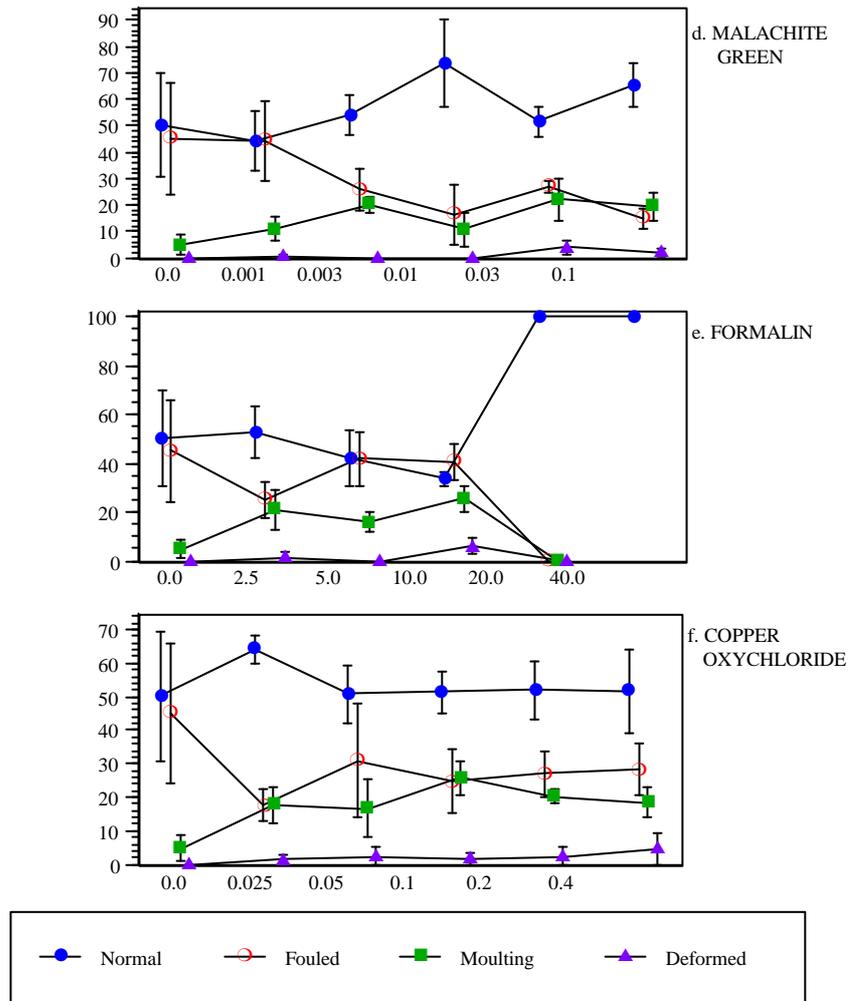


Figure 12. Pattern of mean percentage survival (\pm SE, $n=4$) in control and survival treatments of larvae to each stage.

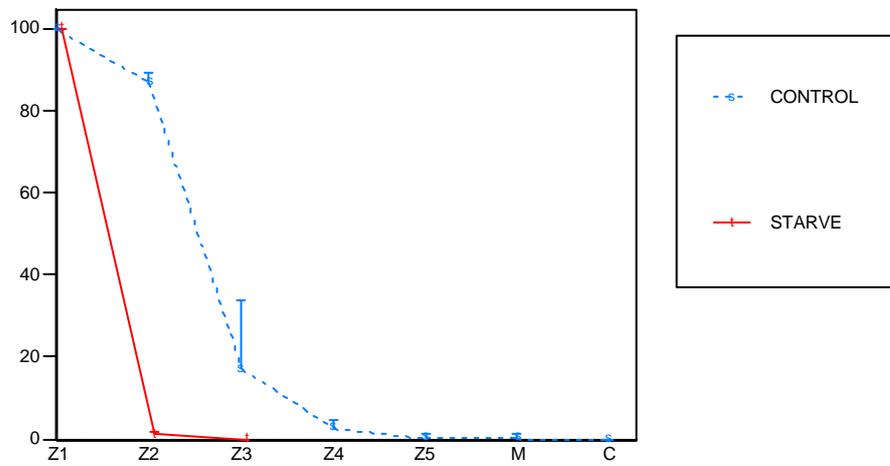


Figure 13. Effect of oxytetracycline and carbendazim concentration on mean percentage survival (\pm SE, $n=4$) of larvae to each stage. Survival plotted for larval stages past zoea 4 should be interpreted with caution as replication in these later stages was frequently reduced to less than 4. Z1...Z5 = zoea 1 ...zoea 5; M = megalopa; C = crab 1. Mean survival for each concentration is the mean number of instars that larvae survived to, eg. mean survival of 2.9 implies that most larvae survived to Z3. Superscripts denote significantly different pairs of means (statistical analysis as per chapter: Use of Prophylactic Treatments for Larval Rearing of Giant Crabs *Pseudocarcinus gigas* (Lamarck)).

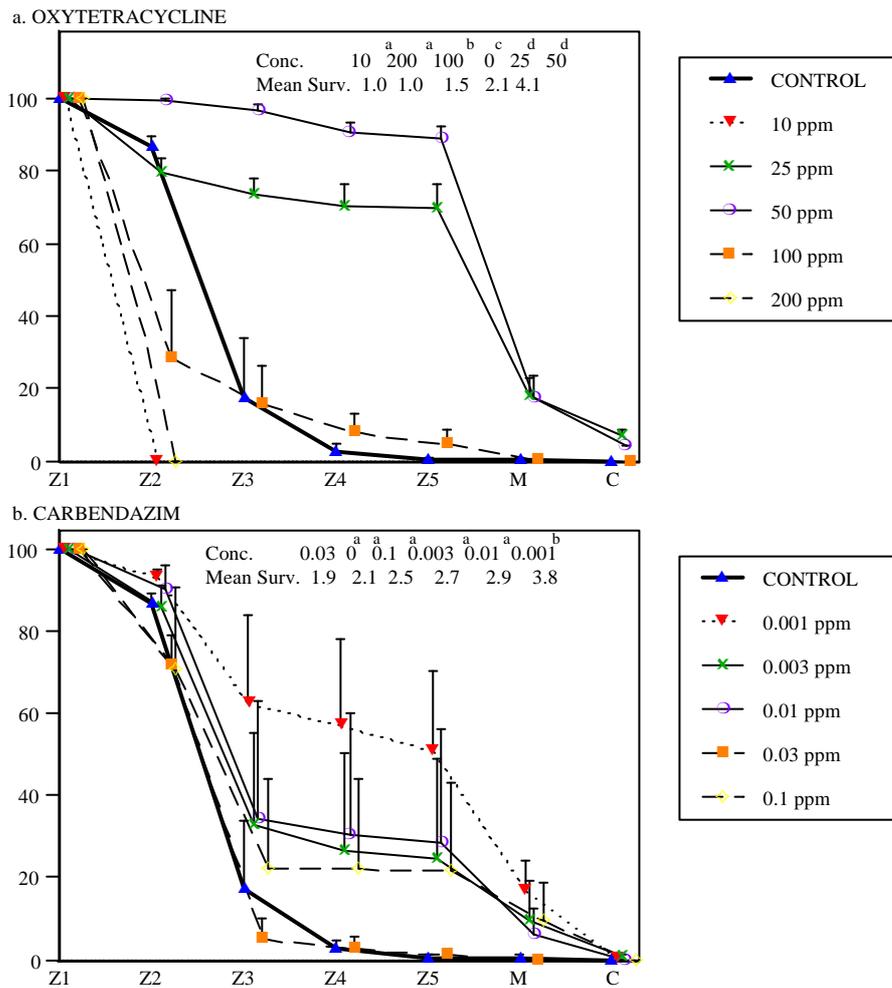


Figure 14. Effect of trifluralin and malachite green concentration on mean percentage survival (\pm SE, $n=4$) of larvae to each stage. Survival plotted for larval stages past zoea 4 should be interpreted with caution as replication in these later stages was frequently reduced to less than 4. Z1...Z5 = zoea 1 ...zoea 5; M = megalopa; C = crab 1. Mean survival for each concentration is the mean number of instars that larvae survived to, eg. mean survival of 2.9 implies that most larvae survived to Z3. Superscripts denote significantly different pairs of means (statistical analysis as per chapter: Use of Prophylactic Treatments for Larval Rearing of Giant Crabs *Pseudocarcinus gigas* (Lamarck)).

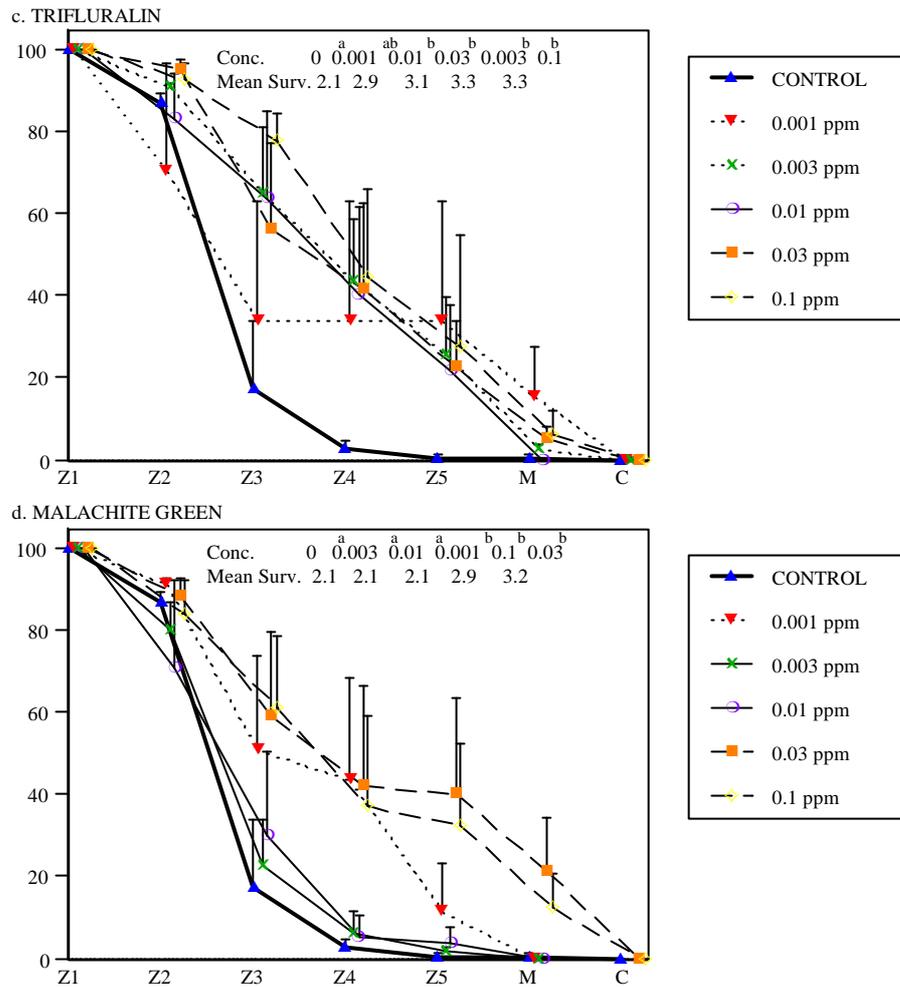
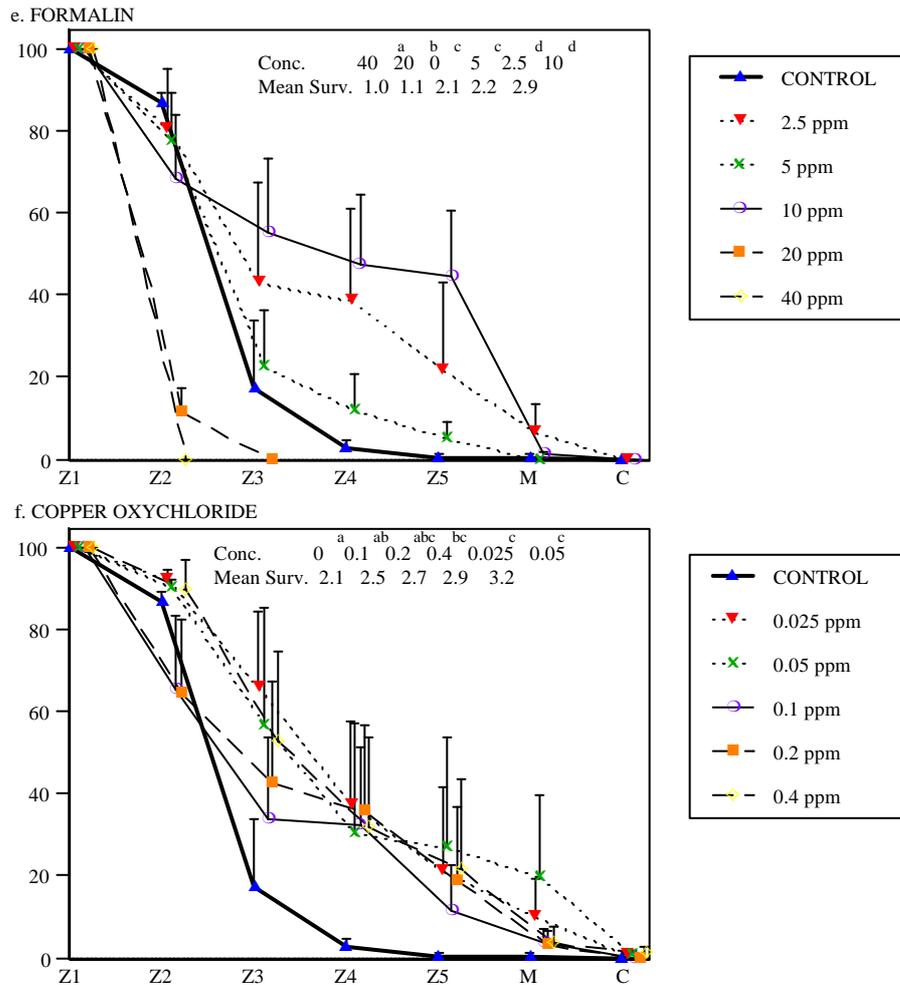


Figure 14. Effect of formalin and copper oxychloride concentration on mean percentage survival (\pm SE, $n=4$) of larvae to each stage. Survival plotted for larval stages past zoea 4 should be interpreted with caution as replication in these later stages was frequently reduced to less than 4. Z1...Z5 = zoea 1 ...zoea 5; M = megalopa; C = crab 1. Mean survival for each concentration is the mean number of instars that larvae survived to, eg. mean survival of 2.9 implies that most larvae survived to Z3. Superscripts denote significantly different pairs of means (statistical analysis as per chapter: Use of Prophylactic Treatments for Larval Rearing of Giant Crabs *Pseudocarcinus gigas* (Lamarck)).



Appendix 10

Biochemical methodology

Method used for the quantification of protein in tissues and eggs

1. Sample preparation

Samples were stored for up to 12 months before analysis in sealed bags at -60° C in darkness. Two samples of the tissue was homogenised with a mortar and pestle and then diluted with distilled water at a ratio of 5 mg/ml. Where the protein concentration of the two samples differed by more than 10 %, the analysis was repeated.

2. Procedure

Protein was assayed by a modified Lowry procedure (Peterson, 1977) with reagents from a commercial kit (Sigma Diagnostics™, protein assay kit #5656).

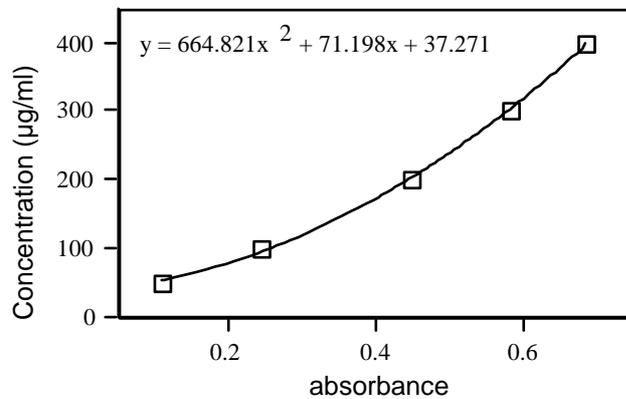
- Standards (50-400 µg/ml) were prepared with bovine serum albumin and blanks were made by substituting distilled water for sample.
- 0.25 ml of samples were added to centrifuge tubes and diluted with 0.75 ml of distilled water to produce a protein concentration within the range of the standards. 1.0 ml of standards and blanks were also added to tubes.
- 0.1 ml of 1.5 mg/ml sodium deoxycholate (DOC) was then added to all tubes, which were mixed and left to stand for 10 min at room temperature.
- 0.1 ml of 72 % w/v trichloroacetic acid (TCA) was then added to all tubes and mixed.

- Tubes were then centrifuged for 10 min at 5000 rpm and the supernatant then discarded.
- The pellet was then dissolved in 1.0 ml Lowrys reagent and 1 ml water, then left for 20 min.
- 0.5 ml Folin and Ciocalteu's phenol reagent working solution was then added to each tube with immediate and repeated mixing.

3. Measurement and quantification of protein concentration.

After 30 min, the absorbance at 600 nm was read in 1 cm cuvettes with a Unicam™ spectrophotometer. Concentration of samples was determined using a 2^o polynomial regression fit to the standard curve (Fig. 1.). Concentration of protein in egg and tissue samples was then calculated by correcting for dilutions. New standard curves were calculated daily.

Figure 1. Calibration curve obtained from standards.



Application of the Folch et al. (1957) method for the measurement of total lipid.

Method

Homogenise 3 grams of tissue with a pestle and mortar, then add 12 ml chloroform and 10 ml methanol. To the mixture, add 6 ml of distilled water.

The mixture is then transferred to centrifuge tubes and spun for 10 minutes at 5,000 rpm to separate into two phases with the solid tissue compressed into a pad between the layers.

The lower phase is removed by passing a long, stainless steel hypodermic needle through the upper layer and pad of tissue into the lower phase. This is then drawn off and the chloroform evaporated in pre-weighed vessels in a fume cabinet for 3 h.

Analyses were duplicated for each sample and the values presented in this thesis are the means. Analyses were repeated where the difference between the two original measures indicated excessive error.

Note: Chloroform poses a human health risk due to its hepatotoxicity and should be used cautiously.

The extraction, identification and quantification of carotenoid pigments from tissues and eggs.

"The real wonder of the world is really these carotenoids" Miriam Rothschild 1994.

1. Sample preparation

Samples were stored for up to 12 months before analysis in sealed bags at -60° C in darkness (Clarke, 1977). Tissue to be analysed was homogenised with a mortar and pestle and then diluted with distilled water at a ratio of 5mg/ml.

2. Extraction of carotenoid pigments

Five ml of acetone was added to 4 ml of diluted sample to extract the pigment. This mixture was shaken and left for 5 minutes. The pigments were then transferred to diethyl ether by the addition of an equal volume of diethyl ether (to acetone) and twenty volumes of 10% (w/v) NaCl solution (van den Enden, 1994). This separated the solutions into two fractions, the upper consisted of diethyl

ether and carotenoids. The upper, diethyl ether fraction was then removed with a pipette, after leaving the mixture for 10 minutes to separate.

3. Thin Layer Chromatography (TLC)

Samples for TLC were extracted in acetone as described for total carotenoid determination, and concentrated by evaporation under gaseous nitrogen.

Carotenoids were separated on C8 octyl silica plates (Merck™) using the following solvents: methanol and water (90:10, v/v); petroleum ether; diethyl ether and petroleum ether (95:5, v/v); and diethyl ether and methanol (95:5, v/v). After development, the positions of pigment zones were recorded. The pigment zones were then scraped from the plates and their absorption spectra recorded in each of the following solvents: petroleum ether, acetone, hexane and diethyl ether.

The presence of esterified forms of carotenoids was investigated by saponifying with the addition of an equal volume of 10% methanolic KOH to the diethyl ether extract (Clarke, 1977). The mixture was left overnight under nitrogen and then TLC was repeated on the saponified extract, with an untreated extract from the same sample running parallel. All chromatography was undertaken in low light or darkness to avoid auto-oxidation (Clarke, 1977).

4. Quantification of total carotenoid

The calculation of total carotenoid relied on the results of thin layer chromatography, which showed that astaxanthin was the major carotenoid present. Consequently, absorption of the carotenoid extracts was measured at 472 nm, the wavelength of maximum absorbance by astaxanthin. Absorbance was measured with a Pye Unicam™ spectrophotometer in 1 cm cuvettes using pure diethyl ether as a blank. The concentration of total carotenoids in µg/ g tissue was then derived using the extinction coefficient of astaxanthin by the formula:

$$x = \frac{E y 10000}{E_{1\text{cm}}^{1\%}}$$

where x=concentration of carotenoid in µg/g tissue

E=extinction at 472 nm

y =dilution factor, and
 $E_{1\text{cm}}^{1\%}$ =extinction coefficient (astaxanthin=2099, in diethyl ether; Clarke,
1977).

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Appendix 11

Assessment of scaling unit for female body size in fecundity analyses

Several measures of female body size were compared against fecundity: whole weight, carapace length, abdomen width, chela length, and chela height. As variation was similar for each measure of size, carapace length was selected for use in subsequent fecundity analyses as this measure of size is already used in the fishery for describing size limits.

Figure 1. Effect of body size on fecundity: comparison of different measures of female size. Fecundity was measured as number of eggs, weight as grams, and length in mm.

