DOCTOR OF PHILOSOPHY

Aspects of the biology of Sacculina carcini (Crustacea: cirripeda: rhizocephala), with particular emphasis on the larval energy budget.

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Award date:
1991

Awarding institution:
Bangor University

ASPECTS OF THE BIOLOGY OF *SACCULINA CARCINI* (CRUSTACEA: CIRRIPEDIA: RHIZOCEPHALA), WITH PARTICULAR EMPHASIS ON THE LARVAL ENERGY BUDGET

A thesis

submitted to the University of Wales

by

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in candidature for the degree of

Philosophiae Doctor

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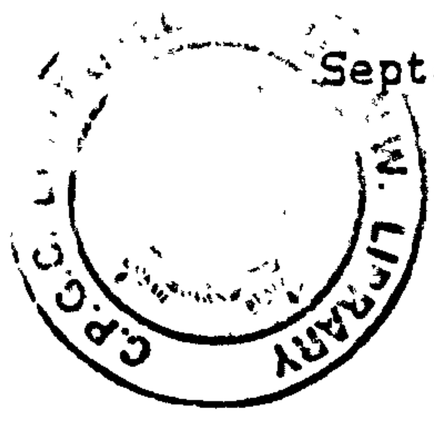
September, 1991



KENTROGON

Kentrogon's a crippled imp, uncanny, lacking kin,
The ' stabbing seed ' a *Cypris* made by shrinking from her skin,-
A *Cypris*? Nay, a fiend that borrowed Cyprid feet and mask,
To cast them off when he had plied his victim-hunting task.

From the cover of a *Nauplius*, one of *Sacculina's* daughters,
Is launched the Cyprid coffin-ship to break beneath the waters:
Then *Kentrogon* emerges from the wreckage on the floor
And his crafty life-line to carry him ashore.

Through the antenna of the *Cypris* on the hairlet of the Crab
His fine proboscis travels and inflicts the needful stab:
Then, gathering all the salvage that he's rescued from the rout,
He slips along the tunnel of his own projecting snout.

So *Kentrogon*, like Charon, carries souls from light to dark,
Himself at once both ferryman and passenger and bark,-
A Phoenix all perverse who on his desperate day of doom
Refanned his flame to start afresh within a living tomb.

From Walter Garstang c. 1922.

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ACKNOWLEDGEMENTS

Many thanks to Dr. Graham Walker for all his enthusiasm, optimism and constant help and advice during the project and the writing of this manuscript.

My thanks also go to Mr. John East for carrying out the T.L.C. and G.L.C. analysis and the gravimetric lipid analysis. I would also like to thank Mr. Malcolm Budd for his custom built temperature-controlled seawater system; Dr. Jim Nott (Plymouth Marine Laboratories) for supplying the animals; Mr. Berwyn Roberts for supplying the food for the animals and Mr. Brian Edwards and Ms. Violante Gomez for allowing me to use some of their data. Also, thanks to Mrs. Eira Gaunt for typing two tables.

I am very grateful to many other people who have readily given up their time for discussion and advice. These include Dr. Dave Bowers, Dr. John Davenport, Dr. Pete Gabbott, Dr. Dave Grove, Dr. Dave Holland, Mr. Lewis LeVay, Dr. Ian Lucas, Prof. Ernest Naylor, Dr. Chris Richardson, Dr. Cliff Warman and Dr. Andy Yule.

I want to thank my family who have been very supportive, long suffering and still recognise me, particularly Sam, Cleo Mum and Jane, also Jonathan who was enthusiastic when I was flagging, helped with the tedious bits and kept me smiling. My thanks also go to the animals which I really enjoyed working with.

I would like to thank the college for contributing funds towards my two trips to Roscoff, which were both essential to my work and very enjoyable. This project was sponsored by a Studentship from the University College of North Wales, Bangor.

SUMMARY

The biology of *Sacculina carcini*, a parasite on the common shore crab, *Carcinus maenas*, was studied with particular emphasis on larval morphology, biochemical content and energetic demands. The prevalence and distribution of the parasite was also investigated.

The field studies carried out on the populations of *S. carcini* on *C. maenas* in northern France and North Wales, together with a review of the literature, demonstrated a disparate distribution. The larvae of *S. carcini* are lecithotrophic and can develop within 5 days to the cyprid and are capable of settlement 2 days later. The highest prevalence for the parasite was observed to correlate with semi-enclosed bodies of water. It is proposed that in this situation, with the short development time, the larvae are retained, thus increasing the probability of successful cypris settlement. Such coastal systems are susceptible to variable conditions, consequently experiments were undertaken to investigate the larval tolerance to temperature and salinity variations.

A morphological study was carried out on the naupliar stages of *S. carcini*. The typical cirripede limbs were simple and lacked gnathobases. There was also a vestigial ventral thoracic process present in the stage III and stage IV nauplius. These observations supported the argument that the rhizocephalan nauplius fits within the cirripede nauplius form.

The energy budget study, involved the investigation of ash-free dry weights per larva, respirometry for each larval stage and analyses of the biochemical constituents using colorimetric and gravimetric techniques. Finally an equation was developed which demonstrates the energy budget for *S. carcini*, from the beginning of larval development to settlement on the host and the subsequent metamorphoses to the inoculation stages.

CHAPTER I : LARVAL BIOLOGY STUDIES : A REVIEW

Within the subphylum Crustacea the class Cirripedia comprises four orders: Thoracica; Acrothoracica; Ascothoracica and Rhizocephala (Barnes 1980). The thoracican order contains free-living and commensal barnacles with six pairs of well developed cirri. The mantle is usually covered with calcareous plates. Its three suborders - Lepadomorpha, Verrucomorpha and Balanomorpha, boast such well studied genera as *Scalpellum, Verruca, Chthamalus, Balanus* and *Tetraclita.*

The acrothoracican order is thought to have stemmed from the lepadomorphs, but there is some debate (Newman 1987) that this order may have preceded them. Acrothoracicans are naked, boring barnacles with 4 ⇒ 6 pairs of cirri and a chitinous attachment disc.

Both ascothoracican and rhizocephalan barnacles are naked and parasitic. Their inclusion in the Cirripedia *sensu stricto* has generally been accepted (Høeg & Lützen 1985), although Newman (1987), has recently queried this general acceptance. Also Grygier (1987), when studying antennular ontogeny of ascothoracid nauplii, reveals proposed homologies of antennular segmentation and setation in the Ascothoracica, Cirripedia and Facetotecta (y-larvae). However, when discussing early planktotrophic ascothoracid nauplii (Grygier 1990), he concludes that the basic ontogenic pattern in these groups is not identical.

The members of the Rhizocephala are highly modified endo-parasites, primarily of decapod crustaceans, tunicates and some cirripedes. Their external morphology is simple, with few distinctive characteristics. Appendages and digestive tract are absent and it is thought that the internal root-like absorptive processes may be the equivalent of a peduncle. (Høeg & Lützen 1985)

There are 31 genera and 231 species in this order and the sac-like reproductive externa does not display arthropodan features, such as segmentation or setae (Newman 1987). However, as with other Cirripedia, the reproductive apparatus and the nerve ganglia of rhizocephalans are located in the visceral mass (Day 1935, Klepal 1987). The latter lies within the mantle cavity and is enclosed by a flexible mantle. A mantle opening is present, but smaller than in non-parasitic cirripedes.

A classification for rhizocephalans was first introduced by Boschma in 1928. In this series of papers (1928 a, b c & d) it was shown that, in order to distinguish between species, especially of the family Sacculinidae, the externa must be serially sectioned. However, once the range of host species had been ascertained, identification of the parasite became easier.

Within the order Rhizocephala (Høeg & Lützen 1985) there are six families; Sylonidae; Clistosaccidae; Peltogastridae; Lernaeodiscidae; Sacculinidae and Chthamalophilidae. These families have been separated into two groups (Newman 1987) using the criterion that kentrogon formation (female larval inoculation stage) has been observed in all but the latter.

Høeg and Lützen (1985) however, consider such a subdivision to be

premature as it is based on insufficient observations. Nevertheless, Peltogastridae, Lernaeodiscidae and Sacculinidae do seem to be more closely related.

Sacculina carcini Thompson belongs to the family Sacculinidae and parasitises the common shore crab, *Carcinus maenas* (Thompson 1836). Délage (1884) carried out a comprehensive study on the life cycle of the parasite. He observed that the young parasite or interna migrans, lives within the host haemolymph. It absorbs nutrients from the haemolymph through its surface (Day 1935, Bresciani & Lützen 1980), which later takes the form of a network of rootlets ramifying throughout the crab (Délage 1884). Smith (1907) observed that the central tumour grows down the host's intestine to the junction of the thorax and abdomen. As it grows the adult's organs differentiate and the tumour arrives at the point of evagination. A primordium, or rudiment of the reproductive body, breaks through the host's abdomen and develops into an externa, connected to the rootlet system via the stalk. Délage (1884) originally thought that the parasite forced its way out by compression of the crab's integument, with the resultant necrosis allowing evagination of the young externa, and Smith (1907) believed evagination only to occur when the host crab moulted. It was noted, however, by Day (1935) that during ecdysis water is imbibed to swell the new cuticle before it is hardened. Thus, any pressure by the internal parasite at this stage would simply push out the soft integument rather than penetrate it. During his observational study of ten intermoult *Carcinus maenas* collected from Plymouth in the Spring,

Day (1935) noted a "pale circular area" under the surface of the crab's abdomen. The following morning the chitinous integument above this area had fallen off, exposing a virgin externa.

Observations of cypris settlement around the mantle opening of this newly emerged virgin externa were repeatedly made by Délage (1884), Smith (1907) and Day (1935), but the significance was not fully appreciated. Smith (1907) thought that these cyprids took no part in fertilisation although Day (1935) termed them complemental males.

Veillet (1943, 1945) observed larval dimorphism in the rhizocephalan *Triangulus galatheae*. He reported that the large cyprid developed into the adult hermaphrodite, whereas the smaller cyprid would function as a larval male. He also suggested that sex determination may occur before fertilisation of the ova. Although he observed no larval dimorphism in *Sacculina carcini* he did surmise that there may be two physiologically different cypris larvae, which would function in a similar way to those of *T. galatheae*. Ichikawa and Yanagimachi (1958) reported a marked size difference in the eggs and subsequent larvae of *Peltogasterella gracilis* Boschma (= *Peltogasterella socialis* Kruger c.f. Reischman 1959). On development to the cypris stage he noted that the penultimate segment on the antennule of the large cyprid was much longer than that of the smaller cyprid. Later experiments showed the large cyprids to be male because they only attached to virgin externae on the abdomen of the host crab. Conversely the small cyprids only attached to the base of plumose setae on the host carapace. For this species it was shown

that a mature externa only ever produced 100% single sex broods. Some externae were male-producing whereas others, although morphologically identical, were female-producing (Yanagimachi 1961). It was reported that the female-producing externa possessed an extra univalent 'F' chromosome. Although all the eggs produced by such an externa were small (Yanagimachi 1990), only 50% contained the extra 'F' chromosome. These eggs, having been fertilized, develop into small cyprids which are capable of attachment and infection of the host, with the resultant externa being female-producing. The small eggs without the 'F' chromosome also result in small cyprids, capable of attachment and host infection. However the resulting externae would produce only large male eggs which develop into male cyprids that can only attach to a juvenile female. Consequently Yanagimachi (1990) commented that sex determination for *Peltogasterella* was due to egg cytoplasm rather than the 'F' chromosome.

Size difference between male and female cyprids was thus established for the Peltogastridae (Yanagimachi 1961) and later also for the Lernaeodiscidae (Ritchie & Høeg 1981), Sylonidae and Clistosaccidae (LÜtzen 1981b, Høeg 1982). However, even though the Sacculinidae contain the largest number of species of the six rhizocephalan families, it was not until 1984 that Høeg reported larval size differences for *Sacculina carcini*.

The small female and larger male cyprids were observed to settle at the base of plumose setae and on the juvenile externae respectively (Høeg 1984). Fixation would only occur after the cyprid had spent at least two days in the free-swimming state,

(Délage 1884, Høeg 1984) and it was originally thought to take place in the dark (Smith 1907). However the latter was shown not to be the case (Høeg 1984). The size of larvae was seen to vary between sampling periods (Høeg 1984, Lützen 1984, Høeg & Lützen 1985).

This seasonal size variation was investigated by Walker (1985), who also carried out light microscope and scanning electron-microscope (S.E.M.) studies on cyprid antennular morphology. The males were not only larger than the females, but also possessed an extra, thin-walled sac, attached to the third antennular segment. This was termed a posterior sac and made identification of the sexes unequivocal. Many larval broods were seen to be mixed, but 100% single sex broods did occur. During the Autumn and Winter these would be male, whereas in the Summer months they were female. Walker (1987) proposed that this progressive changeover of the sex of larvae in a brood from the same externa, may be linked to the physiological state of the externa or due to hormonal influences from the host crab. He proved that it was not related to abiotic factors such as continuous light or temperature changes. Consequently, between June and September in the Plymouth area, *Sacculina carcini* larvae would be predominantly female, thus coinciding with the main recruiting period for young *Carcinus maenas* (Crothers 1967).

Although the minimum internal phase period for this parasite is reported as five months (Walker 1987), it can vary from nine months (Day 1935, Foxon 1940) through twelve months (Orton 1936) and twenty - one months (Délage 1884) to thirty -

four months (Lützen 1984).

At the Isefjord in Denmark, *Sacculina carcini* is close to its northern limit and Lützen (1984) observed that temperature clearly regulates certain events in the parasite's life cycle. He reported that the interna phase, varied from 33 months (Isefjord), to 12 months (The Channel), to 9 months (The Irish Sea), to 2 ⇒ 6 months (The French Mediterranean Coast). He also observed that larvae were incapable of developing to the cypris stage below 6°C. Consequently the maximum emergence of virgin externae in Denmark was between June and July and brood release stopped almost completely between November and April (Lützen 1984).

From observations of crabs received from Plymouth (Walker 1985), it was noted that maximum emergence of virgin externae was between January and June, which coincides with a high percentage of males in the water column. Nevertheless, the coincidence of both virgin externae and male cyprids would appear possible throughout the year in this area, as water temperatures rarely drop below 6°C (Walker 1985).

Female cypris settlement for *Sacculina carcini* was originally described by Délage (1884). He observed and drew figures of the settlement, metamorphosis and injection into the host with the resultant stylet or kentrogon. This work was followed up by Veillet (1964) and Høeg (1985a) using light or electron microscopy to examine this phenomenon for other rhizocephalan species.

Høeg (1984), studied the size and settling behaviour for

both male and female cyprids of *Sacculina carcini* and by 1986 he had observed male cyprid metamorphosis (Høeg pers. comm.). However, it was not until 1987, after observations relating ultrastructure and metamorphosis, for these larvae (Høeg 1987a), that he produced a detailed report of male cyprid settlement (Høeg 1987b) which involved scanning electron microscopy, transmission electron microscopy and light microscopy. He concluded that the trichogon was an instar consisting of several cell types and surrounded by cuticle. He considered it to be the only truly amoeboid crustacean larva with an irregular shape and a pseudopodium-like extension in the direction of movement. As both somatic and reproductive cells were present, Høeg termed the trichogon "an extremely reduced dwarf male". Only a single trichogon can enter and successfully occupy each receptacle (Høeg 1987b). Five to six days after implantation these cells are rearranged and spermatogenesis is initiated. Consequently fertilisation and embryonic development for *Sacculina carcini* were seen to take place within the mantle cavity. A review on barnacle males, by Klepal (1987), noted that this phenomenon, together with the gonochoristic state, are typical of rhizocephalans.

The larvae of *Sacculina carcini* like those of *Lernaeodiscus porcellanae* (Høeg & Ritchie 1987), are lecithotrophic, develop within five days at 20°C and are capable of settling several days later. The four naupliar stages barely increase in volume between successive moults (Walker 1988), whereas planktotrophic balanomorphs almost double their volume at

each stage (Crisp 1986), through to the sixth stage nauplius. However the volume reduction between nauplius IV and the cypris stage, for *S. carcini* was approximately 50% for the female but far less for the male.

Knight-Jones & Waugh (1949), Stubbings (1975), Lang (1979), Egan & Anderson (1986), Moyse (1987) and many other workers have published detailed studies on the larval development of planktotrophic thoracican barnacles. In comparison to these relatively complex larval forms, the lecithotrophic thoracicans show a simple morphology and a reduced development, often with direct release as cyprids from the mantle cavity of the adult e.g, *Ibla idiotica* (Batham 1945a), *Pollicipes spinosus* (= *Calantica spinosa*, Foster 1978), (Batham 1945b), *Tetraclita pacifica* (Crisp 1986)* and *Ibla quadrivalvis* (Anderson 1987).

Tropical thoracican species, such as *Tetraclita squamosa rufotincta* (Barnes & Achituv 1981) also exhibit these traits with naupliar development taking 6 ⇒ 8 days, whether the cultures are fed or starved. The large eggs of this species and *Tetraclita pacifica* develop into almost spherical stage I nauplii and are termed "swimming embryos" by Crisp (1986). Once again, when considering larval form, Crisp showed that the volume remained almost constant between nauplius I and nauplius VI. The trend in this genus is to lose the trophic function in the same way as the non-feeding cypris settlement phase, which is dependent upon a finite energy store for settlement and subsequent metamorphosis to the juvenile. For *Tetraclita divisa*, an island species, the naupliar phase resembles an extended embryogenesis so that larval

* Genus and species names are those used by the original authors in their papers.

release is at the cypris stage.

Achituv & Barnes (1978a), studied the biochemistry of eggs of three cirripede species. For *Balanus balanoides*, which is boreo-arctic, they deduced that the large larval size was probably due to the necessity to feed on large food particles such as cold water diatoms. They surmised that the unusually large egg size of *T. squamosa* was also due to selection for large particulate, non-living matter, relatively abundant at Elat. However, Crisp (1986) thought it more likely that this species did not feed at all. The clear blue waters, at Elat, are low in inorganic nutrients and have few microalgae.

Crisp (1986) considered that a lecithotrophic larva would fail to reach sufficient size, or have enough energy reserves to complete successful settlement and metamorphosis, if it had developed from an average sized egg. However, he did concede that many rhizocephalans do produce numerous small eggs which develop into non-feeding larvae. He argued that, in this case, once the cyprid has found the host there is a plentiful supply of nutrients.

Nevertheless, the settlement target, particularly for a male cyprid of *Sacculina carcini*, is an ephemeral one. This is thought to be the reason for the larger size of the male, thus enabling it to contain increased energy reserves for an extended searching period (Walker 1988). Crisp (1979) considered larval dispersal to be a perverse and expensive adaptation because it must be countered by re-aggregation so that sexual exchange may take place. However such isolated habitats, as the surface of

another animal, make this dispersal essential for survival. Also, whereas planktotrophs must fulfill the functions of growth and dispersal, lecithotrophs function purely for dispersal, including site selection (Crisp 1984). When reviewing the literature on distribution and behaviour of pelagic larvae, Young & Chia (1987), consider predation to be the most significant source of larval mortality. They also discuss larval behaviour, including defence mechanisms, and the ability that larvae have to regulate their vertical position in the water column. This enables them to encounter water masses moving in different directions and at different velocities. Cronin & Forward (1986) note the adaptational significance of vertical migration for the larvae of the estuarine mud crab, *Rhithropanopeus harrisii*. These larvae are able to remain in dimly lit regions of the water column, where they can feed and the risk of predation is reduced. They also ride the residual non-tidal currents so that they are retained in the upper reaches of the estuary. Field data (Cronin 1982) showed that 25% of *R. harrisii* larvae reached the post-larval stage, while remaining in the region where they were first released. These findings support the view that vertical migration must contribute substantially to survival. This view of dispersal differs from that held by DeWolf (1973); he reported a direct correlation between the number of cypris larvae of *Balanus crenatus*, *Balanus improvisus* and *Elminius modestus* in the Western Wadden Sea, and the amount of suspended matter in the water column. Consequently, he deduced that dispersal was solely due to water currents.

Strathmann (1987) notes that the adopted larval strategy is a compromise between parental investment, larval period and mortality. Todd & Doyle (1981), when considering a settlement timing hypothesis for nudibranch molluscs, also include the parameter of dispersal requirement. They suggest that the strategy selected for, would be that which appropriately bridges the period between the optimal time to spawn and settle. Scheltema (1986) however, when discussing models that predict larval dispersal, argues that natural selection eliminates the most maladapted, rather than "chooses" the most adapted individuals. As Thorson (1950) noted, 70% of all sediment-dwelling, temperate and tropical coastal invertebrates have a planktonic development. Scheltema (1986) points out that this infers a planktonic development must therefore be "good enough" for this majority. The other 30%, probably could not return to that mode of development (Strathmann 1978b), remaining captive of their past evolution.

Strathmann (1987) argues that the differences between non-feeding and feeding larvae merely result in a greater dispersal for the latter. As Crisp (1984) states, even a single tidal excursion can carry a larva of short planktonic existence, a useful distance, with extended drift subjecting the larva to a higher risk of mortality from predation or starvation. Strathmann (1987) suggests that the primary function of larval feeding is growth. This allows a decrease in parental investment per offspring and an increased size, or increased energy reserve, at metamorphosis. He comments on the conservative nature of feeding

mechanisms, with similar structures occurring across several related phyla. However Strathmann (1986) notes that the transition between types of larval development are restricted because of a bias against recovery of a feeding larval stage.

In recent years, small scale biochemical techniques have been successfully developed (Holland & Gabbott 1971, Holland & Hannant 1973) and healthy larvae can now be produced in large numbers under laboratory conditions. As a result several studies have been carried out on the energy budgets for invertebrate eggs and pelagic larvae e.g., Achituv & Barnes (1976 & 1978b), Lucas et al (1979), Lucas (1980), Achituv (1981), Achituv & Wortzlavski (1983), Dawirs (1983), Mann & Gallager (1985), Harms (1987) and Lucas & Crisp (1987). The eggs of all invertebrate larvae have a high lipid content even though, as in the case of some bivalves, glycogen is the primary energy source for the adult (Crisp 1984). When considering larval energy metabolism, lipid is far more important than protein, with carbohydrate being utilised at a fairly insignificant level. Lipid is the most energy rich of all nutrients with a high calorific content per unit weight and volume, consequently taking up comparatively little storage space (Crisp 1976). It has a lower density than water, thus providing buoyancy, which is especially beneficial to shelled larvae. In Holland's (1987) review of lipid biochemistry in barnacles, he largely deals with planktotrophic larvae and their adults, but, as the eggs, stage one larvae and cyprids of these species are all non-feeding, the relevance to lecithotrophs is clear. At the cellular level, lipids are involved in cold tolerance, cell

membrane structure, active transport, oxidative phosphorylation, cell organisation and cell replication and as a local hormone. Holland (1987) discusses lipid composition during egg development and points out that the fatty acids present are similar to those of other marine animals with high levels of long chain 20:5ω3 and 22:6ω3 polyunsaturated fatty acids. For *Balanus balanoides*, however, Barnes (1965) discovered that protein catabolism was the main fuel for embryogenesis and this evidence has subsequently been supported by Lucas and Crisp (1987). They concluded that, with the excretion of ammonia, adequate flushing was essential. Therefore, animals living higher up the shore may use lipid in preference to protein as an energy source. Lucas (1980) however, noted an enrichment in the two main energy reserves, protein and neutral lipid, during the last month before egg maturity. He also observed variation in the neutral lipid content from one year to the next, compared to a very consistent phospholipid content. By starving the adult *Balanus balanoides*, which prolongs retention of the eggs (Crisp & Spencer 1958), substantial reduction of their neutral lipid content was recorded. This demonstrated the importance of neutral lipid as an energy reserve.

Holland and Walker (1975), kept free-swimming *Balanus balanoides* cyprids at 8°C for eight weeks in the laboratory and prevented them from settling. At the end of this time these lecithotrophic larvae had utilised 90% of their neutral lipid reserves for maintenance. Changes in minor biochemical components of cirripede eggs for several species have been studied by Barnes (1965) and Achituv and Barnes (1978a and 1978b). However, Achituv

et al (1980) noted gross biochemical changes during the transition from Stage I to Stage II nauplius for *Balanus balanoides* and in 1981 he investigated *Tetraclita squamosa rufotincta* larvae. Although these larvae were not fed, neither were the conditions aseptic, therefore it was possible that some bacteria and protozoans were present. A decrease in larval weight was observed with initial loss of lipids and no loss during naupliar development. At this stage a small amount of protein was utilised and at their metamorphosis to the cyprid there was a marked decrease. Knight-Jones and Crisp (1953) had shown that protein can provide the glucose and nitrogen necessary for the formation of chitin. Consequently it was surmised that this protein decrease was associated with cypris chitin formation. The carbohydrate levels were very low throughout, and the sample for analysis of lipid content was taken before they had expended much energy swimming, so levels were artificially high. Thus, protein was seen to contribute approximately 80% of the total energy utilised by the nauplius for maintenance and development. This use of protein as a major reserve, for these larvae, living in waters which are low in nutrients, would possibly account for their unusually large size.

The methods used by these researchers to determine energy utilisation are related to oxygen uptake, assuming that respiration is fully aerobic. The two extremes under consideration are complete repose (Standard rate) or maximum activity (Active rate). Within this range, typical metabolic rates, including the cost of swimming activity, may be found

(Newell 1979). These have been termed 'routine rates' (and are similar to standard rates) for zooplankton organisms (Vlymen 1970). Thus, energy loss by respiration can be compared with biochemically determined losses. Crisp (1984) correlated the figures obtained by several workers, for metabolic rates of lecithotrophic or starved invertebrate larvae (see Crisp 1984, Table 5.) He concluded that most active larvae consume 2 ⇒ 10 $ml.O_2h.^{-1}g.^{-1}$ dry weight. *Balanus balanoides* cyprids however showed a consistently recurring value, an order of magnitude lower, being approximately $0.5ml.O_2h^{-1}g^{-1}$ dry weight in three independent studies (Zeuthen 1947, Holland & Walker 1975, Lucas et al 1979). This unusually low metabolic rate lengthens the survival time in the laboratory and, presumably also in nature. The dramatic increase in metabolic rate following cypris settlement occurs during metamorphosis and the speed of the latter can be governed by oxygen availability of the surrounding medium (Lucas et al 1979). Consequently it is important when considering an energy budget for larval development of a particular species, to take into account the physiological and ecological conditions under which the larvae exist. Dawirs (1983) and Harms (1987) carried out studies for the larvae of *Carcinus maenas* and *Elminius modestus* respectively. Measurements for all stages were taken at three different temperatures and, as both species are planktotrophic, Dawirs (1983) also considered starved zoea. In this case he concluded that an observed dry weight gain of 20% throughout the first day was caused by inorganic salts entering the organism after the moult of the pre-zoea. The dry

weight then decreased as starvation continued. His observations showed an approximate energy loss of 65% occurring during the first four days and this remained constant thereafter if starvation continued. When considering energetics for crustaceans the state of the moult cycle is also relevant, as Hagerman (1976) noted whilst studying *Crangon vulgaris*. During late pro-ecdysis the increase in oxygen uptake exceeded the standard rate by 2 ⇒ 4 times, possibly due to energy needed for regulation of physical processes within.

For *Elminius modestus* (Harms 1987), the dry weight, elemental composition (C, H, N) and energy content were all at their highest values at 18°C and the lowest biomass loss by exuviae was also found at this temperature. During larval development the energy loss due to investment in growth was found to be much greater than that for metabolism. Thus, it was deduced that a higher percentage of accumulated energy was converted into growth of the larvae at 18°C compared to that at 12° or 24°C.

Planktotrophic larvae have to endure variable food availability and the consequent threat of short-term starvation, but the larvae of *Sacculina carcini* are lecithotrophic. Although they have their own energy resource it is finite and therefore the length of pelagic life is limited. Recent studies however, have been carried out on the uptake of dissolved organic material from seawater, involving mainly molluscan and echinoderm eggs and larvae (Manahan 1983, 1989; Manahan et al 1989; Jaeckle & Manahan 1989a, 1989b, 1989c). Manahan (1983) observed the uptake of dissolved glycine, following egg fertilization in *Crassostrea*

gigas and Jaeckle & Manahan (1989b) detected an energy imbalance and weight increase, during the development of the lecithotrophic molluscan larvae of *Haliotis rufescens*. It had previously been shown (Jaeckle & Manahan 1989a) that *H. rufescens* can take up and metabolise dissolved free amino acids from seawater. Nevertheless Shilling & Manahan (pers. comm.), noted that the crustacean *Artemia salina* was unable to utilise this resource. (Also see Stephens 1986)

Not only do the larvae of *Sacculina carcini* have to find the ephemeral settlement target, but also the conditions to which they are subjected, in different habitats, can be extreme. Pechenik (1987) reviewed the literature on the influence of environmental factors on larval survival and development. He commented that changes in conditions will alter the rate of utilisation of finite energy reserves for lecithotrophs. For planktotrophs, there would be an increase in length of time spent in the plankton. He notes that temperature has a greater effect than salinity for example, but often, variable factors interact making the overall effect more difficult to assess. Lützen (1984), working at the Isefjord, Denmark, noted salinity varying from 18‰ to 21‰ and the temperature range was 0°C ⇒ 18°C. Bourdon (1963) found *Sacculina carcini* on three species of crab at Roscoff. Two of these host species lived sublittorally, thus exposing the parasite to full sea water salinity of 34‰. This is also the case for the sublittoral population at Plymouth where surface water temperatures vary between 6°C and 16°C (Walker 1985).

In 1959, Rasmussen, also working at Isefjord, noted that

sacculinised male and female *Carcinus maenas* bearing mature externae migrate into deep water where they remain for up to one year. In the same way, berried females leave the littoral zone in early Summer for the deeper, more saline offshore environment. These normal crabs remain until their larvae hatch, then return to moult and copulate with the males. The egg cleaning behaviour carried out by such berried females, is also mimicked by parasitised crabs and periodic flapping of the abdomen was seen to increase when oxygen tension of the water was low (Rasmussen 1959). This response was not a simple tactile one as the externa had to be living, thus it was probably due to feminisation of the host. Such observations were also made by Veillet (1945) and by Bishop and Cannon (1979) studying sacculinised *Portunus* (= *Liocarcinus*) species. Rainbow (1979), noted increased infection levels, relative to habitat depth, among *Carcinus maenas* in the Pembroke area. The young intertidal crabs were 16% infected: at 3 metres subtidally, infection was 36.2% and at 10 ⇒ 15 metres there were older crabs 80.4% infected. Sloan (1985), studying the infection of *Lithodes aequispina* by the rhizocephalan *Briarosaccus callosus* concluded that the levels of infection were related to a combination of physical and biological factors. Local topography may restrict crab population distribution, together with modified behaviour of parasitised hosts. Unlike the latter case ,many reseachers have observed that parasitism of *C. maenas* by *S. carcini* also prevents the host's ability to moult, although Lützen (1981a) has recorded scarred crabs which do moult. Veillet (1945) compared parasitised with non-parasitised

C. maenas stating that there was a reduction in relative moult increment from 30% to 10% over six months during interna development. At this stage the male is feminised by allometric growth of the abdomen, thus making young parasitised males recognizable before the externa is visible. Once the externa has emerged parasitic anecdysis follows. These effects on the secondary sexual characters of the host were assessed by Reinhard (1956) to possibly result from destruction of the host endocrine glands by the parasite.

A literature survey of the effects that crustacean parasitic castrators have on the growth of their crustacean hosts was carried out by O'Brien and Van Wyck (1985). They considered hormonal and nutritional drain to influence host growth. As there was minimal data available comparing parasitised to non-parasitised host size they suggested a more relevant parameter. By dividing the number of parasitised hosts by the total number of hosts within a size class, this would determine parasite prevelance as a function of host size. The increase, decrease, or constancy of size prevalence would infer whether different parasites were affecting host growth in different ways. However, size prevalence curves would also reflect dynamic equilibrium processes simultaneously occurring in the population, e.g. recruitment, infection rate, mortality and differential growth. Thus, backup data by measurement would be essential to produce meaningful results. Nevertheless, they do infer that for *Sacculina carcini*, smaller adult host size classes harbour the highest prevalence of parasites.

The energetic cost of any rhizocephalan parasite-host relationship is not known, but work on parasitism of the bopyrid *Palaeomonetes pandalicola* on the grass shrimp *Palaeomonetes pugio* was carried out by Walker (1977) and also by Anderson (1977). Walker found that 25% of the host haemolymph volume went daily to the parasite and Anderson assessed that 10% of the daily energy intake for the host was lost to the parasite.

O'Brien and Van Wyck (1985) discussed the enhancement of somatic growth by re-apportionment of host energy reserves due to the presence of a parasitic castrator. However, in those rhizocephalan families studied there was no significant difference between parasitised and non-parasitised host size (Lernaeodiscidae - Brinkman (1936); Clistosaccidae - Høeg (1982); Sylonidae - Lützen (1981b); Thompsonidae - Potts (1915), Reverbei (1942), Phang (1975).

Considerable work has also been carried out on hormonal activity. Andrieux et al (1981), noted that "parasitised" haemolymph injected into non - parasitised crabs increased the moult cycle length. Herberts (1982) discovered two unidentified protein factors in the haemolymph of sacculinised *Carcinus maenas* that were not present in the non-parasitised crab. Consequently it is possible that moulting ceases due to hormonal input from *Sacculina carcini*. Alternatively the increase in rate of energy utilisation linked to the rhizocephalan reproductive effort may affect the duration of the moult cycle. Haematophagy would lengthen the time required to absorb sufficient energy reserves before ecdysis was possible. Ecdysis is also a hazardous period.

The host is more susceptible to predation or may not withdraw successfully and host death would lead to the parasite's death as rhizocephalans are unable to abandon and re-infect.

From the evolutionary standpoint it would seem that greater host size would increase survivorship and improve intraspecific competitiveness for food and space. Normally it would also increase host fecundity but not in this case. However the reduced size of sacculinised *C. maenas* may increase parasitic fecundity by reducing generation time. Cole (1954) thought that selection should push the time of the first larval release to the minimum physical host size. O'Brien (1984) studying *Pugettia producta* parasitised by *Heterosaccus californicus* noted that this may be the case because there would be fewer instars before allometric growth began.

Some early work was carried out on the fat content of crabs infected by rhizocephalan parasites but the evidence tends to be conflicting, largely because the parameters measured were different and therefore not truly comparable (Smith 1911, Robson 1911, Fischer 1928, Dornesco & Fisher-Piette 1931 & Reinhard 1944).

Thus it can be seen that when determining the total energetic strategy of parasitism by *Sacculina carcini* on *Carcinus maenas*, many factors are brought into play. For the study of the larval energy budget a proportion of these must be considered. When reproductive energy is low the number of propagules, rather than the size or quality tend to be reduced (Crisp 1984). The size and composition of each individual reproductive body

produced by a species is determined within strict limits and fitness depends on correct investment with excess being wasteful and too little leading to extinction.

CHAPTER II : FIELD STUDIES ON THE DISTRIBUTION OF *SACCULINA CARCINI* ON *CARCINUS MAENAS*

Parasitism of *Carcinus maenas* by *Sacculina carcini* has been reported by different authors in several geographical areas (see Table 1). Unfortunately methods of capture, times of sampling, intervals between sample periods and methods of recording the resultant catch, often vary. This makes any correlation of results from the literature very difficult. Consequently, there are many sites of known parasitism but little is known of the temporal or spatial variation that may occur within the populations.

In May 1988, the opportunity arose to visit the Station Biologique in Roscoff, northern France. These are the laboratories where Yves Délage produced his works (1884) on *Sacculina carcini* and where the parasite is said to be abundant locally on *Carcinus maenas* (Jens Høeg, pers. comm.). An initial survey was carried out within the limited time available and in May 1989, a return visit allowed a more extensive survey to be undertaken.

In November 1989, data were received from M.A.F.F., Conwy (Brian Edwards, pers. comm.) regarding sacculinised *Carcinus maenas* at Tal-y-foel, on the Menai Strait. As a result of this information, an investigative survey was carried out in the area. Observations obtained from this field work and, from experiments to assess the temperature and salinity tolerance of

Figure 1.

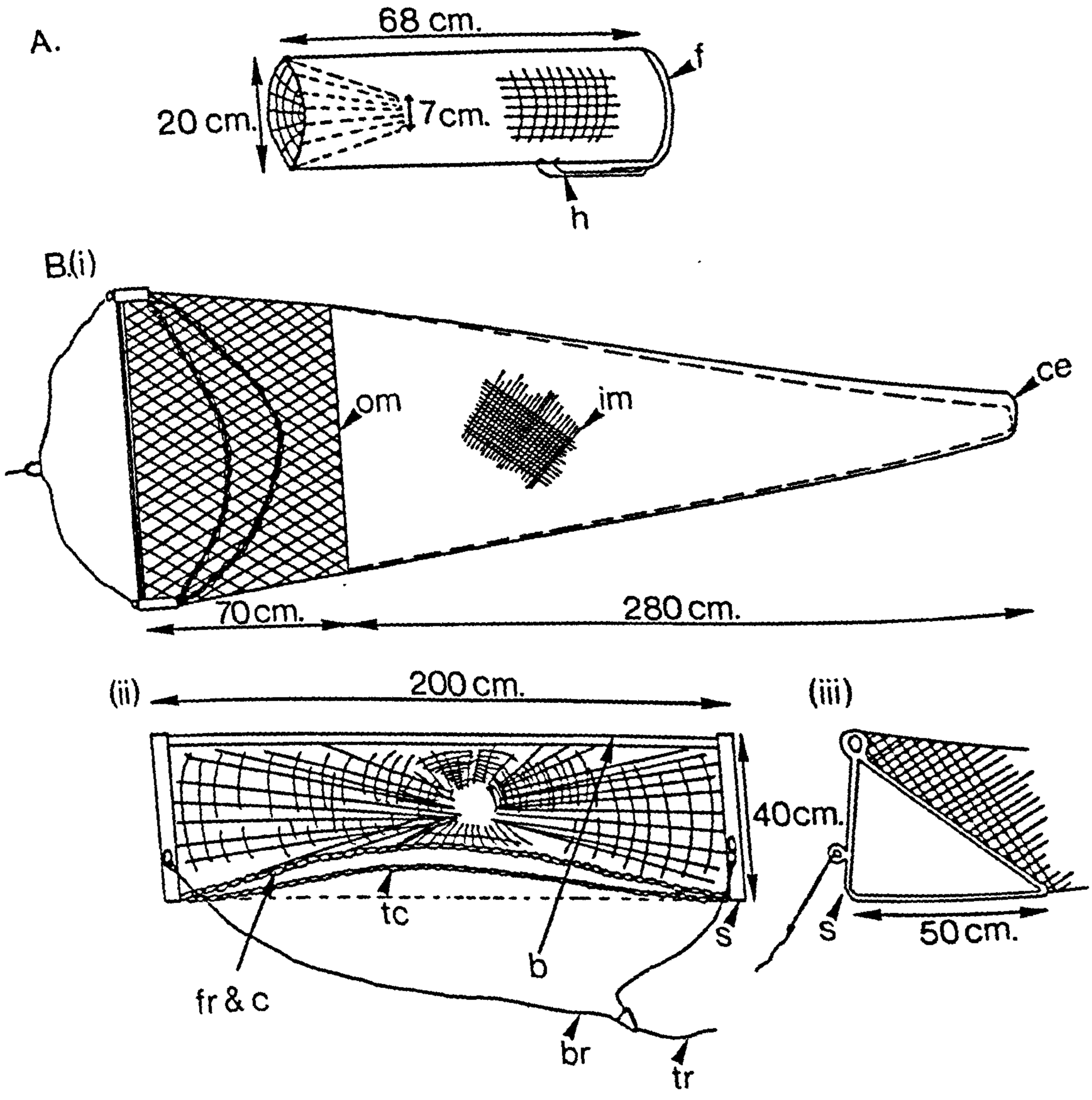


Figure 1. Diagrams to show the sampling gear used in this study.

A. Netlon crab trap - 5 mm. mesh.

B. Beam trawl from (i) dorsal (ii) frontal and (iii) lateral views (after Yee Ting 1990).

b = beam; br = bridle; ce = cod end; f = flap; fr & c = foot rope & chain; h = hooks; im = inner mesh (5 mm. diam.); om = outer mesh (20 mm. diam.); s = skids; tc = tickler chain (30 mm. diam); tr = towing rope.

the parasite larvae, together with information gleaned from the literature, have combined to enable a discussion on the distribution of *Sacculina carcini* on *Carcinus maenas*.

MATERIALS AND METHODS

Common shore crabs (*Carcinus maenas*) were captured by three methods; hand collection - over a 30 minute period; beam trawl - for thirty minute periods and, crab traps - left over two tidal cycles (see Figure 1. The remaining figures, tables and maps are at the end of the text in this chapter.). Traps were baited with mackerel, octopus or horse mackerel. Carapace width was measured for the captured crabs and they were also sexed. The presence or absence of *S. carcini* as a mature externa, virgin externa or a scar, was noted for each crab. The initial survey, using traps and hand collection, was carried out in Roscoff, Brittany, northern France in May 1988 and took place over three days, 10th ⇒ 12th May. Two traps were laid and 30 minute hand collections made, on two occasions and at two sites (Map 1 & Table 2). In the aquarium of the Station Biologique, Roscoff, a sample of shore crabs was held. These crabs had been trawled, the previous week, from the littoral zone during high water (Site 3, Map 1). The percentage of parasite incidence and the state of the parasite were recorded.

The following year, in May 1989, the sampling period lasted seven days, from the 9th ⇒ 15th May inclusive and covered

six sites, from Roscoff harbour westwards to Porz ar Stréat (Map 2). At each site 30 minute hand collections were made and two baited traps were left over two tidal cycles. The captured crabs were measured and sexed and the state of the parasite noted (Table 3). Once again, a sample of shore crabs, trawled from the littoral zone during high water (Site 3, Map 2) was available in the aquarium. These crabs were measured, sexed and the state of the parasite recorded.

In order to investigate information received from M.A.F.F., Conwy, common shore crabs were hand collected, trapped and trawled along the Menai Strait, from November 1989 to February 1990 (Map 3, Table 4). During this period, catches of crabs, collected locally for two other independent surveys, were also monitored (Table 5).

From February 1988 to February 1990 experiments were carried out to monitor the development of male and female *S. carcini* larvae at low temperatures. The larvae were collected from parasitised *Carcinus maenas* maintained at 18°C (see Walker 1987). Approximately 300 larvae were pipetted into two, sterilised 500 ml. glass beakers, containing U.V.-irradiated fine filtered seawater and placed at room temperature. The mouth of each beaker was covered with a piece of card to avoid air-borne contamination and minimise evaporation. These beakers were used as the control for the experiment. In the same way, approximately 200 larvae, were pipetted into two sterilised 500 ml. glass beakers full of U.V.-irradiated fine filtered sea water and placed in a water bath at the required temperature. Compressed

air was bubbled through the water in each beaker. The larvae were monitored regularly, using a Wild M5-97410 binocular microscope and any changes in the stage of development were noted. Samples of larvae were taken off daily and viewed under a Leitz dialux microscope, to measure them and verify their developmental stage by measurement. When observations under the low power microscope showed no change in stage, samples of larvae were not taken daily, to minimise contamination. Relevant photographs of larvae were taken with a Leitz Orthoplan photo microscope and cyprids from the control group were measured and sexed (Walker 1987).

To assess the salinity tolerance of the larvae, seawater samples of different concentrations, ranging from 17‰ to 32‰, were made up, using U.V.-irradiated fine filtered seawater and distilled deionised water, and the solutions were placed in sterilised 200 ml. glass beakers. The salinities were measured using an American Optical Corporation hand refractometer. Initially, the larvae were acclimated to reduced salinities (see acclimation times on Table 8) and two hundred larvae were pipetted into each beaker. The beakers were then covered and kept at room temperature. Daily room temperatures were recorded, together with larval mortality, activity and developmental stage. The control group was maintained as previously described in 500 ml. glass beakers, and the cyprids measured and sexed (see Walker 1987).

RESULTS

The data collected in Roscoff in 1988 and 1989 are given in Map 1, Table 2 and Map 2, Table 3, respectively. Figure 2 shows the overall percentages of parasitised crabs captured at each site by the two methods of hand collection and trapping, during 1988 and 1989 combined. Figure 4 gives the total number of sacculinised crabs which were retained at the end of the sampling period in 1988. A size frequency distribution for crabs trawled at Roscoff in 1989 is shown on Figure 3.

Results from the local survey along the Menai Strait are given on Map 3, Table 4 and results of the crabs examined from the other two independent surveys are in Table 5. Information received from M.A.F.F. Conwy is shown on Figures 5 and 6.

Results from the temperature experiments are given in Tables 6 & 7. The acclimation times used to place the larvae at different salinities are shown on Table 8 and the results for the salinity experiments with male and female larvae are given in Tables 9 & 10.

DISCUSSION

During the initial survey at Roscoff in May 1988, it immediately became evident that the rate of infection recorded for *S. carcini* on *Carcinus maenas* related strongly to the method of capture used (Table 2). A particularly high infection rate of

68% was observed from one hand collection that year in Roscoff harbour (Site 1). The area next to the harbour wall, adjacent to this sampling site, was dredged in the autumn of 1988 to provide fishing boats with a suitable channel. Such dredging activity did not markedly affect the population of parasitised crabs as the average infection rates, for both years at Sites 1 & 2 were approximately 60% ⇒ 50% using hand collection, compared with 10% ⇒ 6% using traps. On two occasions the traps were baited with octopus, as horse mackerel was not available. Both times this resulted in a poor catch (Site 1, Table 2 & Site 1, Table 3). The trawled crabs, which were examined from Site 3, showed a consistent rate of approximately 6% parasitism in both 1988 and 1989. The infection rates recorded at Sites 4 to 8, going westwards along the Brittany coast, dropped dramatically within a 25 kilometre range. Nevertheless the different percentages recorded at each site, once again reflect the method of capture used, with a far higher rate of parasitism in the hand collected samples (Figure 2). This was not the case at Site 8, where parasitism was found to be less than 2% using both methods. At Site 7, only two crabs were caught in the trap. This was probably because it was not sufficiently weighted down and consequently moved too much to be effective. It should be noted here that no berried crabs were captured in the traps, whereas, amongst the 252 female crabs caught by the two other methods in May 1989, approximately 12% were berried. Only two of all the sacculinised crabs studied showed double infections (i.e. two externae). In 1988 13% of the crabs with mature externae had not released their

first brood of larvae but the number of virgin externae bearing male cyprids was not noted. However, in 1989, 30% of the mature externae had not released a brood and out of the 13 virgin externae observed, 7 had cyprids around the mantle aperture. Thus, of all the externae observed in 1989, 47% were mature, 38% were immature (i.e. had not released a brood, or bore male cyprids), and 15% were virgin. Høeg (pers. comm.) collected *Carcinus maenas* bearing *S. carcini* at Pte. de Perharidi (Map 1), west of Roscoff, in May to June, and again in September of 1986. In the May to June period he observed 37% were mature externae, 19% were immature externae and 44% were virgin externae. These figures changed to 92%, 7% and 1% respectively for the September period. The apparent fall in mean annual sea temperature at Roscoff, since 1970, remained during 1983-1987 (Dauvin et al 1989). However, the more rapid maturation of externae in May 1989 may be due to increased temperatures resulting from the mild winters and warm summers of 1987 and 1988.

The crabs which were trawled at Roscoff in 1989, were approximately 60% male and many of the females had recently released their eggs. No berried females were captured. The size frequency distribution is shown on Figure 3 with most crabs measuring between 40 and 70 mm. carapace width. The 11 parasitised crabs ranged between 43 and 67 mm. carapace width. From Figure 4, it can be seen that the majority of parasitised crabs, collected in 1988, ranged between 20 mm. and 45 mm. carapace width. During high water, the small crabs are more susceptible to predation by large crabs which have migrated up

the shore to feed (Naylor 1962). Consequently, during this period, many of the smaller crabs, which are more heavily parasitised, and therefore likely to adopt the "nursing" behaviour of berried females (Rasmussen 1959), may shelter beneath rocks in the intertidal and in the deeper sublittoral zones. These are possible reasons for the very low percentage parasitism recorded for the trawled crabs in 1988 and 1989 at Roscoff.

The data collected along the Menai Strait in North Wales showed *Sacculina carcini* to be present on *Carcinus maenas* at only two of the sites visited (Sites 6 & 7, Map 3). The infection rate observed at both these sites was less than 1%. One male with a virgin externa and one female with a mature externa were noted at Site 6, whereas the infected crab at Site 7 was a scarred female with live rootlets within. All three animals were captured by traps. The figures produced by M.A.F.F. for 1975 ⇒ 1977 at Site 6 gave a maximum infection rate of nearly 6% (Figure 5). They used a beam trawl of the same dimensions as that used in the present study, towed along the same sublittoral area for two hours either side of low water. The other method of capture employed in the 22 month study was that of funnel traps, set at low water neaps and fishing on the ebb tide. Recognition of the parasite was marked by the presence of an externa and over the first two years a pattern emerged showing the main incidence peak to be in August with a smaller peak earlier in the spring. It can also be seen from Figure 5, that the numbers of parasitised crabs captured by traps were consistently fewer than those captured by the beam

trawl. Once again this showed the method of capture to be an important consideration.

In the Menai Strait the megalopa larvae settle out of the plankton and develop to first crab stage by June. It would appear that the megalopa is too efficient in its cleaning capabilities to enable *S. carcini* cyprids to settle successfully anywhere on its surface (Walker, pers. comm.). However this is not the case for the first stage crab which may become infected from June onward, throughout the summer, with the resultant virgin externa emerging the following spring (Lützen 1981a). At this time of the year, male larvae will predominate in the broods of *Sacculina carcini* which are released into the plankton (Walker 1987). Once the virgin externae have been infected by male cyprids, the development of the parasite may cause the host to migrate to the deeper sublittoral zone, with the normal berried females (Rasmussen 1959) and may account for the trough shown on Figure 5 in March and April. The decrease in the number of externae recorded in September to November may illustrate the main period when externae which have survived the previous winter, or winters, finally drop off their host.

During the period of study at Tal-y-foel the overall percentage of crabs parasitised by *S. carcini* decreased. Within the Menai Strait there is a residual south-westerly flow of water (Simpson et al 1971). Although the decrease in parasitism may have continued in this area, it was thought that there may be evidence of *S. carcini* on *C. maenas* at Site 8 (see Map 3). However, this was not the case as over 2,000 crabs were examined from this

site alone and no signs of parasitism were observed. It is possible that the main parasitised crab population occurs outside the Menai Strait. Perhaps an estuary such as Maltraeth (see Map 3) would be a better site for retention of larvae and consequent infection. Unfortunately there was insufficient time to carry out a survey in this area.

From experiments undertaken in the laboratory to monitor the temperature and salinity tolerance of *S. carcini* larvae, it became clear that both males and females could tolerate very low temperatures. When male larvae were transferred directly from a system at 18°C to one at 2°C they survived for 25 days but did not develop beyond stage II. Most broods of *S. carcini* larvae consist of a high proportion of stage II's on release. When the system was maintained at 5·5°C ± 0·5°C, the female and male larvae survived for 44 days and 35 days respectively. Once again they did not develop beyond stage II. The main problem when keeping larvae in a beaker for any length of time, at temperatures greater than 5°C, was due to contamination by protozoa. Although the beakers were sterilised before use, cessation of an experiment was usually due to such contamination and not because the larvae reached the limit of their energy resources. Nevertheless, even if these larvae could survive for longer periods at low temperatures in the wild, it is not known whether they would have sufficient energy reserves to settle and metamorphose successfully (see Lucas et al 1979 for *Balanus balanoides*). From these experiments it appeared that male larvae could develop to cyprids within 16 days at 7°C - 8°C (Table 6),

whereas females developed within 17 days at 8°C - 9°C (Table 7). These were the lowest temperatures at which these larvae completed development. The control group, which were kept at a room temperature of 17·0°C ± 2·0°C, developed to cyprids within 6 days. Activity of the larvae, at the lower temperatures, consisted of the usual bursts of swimming but with longer periods spent resting on the bottom of the beaker. Contamination by protozoa, with the consequent attack on larval limbs in particular, resulted in reduced larval movement and appeared to lessen larval capability to moult successfully.

When the experiments on larval salinity tolerance were carried out, it was necessary to acclimate the larvae gradually to decreasing salinities (Table 8). The lowest level tested was at 17‰ and after 6 days, the control group, at 32‰, developed to cyprids with normal swimming activity and only a 3% mortality rate. At 17‰, there was a 13% mortality rate, over the same period of time. However the activity was greatly reduced, the larvae merely twitching their limbs in short bursts. Such low activity promoted protozoan contamination and by Day 6 they were still at stage II.

Development to cyprids was achieved within 6 days at 22‰ for female larvae (Table 9) and within 9 days at 21‰ for males (Table 10). In both cases the swimming activity of the nauplii was greatly reduced and consequently their ability to moult successfully was restricted. The stage III and stage IV cuticles together with developing cyprid, were visible inside the stage II carapace. Once the cyprid was fully developed, its greater bursts

of swimming activity, allowed it to free itself from the other cuticles and continue normal activity at these salinities.

A third experiment with male larvae was carried out at 17·5°C ± 0·5°C. Those larvae at 18‰ and 19‰ developed to stage III by Day 4 but, partially due to low activity, they were incapable of moulting properly and remained within the stage II cuticle. Many larvae were seen to lose body fluid through their frontal horns at these low salinities, by Day 6 they were heavily contaminated with protozoa and by Day 8 they were all dead. The control group however, at 32‰, reached the cypris stage by Day 6.

From these results it appeared that there is little difference between the temperature and salinity tolerances of male and female *S. carcini* larvae. A greater capability to reach the cypris stage at low temperatures would enhance the chances of successful settlement for male larvae, the majority of which are released over the winter months (Walker 1987). The cut-off point, restricting complete larval development, was seen to be marginally lower for males than females. The male larvae which developed to stage III by Day 10 at 7·0°C ± 0·25°C, were still at stage III by Day 21. This experiment had to be stopped because the dip-cooler broke down. However, if it had continued and the larvae had reached the cypris stage, it would have been interesting to observe whether they could achieve settlement on a virgin externa and the subsequent metamorphosis.

Reduced salinities did extend the larval developmental period. However, the reduced larval activity and consequent protozoan contamination observed in the laboratory situation,

would not be the case in the wild, where larvae would be subjected to infinite water massess. Nevertheless, it is uncertain whether they could maintain their position in the water column.

Høeg and Lützen (1985), mapped out the distribution of *S. carcini* from the west coast of Sweden down to northern Spain including the patchy distribution around the British Isles, the Mediterranean coast of France, the northern Adriatic and the Black Sea. The parasite was also introduced to Burmese waters by Boschma in 1972. Not surprisingly, the clearest records are in areas close to marine stations. However, in reviewing the literature it appears that parasite distribution is not continuous and that certain conditions encourage parasite establishment and maintainence.

The larvae of *S. carcini* develop to cyprids within five days at 18·0°C and are competent to settle three days later (Høeg 1991). This rapid lecithotrophic development seems to thrive under conditions where the ambient temperature is c. 18·0°C, and both the larvae and their prospective hosts are not widely distributed. This is particularly important for the male cyprids seeking ephemeral virgin externae. Such conditions result in very high infection rates. Veillet (1945), studied *S. carcini* on *Carcinus maenas* in L'Etang de Thau, a lagoon connected to the Mediterranean sea by a series of canals. Temperatures within the lagoon were seen to drop to 5·0°C ⇒ 0·0°C in the winter but minimum open sea temperature was 12·0°C. Salinity varied greatly, depending on precipitation and consequent land run-off, but was

generally lower than full strength seawater. In this area, the larvae of *C. maenas* settle out of the plankton by April or May, they then remain in the shallow intertidal becoming sexually mature by July to August and vitellogenesis commences in October. To avoid low salinity conditions, under which eggs may develop abnormally (Broekhuysen 1936), berried females and those crabs which are parasitised, migrate towards the sea, entering the canal system at the end of November. Only males, a few non-ovigerous females and the non-parasitised crabs, remain in the deep channels of the lagoon. At the end of winter, warm seawater rushes into the canals and the females and parasitised crabs return to the lagoon. Under these conditions, cyprids were found in the plankton throughout the year and crabs were infected at all stages of development. Parasite prevalence (i.e. the percentage of the total number of crabs examined which were parasitised - Margolis et al 1982) was regularly recorded as 70% ⇒ 90% within the lagoon. Such an enclosed system, with crabs able to migrate to waters of tolerable temperature and salinity, must enhance the persistence of the parasite.

On the west coast of Brittany, Bourdon (1960), reported *Sacculina carcini* on *Carcinus maenas* in the Baie de Quiberon. This is an area of coastline partially enclosed by a peninsula and consisting of many smaller bays and estuaries. St. Julien is a site sheltered by the peninsula and the incidence of parasitism observed here was greater than 50%. Push nets were used to capture the crabs studied. Thirty sites were monitored and within 10 ⇒ 15 km. of St. Julien, parasite prevalence was seen to drop

suddenly. These results compare closely to those found at Roscoff in 1989. Simultaneous infestation with the entoniscid *Portunion maenadis* was also noted by Bourdon at only two of the sites tested. Both sites were on the Rivière de la Trinité and infestations were numerous. A similar situation was recorded at L'Etang de Thau (Veillet 1945). The sites, at which parasitism by *S. carcini* was seen to decrease rapidly (Bourdon 1960), were at the point where the bay became more exposed to the open ocean. It seems logical that dispersal of these non-feeding larvae out to sea, would minimise their chances of inoculating a suitable host.

Another semi-enclosed system where *C. maenas* is parasitised by *S. carcini*, was studied by Rasmussen at the Isefjord, Denmark, from 1953-1960 (Rasmussen 1973). Although the whole fjord was monitored the main study area was close to the laboratory at Vellerup Vig, where 19,000 crabs out of a total 21,000, were examined. The methods of capture were prawn pots, eel traps and push nets. Distinction was not made between these methods, but Rasmussen did note that females were under-represented in the hauls and therefore parasite prevalence was probably higher than that recorded. Simultaneous infestation with *Portunion maenadis* was observed but as *P. maenadis* also induces feminisation, this was not a criterion used to identify parasitism by *S. carcini*. Occasional observations were made where both species had infected a host, but no feminisation was apparent.

Close to Vellerup Vig, parasite prevalence was much greater in the sublittoral than the littoral zone. From 1956-58,

90% of crabs with "small" externae were caught in the shallow water, whereas those with "large ripe" externae were from deeper water. Once again this was a similar situation to that reported in L'Etang de Thau (Veillet 1945). From 1954 (Rasmussen 1973), the infection rate appeared to decrease gradually reaching a low point in 1957. These figures rose again in 1958 with 20% (i.e. the highest infection rate ever recorded in the fjord), reported in the October. However, by July 1960, 0% was recorded at the same site. The high numbers of small crabs observed in 1951-52 may correlate with the high infection rate in 1953-54. The latter was also a good "crab year" but the parasite prevalence was seen to decrease after this period. The summer and autumn of 1955 were unusually warm with a mean temperature of 17·2°C and the emergence of virgin externae was observed from June to October, whereas emergence had only lasted throughout June and July in other years. In the September of 1956, an unusually warm month, a high number of virgin externae was again recorded. One would assume that under such conditions the parasite would thrive. Nevertheless, for some reason the male cypris settlement may have failed in that period. Winter temperatures were not published (Rasmussen 1973) but Lützen (1984), recorded temperatures of 0°C ⇒ 18°C from February to August and salinities varying from 18‰ ⇒ 21‰ in the Isefjord. During his three year study in this area, Lützen (1984) concluded that between November and April parasitised crabs overwinter by burying themselves into the substratum, for he reported sand in the mantle cavity of the externae he examined. Lützen (1984) noted that the larvae of

S. carcini could not develop through to cyprids at temperatures less than 6°C, therefore the winter was spent in a non-breeding state. He observed two breeding periods. The first began in May, with a peak in June, and the second was from mid-July to October, which were the warmest months. He noted that at least six broods of eggs could be released in this period and the internal phase took 2 years and 9 months in these waters compared to 9 months in the Irish Sea (Day 1935). Walker (1987) reported that virgin externae are no longer capable of accepting male cyprids after 8 months. Consequently, if temperatures had remained low in the winter of 1956, or if high precipitation had caused low salinities in the fjord, male cyprids may not have developed in time.

More recently, a study has been carried out in Portugal. In October and December of 1987 and in July 1988, observations including the parasitism of *C. maenas* by *S. carcini* were made in the lagoon of Aveiro, west Portugal (Gomez, pers. comm.). Temperature, salinity and percentage parasitism were recorded at over sixteen sites each month. In October the temperatures were between 17·0°C and 19·1°C, in December they had dropped to 11·0°C to 13·5°C, with one site at 15·0°C. In July the temperatures ranged from 18·6°C to 23·7°C. Compared to these relatively stable temperature distributions, salinities varied greatly. For example, the first six salinity readings in October were 18‰, 25‰, 12‰, 1·5‰, 26‰, & 4·8‰. The number of crabs caught at each site varied between 100 and 500 but, below a salinity of 12‰, no parasitism was recorded (Figure 7). Also, out of the 24

recordings of parasitism made, 20 were at sites where the salinity was greater than 18‰. Once again an enclosed system exists where both the larvae of host and parasite would remain entrained, but the controlling factor here may be salinity, with the host's tolerance being far greater than that of the parasite.

Carcinus maenas is widely distributed intertidally but rarely reported below 10m. depth. It is often found in estuarine conditions with salinities ranging from 35‰ ⇒ 10‰ (Crothers 1968). Temperatures up to 26°C can also be tolerated by the shore crab but Naylor (1965), noted that a drop in temperature was necessary to stimulate breeding. From the literature, some of which has already been cited, the presence of *S. carcini* on *Carcinus maenas* has been recorded at high infection rates in estuaries and other semi land-locked bodies of water. Rainbow (et al 1979) reported 80·4% and 36·2% infection rates using a beam trawl in the sublittoral zones of Milford Haven and Pembroke Dock respectively. From the intertidal zones at West Angle Bay and Pembroke Ferry he recorded parasite prevalence of 6·7% and 18·2%, however the method of capture used intertidally was not specified. If crab traps were used it is possible that these figures under-represent the actual percentage incidence of the parasite. This study (Rainbow et al 1979) was carried out in September, a period of the year when the majority of crabs infected with *S. carcini* would bear a mature externa. This may be another reason for the higher percentage incidence in the sublittoral zone. Rainbow (pers. comm.) trawled the same area two years later and reported the parasite prevalence to be 5%. This

variation between years is similar to the situation found by Rasmussen (1973) at Isefjord and from this study carried out in the Menai Strait. It seems possible that, because *Carcinus maenas* migrates readily, the centres of high parasite concentration may differ between years. This is probably not the case for *S. carcini* on *Liocarcinus holsatus*, which also migrates over very long distances but is a species that appears to aggregate more than *C. maenas*.

Since 1964, observations of *S. carcini* on *Carcinus maenas* and *Liocarcinus holsatus* have been noted in Milford Haven and the Severn Estuary (Crothers, pers. comm. and Rainbow, pers. comm.). It appears that the parasite occurs on one or the other host but not on both, in any single location. Fauna lists from other areas such as, Plymouth, Port Erin and Strangford Lough, also support these observations. In North Wales there is a population of *S. carcini* on *Liocarcinus holsatus* in the sublittoral zone of Red Wharf Bay, Anglesey (Map 3)(pers. obs.), but *S. carcini* on *Carcinus maenas* has not been found in the same area.

Day (1935) studied *S. carcini* on *Liocarcinus holsatus* in the Mersey Estuary, between November 1931 and October 1932. The crabs were collected by shrimp trawl and he observed a high infection rate of 73% in May (Figure 8). This was also the month in which most berried females were captured. He considered the fate of crabs after the externa had dropped off and observed crabs which did not moult, some of which died and others produced an externa within 5 months. He also reported crabs which did moult, with the males losing their abdominal characteristics of

feminisation and becoming a more normal shape. In this case, some of the roots had not necrosed whereas others had melanised, being cut off from the healthy tissue by a layer of chitin. Similar observations have been noted for *Carcinus maenas* in this study. Also the fate of the externa can vary. A large male crab which bore a mature externa close to the end of its abdomen was seen to rip the externa with its chelae and consequently remove it.

Another observation was made when a mature externa, due to release its brood, was accidentally knocked off the host. The externa was placed in a beaker of clean sea water and one hour later, began to pulsate. This continued until all the larvae were released. The externa was maintained in clean sea water at 17·0°C ± 2·0°C and daily observations showed it to be undergoing gentle waves of contraction. After 11 days it was still moving but became covered by a fungal infection and later no movement was noted.

The final region to be discussed is around the Clyde Sea area where Foxon (1940), Pyefinch (1946) and Heath (1971) have reported *S. carcini* present on *Carcinus maenas*. Working at Millport, Heath (1971) observed scarred crabs which later bore virgin externae arising from a separate break in the cuticle. Although he used two methods of capture, (i.e. creels, subtidally and hand collection intertidally) he did not differentiate between the two and amassed the resultant catches. The survey lasted from September 1965 to September 1966 and the captured crabs were separated into four categories; modified males with no externae; scarred crabs; crabs bearing a large externa and those

bearing small externae. Throughout the year he was able to collect crabs from all categories. Each group, except for those crabs with small externae, displayed a marked peak during the year and parasite prevalence was recorded (Heath 1971) at less than 7%. Temperatures at Millport from 1959 ⇒ 1979 (Moore 1980), showed an annual mean of approximately 10°C. Each year the general trend was towards a minimum of 6°C ⇒ 7°C occurring in February and March, and a maximum of 13°C ⇒ 14°C in August. Moore (1980) also noted that sea surface temperatures for the decade 1969 ⇒ 1978 were warmer than the preceding twenty years. At such temperatures the successful development of *S. carcini* nauplii through to the cypris stage, could be achieved during most months of the year. In recent years (Moore, pers. comm. and Rainbow, pers. comm.), the incidence of parasitism in this area, appears higher than it was 15 - 20 years ago, although there are no data to corroborate these opinions. Consequently, the distribution of *S. carcini* on *Carcinus maenas* appears not only to be patchy but also to vary both temporally and spatially.

In the laboratory situation, larvae of *S. carcini* were capable of withstanding a dramatic drop in temperature from 18°C to 2°C. However the males could only complete development to the cypris stage at 7°C ⇒ 8°C and the females at 8°C ⇒ 9°C. The developmental period at these low temperatures was approximately 3 times that required at 18°C. When considering low salinities, the larvae developed to cyprid inside previous stage cuticles. This occurred within a similar time scale to the development of the control group at 32‰. The reduced mobility experienced by the

larvae at low salinities appeared to hamper the moulting process. This may not be the case in the wild where the larvae are subjected to water currents and from the study at Aveiro (Gomez, pers. comm), it appears that they can withstand low salinities. Thus, the distribution would seem to be the result of a compromise. The water circulation in a relatively enclosed body of water, such as a harbour, estuary, lagoon or fjord may result in retention of larvae for both the host and the parasite. At L'Etang de Thau (Veillet 1945), with parasitism recorded up to 90%, the lagoon is connected by narrow canals to a sea with a very small tidal range. The lagoon at Aveiro, which is only 1 ⇒ 6 metres deep, has a similarly restricted circulation. However within such areas the temperature and salinity can fluctuate greatly. In an estuary for example, there is a seaward flow of lower salinity water in the upper layer, balancing a landward flow of higher salinity water at the bottom (Bowden 1962). This situation is utilised by *Rhithropanopeus harrisii* (Cronin & Forward 1986), the mud crab which has been reported with a parasite prevalence of 33% by the rhizocephalan *Loxothylacus panopaei*. These crab larvae undergo regular vertical migrations to maintain their position in the dimly lit regions of the water column, where they are able to feed, but there is little risk of visually directed predation. This also enables the larvae to ride the residual non-tidal currents, so they are retained high up the estuary. In Milford Haven, the volume of freshwater entering the Haven is small compared with its tidal volume (Nelson-Smith 1965). Consequently salinities at substratum level, in the upper

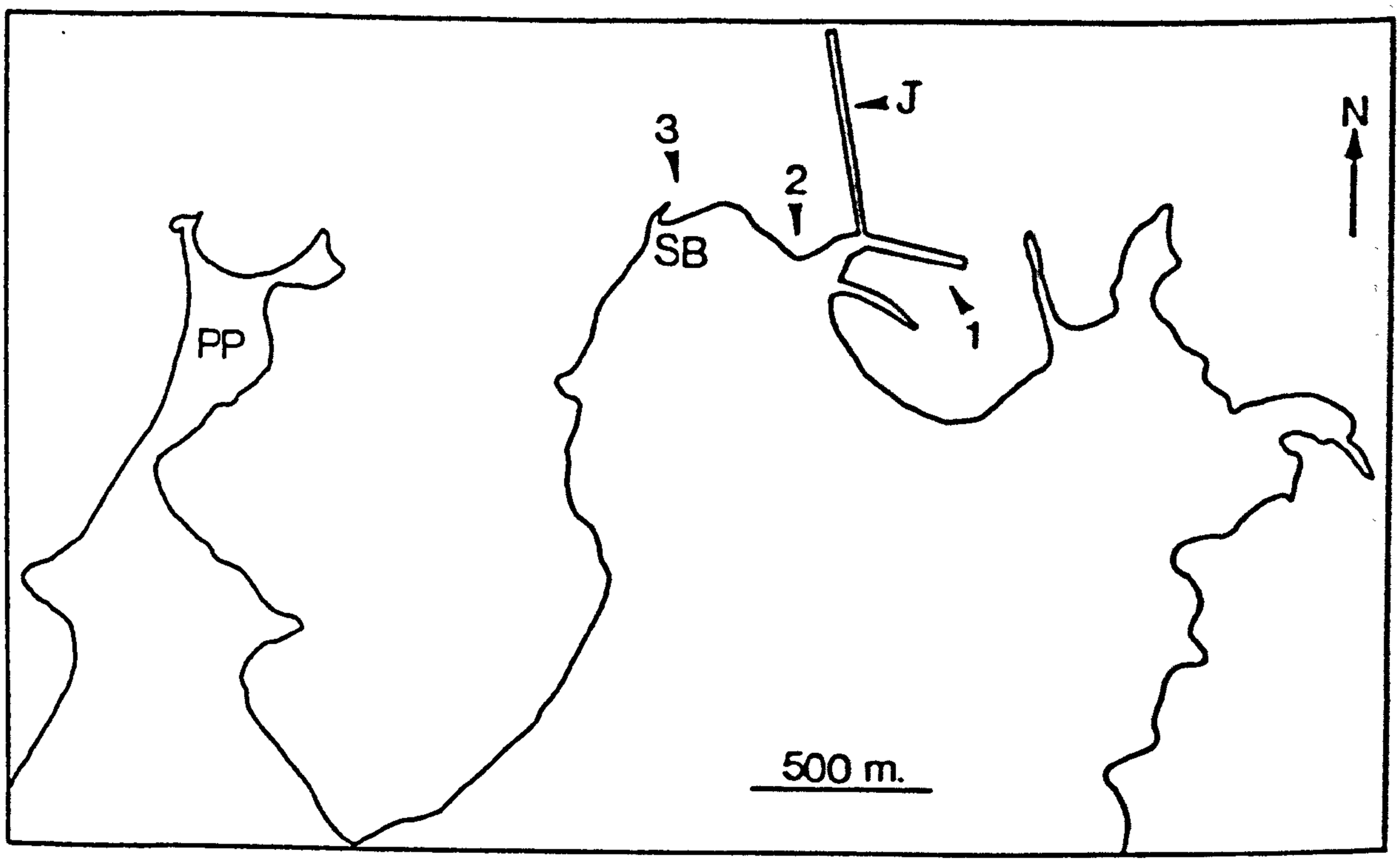
reaches of the estuary, are high. At low slack water of a neap tide, the salinity at Landshipping Quay, was recorded as 26‰ at the bottom and 21‰ at the surface. It is further up the estuary, at the confluence of the East and West Cleddau Rivers that salinity drops to 10‰. Dr. H. A. Cole, in a mimeographed report (1956), referred to the "unexplained mechanism" which tends to hold oyster larvae in the upper reaches of Milford Haven. In the same way, it would seem that *Sacculina carcini* larvae, swimming in short bursts and spending periods lying on the bottom, could also be retained within such a system.

The flushing time, for a body of water, represents the time taken to completely renew the water within that volume. This may be calculated for a harbour, for example, by dividing the volume of the harbour by the tidal prism. (Dyer 1973). It is possible to calculate flushing times for the different semi-enclosed bodies of water described here. However, a comparison of flushing times with parasite prevalence would only be valid if the method of capture was consistent in each case. From the literature cited and from the surveys reported, it appears that a longer flushing time would coincide with higher parasite prevalence, but further research would be necessary to investigate this theory. It is true that the regions of high concentration of *S. carcini* on *Carcinus maenas* do tend to occur within estuaries or other semi land-locked bodies of water. However, within such areas temperature and salinity can fluctuate greatly. It is not known whether the parasite can acclimate to very low salinities if it infects a host which lives under such conditions. The experiments

in the laboratory were carried out on larvae released from hosts maintained at 18·0°C ± 1·0°C. Rubiliani (1985) discussed the response by two species of crab to an extract from the rhizocephalan parasite, *Loxothylacus panopaei*. He suggested that the sacculinid has a considerable capacity to evolve, adapting its physiology to that of its new host. The concept of a new "physiological race" of parasite, particularly among estuarine fauna, is not a new one (Muus 1967). However the extension of the larval development period, under low temperature conditions may be expensive in terms of energy resources.

Table 1 : A table of some authors who have recorded *Sacculina carcini* present on *Carcinus maenas*.

Author	Date	Site	Region	Method of capture
Foxon	1940	Millport	S. W. Scotland	Creels
Veillet	1945	L'Etang de Thau	S. France	Dredges and set nets
Bourdon	1960	Baie de Quiberon	N. W. France	Push nets
Crothers	1964	Milford Haven	S. W. England	Traps
Heath	1968	Millport	S. W. Scotland	Creels
Rasmussen	1973	Isefjord	E. Denmark	Pots, push nets and eel traps
M. A. F. F. pers. comm.	1975	Tal-y-foel	N. Wales	Traps and beam trawl
Rainbow et al.	1979	Milford Haven	S. W. Wales	Beam trawl and "collection"



Map 1 : Map of the Roscoff coastline to show the sampling sites for the May 1988 survey.

Key : SITES

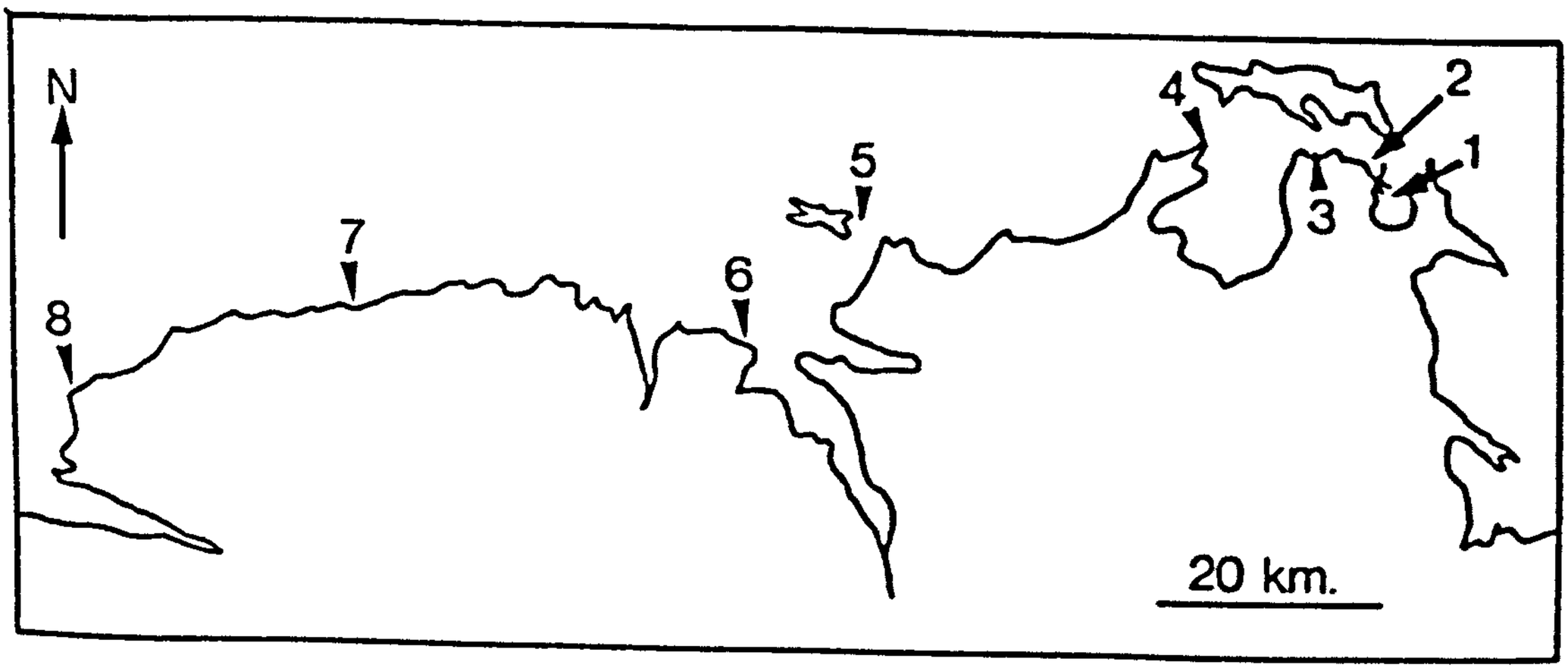
1 The outer harbour;
2 Roscoff beach;
3 The region trawled during high water;

J = Jetty for the Ile de Batz ferry;
PP = Pointe de Perharidi;
SB = Station Biologique.

Table 2 : Table of the crabs captured in Roscoff, May 1988, to show the incidence of parasitism and the state of the parasite.

SITE	METHOD	TOTAL CATCH	INFECTED CRABS	% I.	M. E.	V. E.	SCAR
1	Hand◀	22(16M 26F)	15(12M 3F)	68·2	13	2	0
1	Hand▼	59(33M 26F)	29(13M 16F)	49·2	26	2	1
1	Trap◀	0					
1	Trap▼	108(63M 45F)	11(7M 4F)	10.2	5	2	4
2	Hand◀	15(10M 5F)	7(6M 1F)	46·7	6	0	1
2	Hand▼	23(20M 3F)	11(11M 0F)	47·8	11	0	0
2	Trap▼	49(25M 24F)	3(2M 1F)	6·1	3	0	0
3	Trawl▼	219 (n.s.)	12 (n.s.)	5·5	6	2	4

Key : F = Female; M = Male; n.s. = not sexed; M.E. = Mature Externa;
% I. = Percentage Infected; V.E. = Virgin Externa;
◀ = Mid Water; ▼ = Low Water.



Map 2 : Map of the coastline, west of Roscoff, to show the sampling sites for the May 1989 survey.

Key : SITES

1 Roscoff Harbour;
2 Roscoff Beach;
3 Trawl Site;
4 Pointe de Perharidi;
5 Ile de Siec;
6 Morguériec Harbour;
7 Kerfissien Harbour;
8 Porz ar Stréat.

Table 3 : Table of the crabs captured in Roscoff, May 1989, to show the incidence of parasitism and the state of the parasite.

SITE	METHOD	TOTAL CATCH	INFECTED CRABS	% I.	M. E.	V. E.	SCAR
1	Hand▼	40(23M 17F)	21(12M 9F)	52·5	15	3	3
1	Trap▼	11(9M 2F)	0	0			
3	Trawl▼	177(103M 74F)	11(8M 3F)	6·2	2	4	5
4	Hand▲	14(8M 6F)	0	0			
4	Hand◀	23(11M 12F)	4(2M 2F)	17·4	2	1	1
4	Hand▼	28(16M 12F)	7(5M 2F)	25·0	5	2	0
4	Trap◀	21(17M 5F)	2(1M 1F)	9·5	1	1	0
4	Trap▼	3(2M 1F)	1(0M 1F)	33·5	0	0	1
5	Hand▼	17(4M 13F)	1(0M 1F)	5·9	0	0	1
5	Trap▼	24(16M 8F)	1(0M 1F)	4·2	1	0	0
6	Hand◀	16(12M 4F)	0	0			
6	Hand▼	23(9M 4F)	1(1M 0F)	4·4	0	0	1
6	Trap◀	10(10M 0F)	0	0			
6	Trap▼	10(9M 1F)	0	0			
7	Hand▼	26(8M 18F)	2(1M 1F)	7·7	0	2	0
7	Trap▼	2(2M 0F)	0	0			
8	Hand▼	85(43M 42F)	1(0M 1F)	1·2	1	0	0
8	Trap▼	53(20M 33F)	1(1M 0F)	1·9	0	0	1

Key : F = Female; M = Male; M. E. = Mature Externa; %I. = Percentage Infected; V. E. = Virgin Externa; ▲ = High Water; ◀ = Mid Water ▼ = Low Water.

Figure 2. **Combined data (1988 & 1989) to show percentage parasitism at each site.**

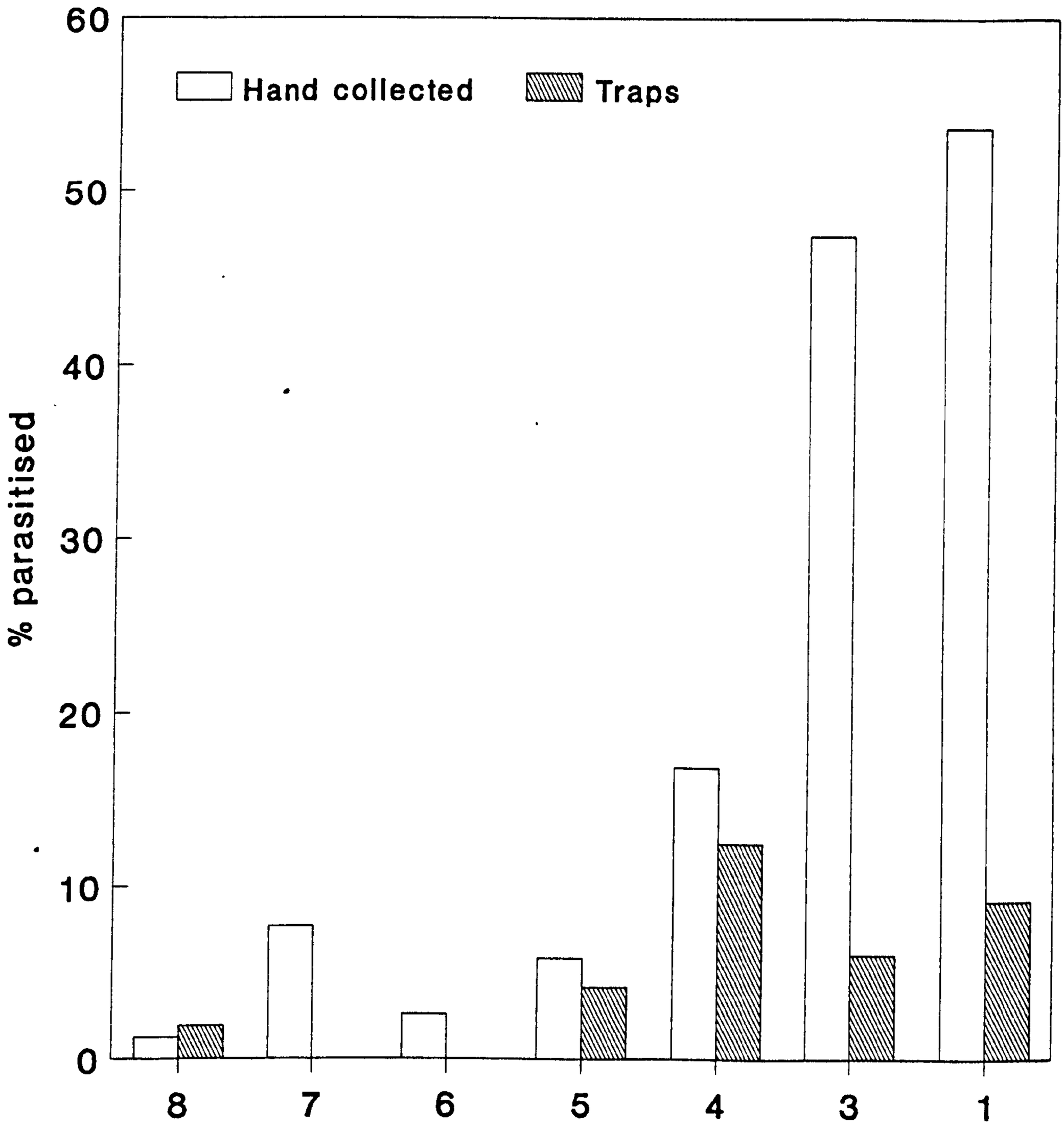


Figure 3. **Size frequency distribution for crabs trawled at Roscoff, 1989.**

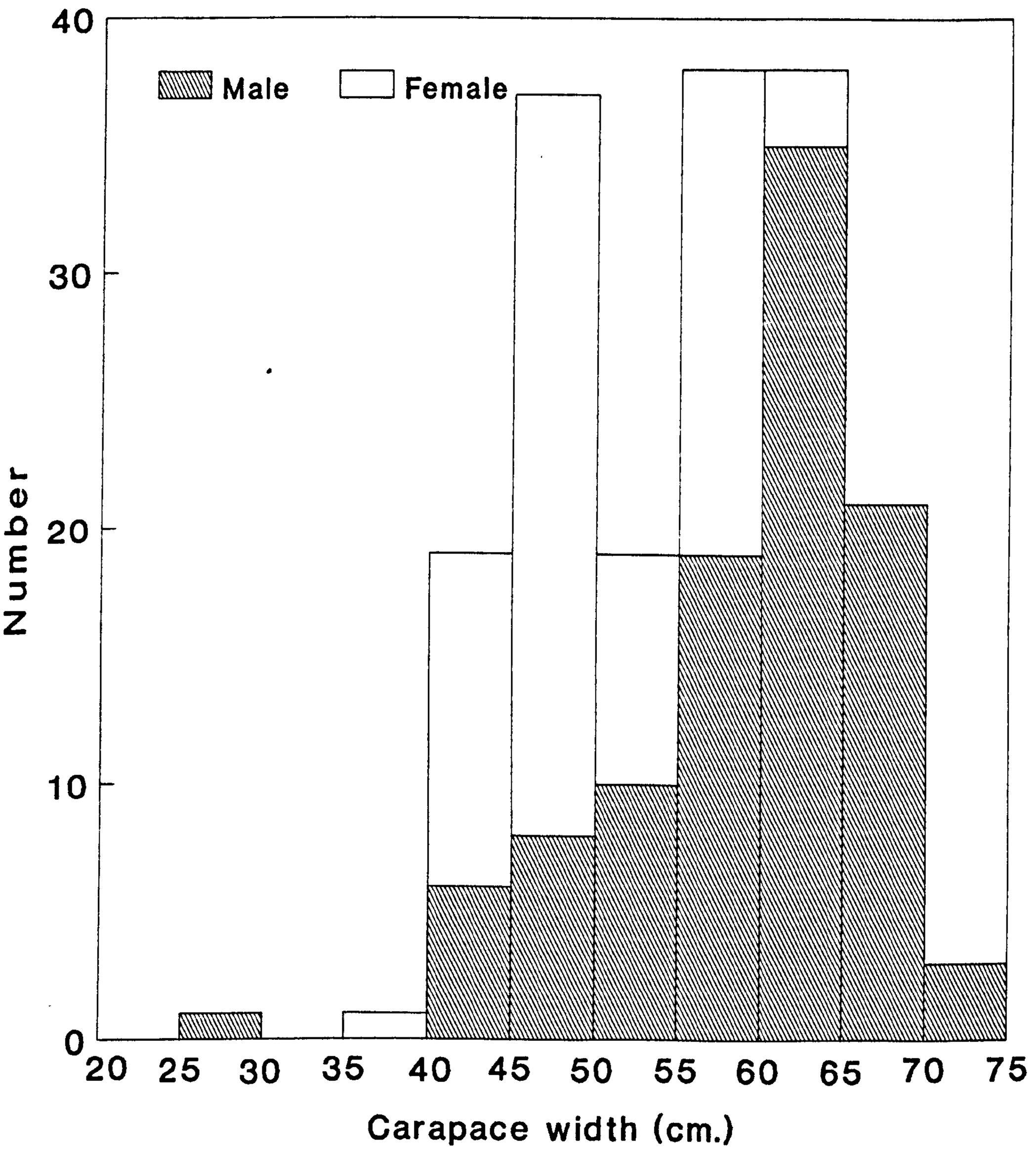
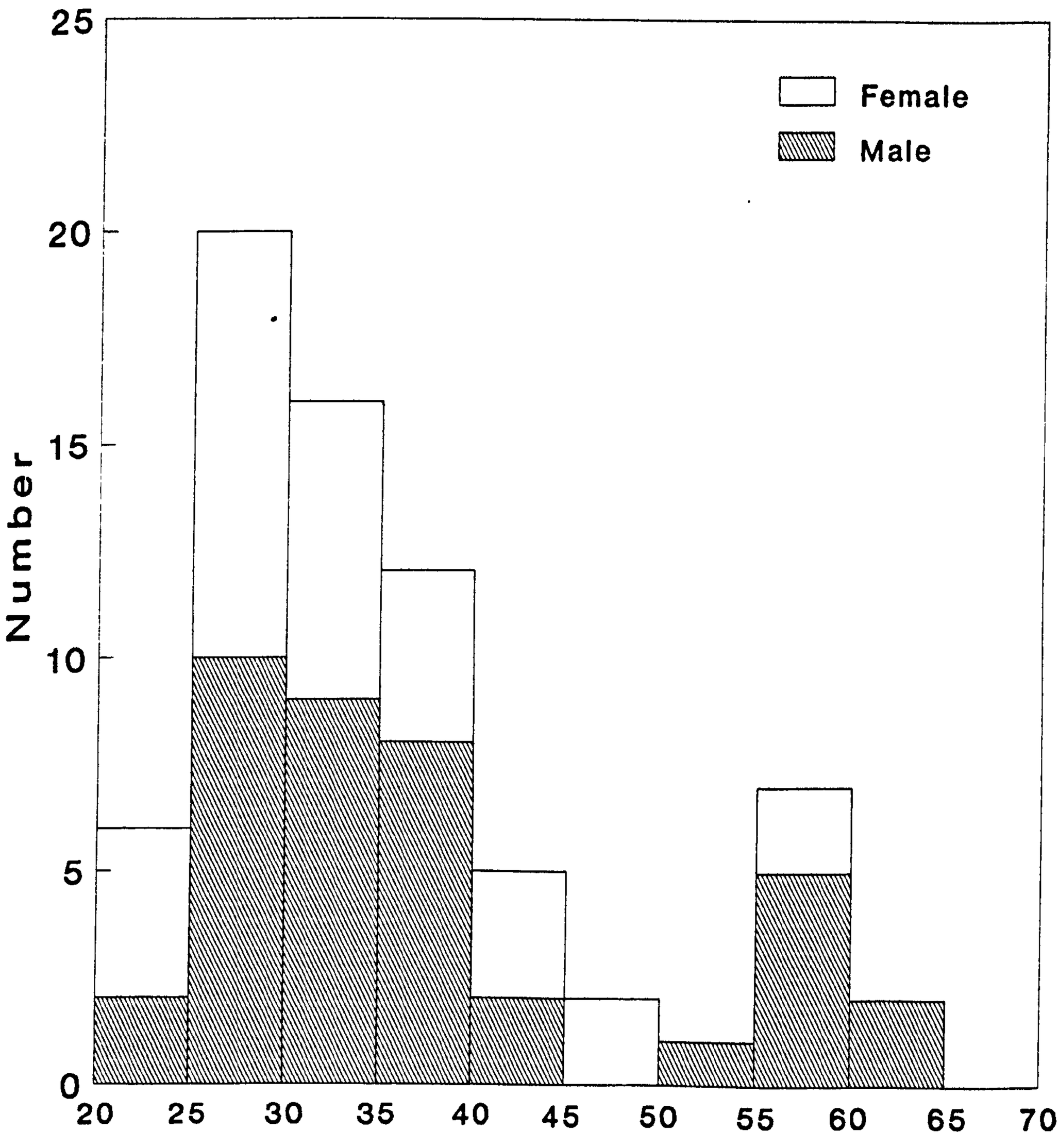
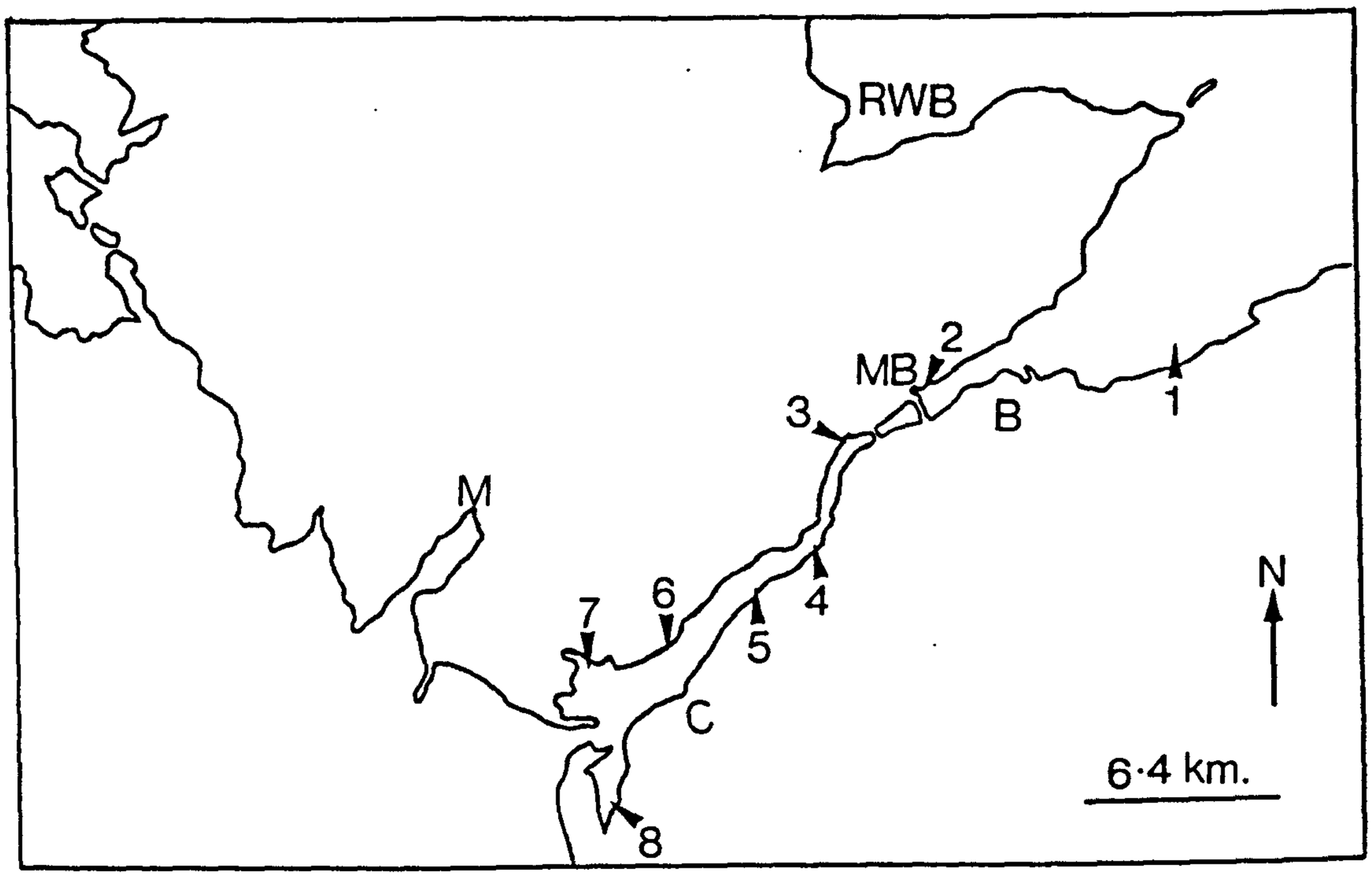


Figure 4. **Size frequency distribution of the crabs collected in Roscoff, 1988.**





Map 3 : Map of the Menai Strait to show the sampling sites.

Key : SITES

1 Traeth Lafan;
2 Ynys Faelog;
3 Pwll Fanog;
4 Port Dinorwic;
5 Plas Menai;
6 Tal-y-foel;
7 Traeth Melynog;
8 The Foryd;

B = Bangor; C = Caernarfon; M = Maltraeth;
MB = Menai bridge; RWB = Red Wharf Bay.

Table 4 : Table of crabs captured along the Menai Strait between November 1989 & February 1990, to show the incidence of parasitism.

SITE	TRAP	HAND	TRAWL	TOTAL CATCH	INFECTED CRABS
3		✓		4(2M 2F)	0
3	3▼			229(168M 61F)	0
4		✓		22(12M 10F)	0
4	3▼			134(107M 27F)	0
5		✓		4(2M 2F)	0
5	3▼			148(105M 43F)	0
6	6▼			236(119M 117F)	0
6	18⇓			128(82M 46F)	0
6	18⇓			1079(717M 362F)	2
6			×2	16(9M 7F)	0
6			×3	266(223M 43F)	0
8	8▼			67(40M 27F)	0
8	8▼			229(176M 53F)	0
8	8▼			136(94M 42F)	0
8	8▼			76(64M 12F)	0
8	8▼			57(47M 10F)	0

Key : ▼ = Low Water; ⇓ = Sublittoral.
Each trawl lasted 20 minutes;
M = Male; F = Female.

N.B.: The two parasitised crabs were, one male bearing a virgin externa and one female bearing a mature externa.

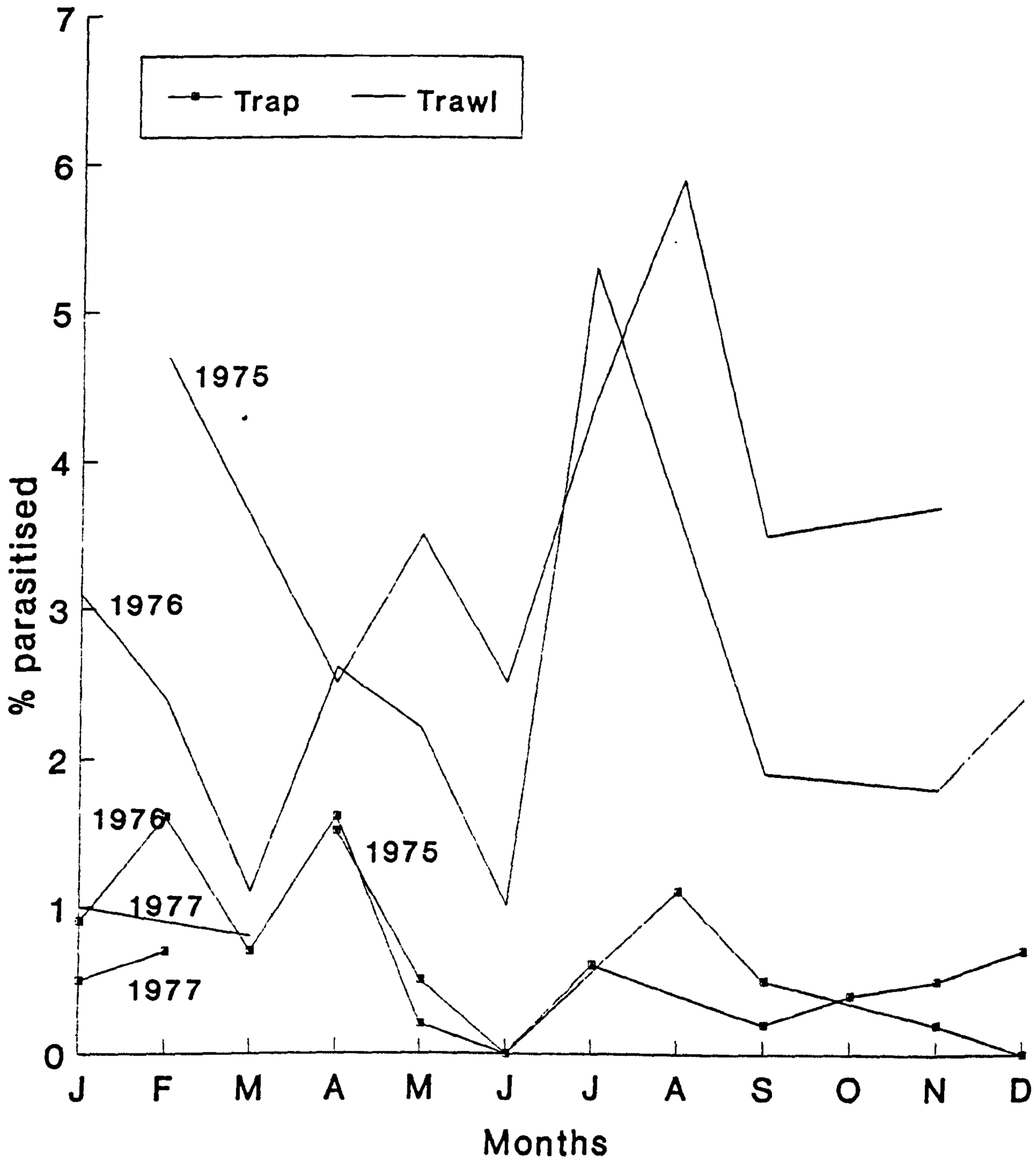
Table 5 : Table of crabs examined, from two independent surveys along the Menai Strait between November 1989 and March 1990.

SITE	TRAP	TRAWL	TOTAL CATCH	INFECTED CRABS
1	8▼		162(86M 76F)	0
1	8▼		368(186M 182F)	0
2		✓	125(92M 33F)	0
7	18▼		131(40M 91F)	1
7	18▼		31(15M 16F)	0
7	18▼		42(21M 21F)	0
7		✓	13(8M 5F)	0
7		✓	30(16M 14F)	0
8		✓	416(265M 151F)	0
8		✓	189(115M 74F)	0
8		✓	220(148M 72F)	0
8		✓	499(354M 145F	0
8		✓	289(199M 90F)	0

Key : ▼ = Low Water; M = Male; F = Female.

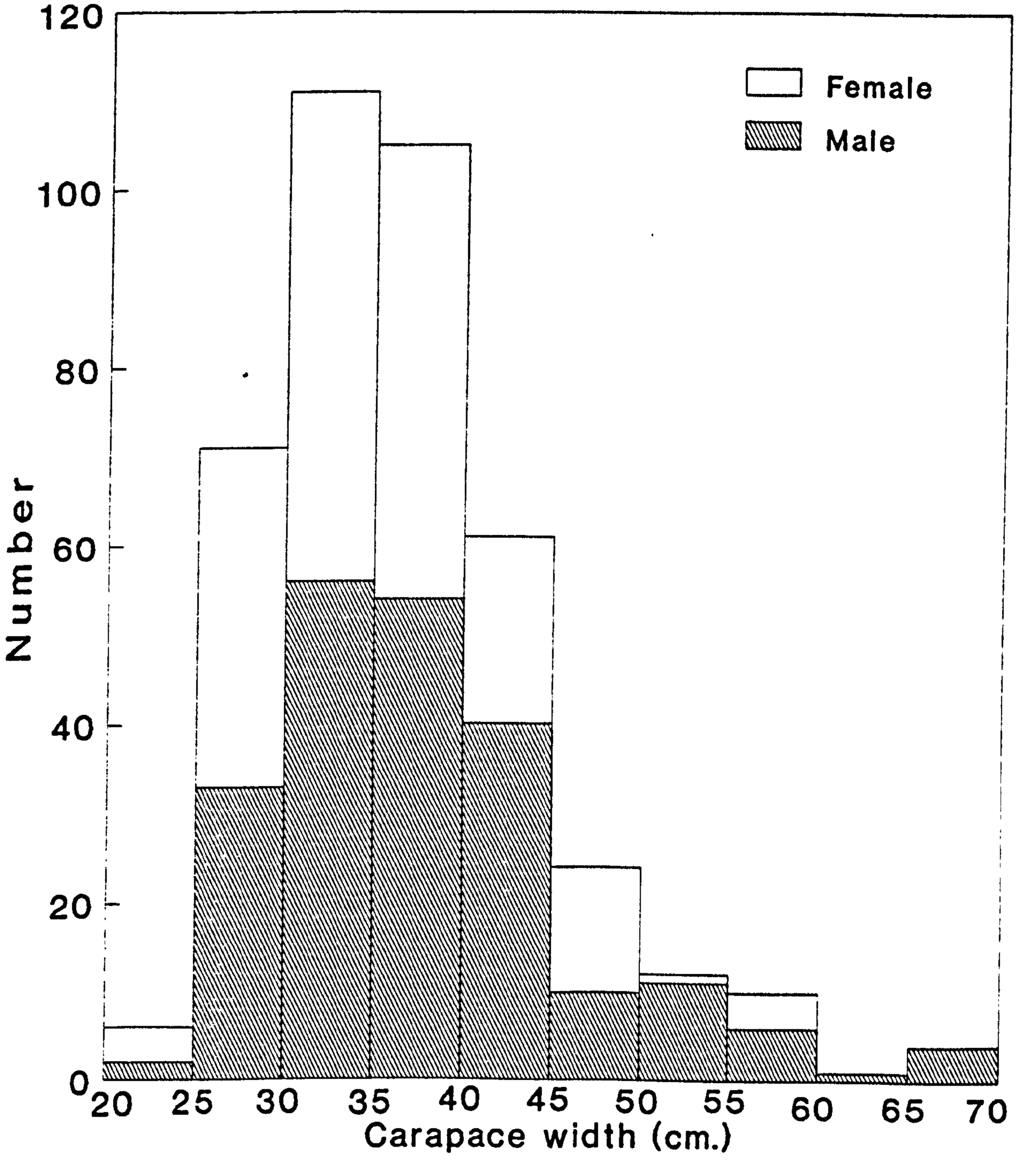
N.B. The one parasitised crab was a female bearing a scar and live rootlets within.

Figure 5. **Percentage infection of crabs at Tal-y-foel, 1975 - 1977.(M.A.F.F.)**



(Edwards, pers.comm.)

Figure 6. **Parasitised crabs collected at Tal-y-foel, 1975-1977.(M.A.F.F.)**



(Edwards, pers.comm.)

Table 6 : Results from the experiments to monitor the development of male *S. carcini* larvae at low temperatures.

DAY	STAGE	TEMPERATURE(°C)	OBSERVATIONS / COMMENTS
1	I & II	2·0 ± 0·5	Control group, cyprids by Day 6 at 17·0°C ± 2·0°C
25	II	ditto	
1	I & II	5·5 ± 0·5	Control group, cyprids by Day 6 at 17·0°C ± 2·0°C
35	II	ditto	
1	I & II	7·0 ± 0·5	Control group, cyprids by Day 6 at 17·0°C ± 2.0°C.
10	III	ditto	The coolers on the tank broke on Day 21 and the tank rose to 10·0°C.
21	III	ditto	
1	I & II	7·5	Control group, cyprids by Day 6 at 17·0°C ± 2·0°C. On Days 11 & 12, temperature fell to 6·0°C Normal swimming activity, protozoa present by Day 7 and Stage IV's visible inside Stage III's by Day 8.
2	II	8·0	
3	II	7·5	
4	II	7·5	
7	III	7·5	
8	III & IV	8·0	
9	III & IV	8·0	
10	III & IV	8·0	
15	III & IV	8·0	
16	III, IV & cyprid		

Table 7 : Results from the experiments to monitor the development of female *S. carcini* larvae at low temperatures.

DAY	STAGE	TEMPERATURE(°C)	OBSERVATIONS / COMMENTS
1	I & II	5·5 ± 0·5	Control group, cyprids by Day 6 at 17·0°C ± 2·0°C.
44	II	ditto	
1	I & II	6·0 ± 1·0	Control group, cuprids by Day 6 at 17·0°C ± 2·0°C.
42	II	ditto	Protozoa present by Day 12.
1	I & II	8·5	Control group, cyprids by Day 6 at 17·0°C ± 2·0°C.
2	II	8·5	Longer periods of time spent resting on the bottom of the beaker.
3	II	8·5	
4	II	8·5	
5	II	9·0	
7	II	9·0	
10	II	8·5	
13	II & III	8·5	
14	II, III IV	8·5	
17	II, III IV & cyprid	8·5	

Table 8 : Acclimation times for larvae of *S. carcini* subjected to low salinities.

SALINITY DROP(‰)	TIME SPENT AT LOWER SALINITY(mins.)
32 ⇒ 27	30
27 ⇒ 25	15
25 ⇒ 22	15
22 ⇒ 21	5
21 ⇒ 20	5

N.B.

The control groups for all the salinity experiments were maintained at 17·0°C ± 2·0°C and 32‰. The larvae developed to the cypris stage by Day 6. The normal behaviour of swimming in short bursts interspersed with sinking and intermittent periods spent on the bottom of the beaker was observed.

Table 9 : Results from the experiment to monitor the development of female *S. carcini* larvae at low salinities.

DAY	SALINITY (‰)				TEMP. (°C)
	17‰	22‰	27‰	32‰	
1	I & II S	I & II N	I & II N	I & II N	18·0
2	I & II T, 2D	II & III S & T	III N	III N	18·0
3	I & II T, 4D	III & IV S & T	IV N & S	IV N	19· 0
4	I & II T, 4D	III & IV S & T, 2D	IV N & S	IV N & S, 2D	16·0
5	I & II T, 5D	IV inside III, T, 3D	IV N & S	IV N & S, 3D	15·5
6	I & II T, 11D	IV(50%) S CYPRID N 5D	CYPRID N, 2D	CYPRID N, 1D	18·0
	13%	5%	1%	3%	MORTALITY

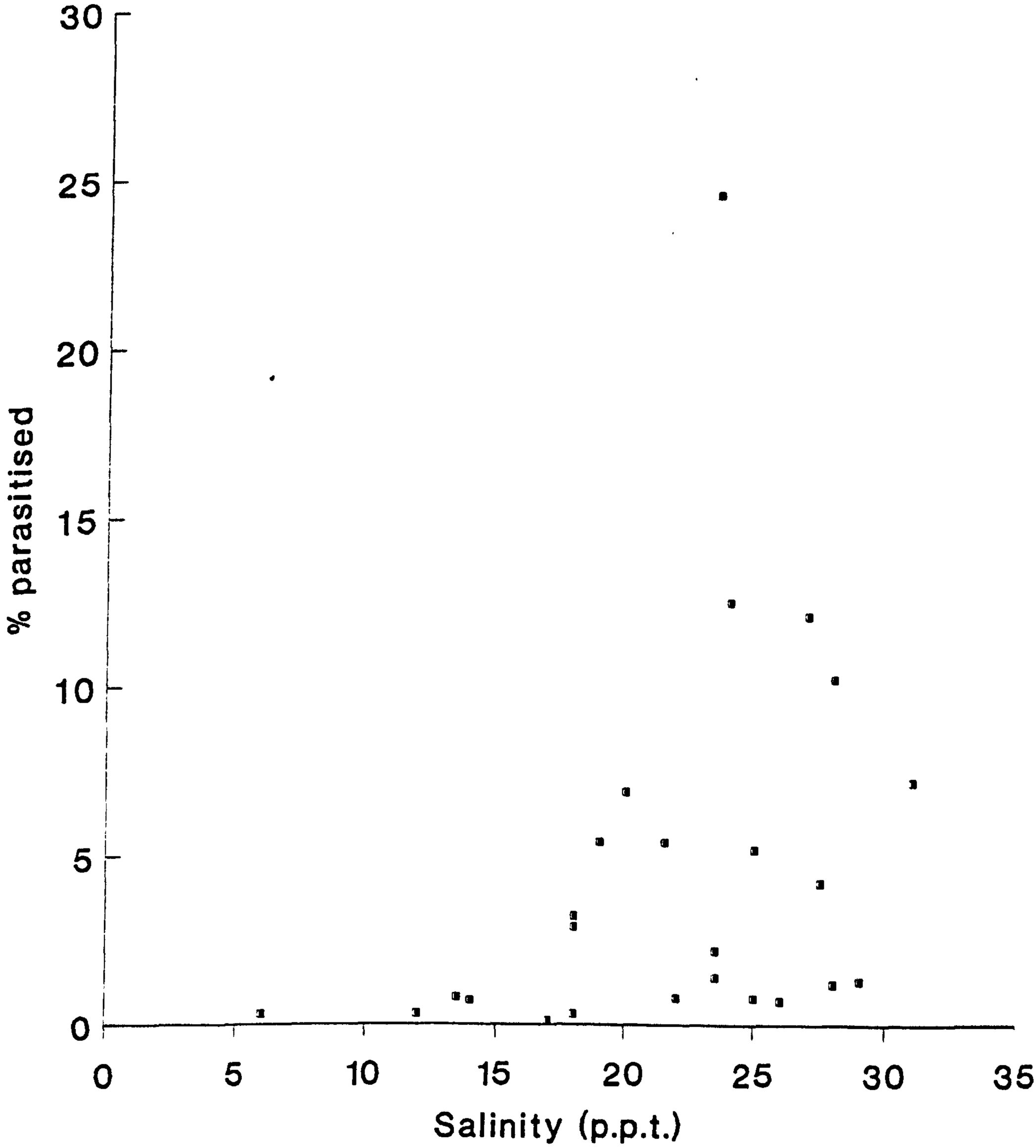
Key : I, II, III, IV = Stages of larval development; D = Dead; N = Normal swimming activity; S = Swimming, with longer periods on the bottom of the beaker; T = Twitching limbs, lying on the bottom.

Table 10 : Results from the experiment to monitor the development of male *S. carcini* larvae at low salinities.

DAY	SALINITY (‰)					
	20‰	21‰	22‰	25‰	27‰	32‰
1	I & II T	I & II T	I & II S & T	I & II S	I & II S	I & II S
2	II, T	II & III T	II, S	II, N 1D	II & III N, 3D	II & III N, 5D
3	II, T	II & III T, 4D	II S, T, 5D	III N, S, 4D	III N, S, 8D	III N, S, 10D
4	II, T 6D	III & IV inside II T, 5D	III & IV inside II T, 4D	III & IV N, S, 2D	IV inside III, N, S, 6D	IV N, S, 6D
5	IV inside II, T, 7D T, 7D	IV inside II, CLV, T, 13D	III & IV inside II T, 4D	III & IV N, S, 4D	III & IV N, S, 12D	IV N, S, 7D
6	IV inside II, CLV T, 2D	IV inside II, CLV T, 4D	IV inside II CLV T, 10D	III & IV N, S, 5D	III & IV N, S, 7D	CYPRID N, 6D
7	CYPRID inside II, B, 4D	CYPRID inside II B, 6D	CYPRID inside II	CYPRID N, 7D	CYPRID N, 10D	CYPRID N, 5D
8	CYPRID inside II B, 2D	CYPRID inside II B, 4D	1 CYPRID CYPRID inside II B, 3D	CYPRID N, 2D	CYPRID N, 6D	CYPRID N, 3D
9	CYPRID inside II B, 6D	1 CYPRID CYPRID inside II B, 5D	6 CYPRID N, CYPRID inside II B, 6D	CYPRID N, 5D	CYPRID N, 2D	CYPRID N, 3D
10	CYPRID inside II all D	2 CYPRID N, CYPRID inside II B, 24D	50% CYPRID N, 10D	CYPRID N, 5D	CYPRID N, 7D	CYPRID N, 4D

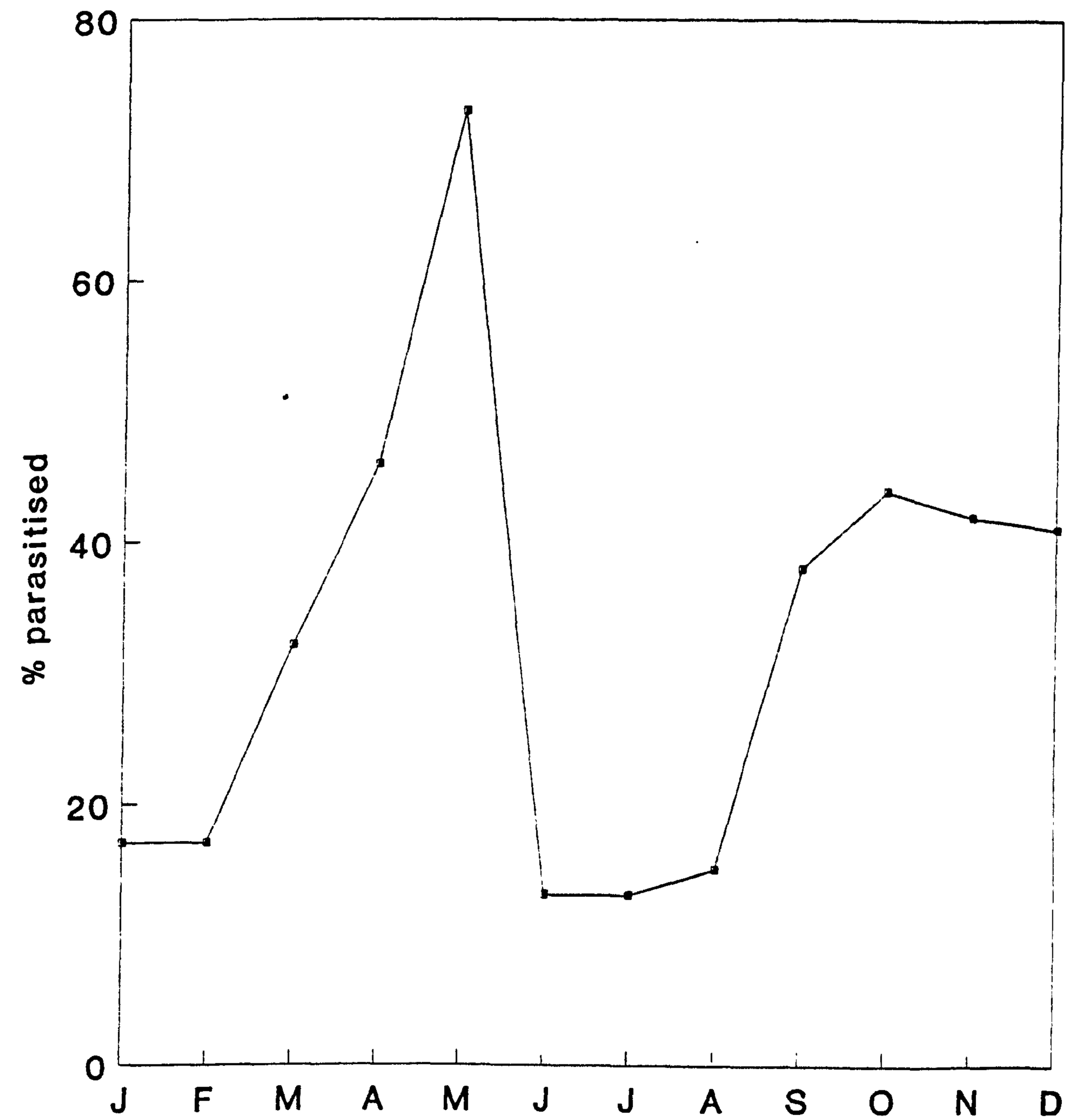
Key : B = Beating; CLV = Cyprid Limbs Visible; D = Dead; N = Normal swimming activity; S = Swimming with long periods on the bottom; T = Twitching limbs, lying on the bottom.

Figure 7. **Percentage parasitism relative to salinity, Aveiro, 1987/88.**



(Gomez, pers.comm.)

Figure 8. **Percentage parasitism of crabs in the Mersey Estuary (Day 1935).**



(*S.carcini* on *Liocarcinus holsatus*)

CHAPTER III : THE MORPHOLOGY OF THE NAUPLIUS STAGES OF *SACCULINA CARCINI*

Délage (1884) carried out the first comprehensive study on the life-cycle of *Sacculina carcini*, a parasite of the common shore crab, *Carcinus maenas*. He revealed this parasite to be a crustacean belonging to the Class Cirripedia. Although his work was initially questioned (Giard 1887; Coutière 1902), Smith (1907) was able to produce confirmation of Délage's findings.

Since then, work on this species has continued at odd times and particularly in recent years, when significant discoveries have been made on that part of the life cycle involving cypris larvae. Høeg, (1984) reported that *S. carcini* larvae exhibit sexual dimorphism, with the male cyprid being generally larger than the female. Their respective settlement sites were also very specific. This knowledge was extended by Walker (1985), when he carried out a scanning electron microscope study of the cyprids with particular emphasis on their antennular morphology. He observed the long posterior sac emerging from the third antennular segment of the male cyprid alone. Thus, unequivocal identification of larval sex could be made.

The description of the life-cycle by Délage was, for its time, a classic monograph which is still widely reported in textbooks. However it has since been amended by Høeg (1984), Lützen (1984) and Høeg & Lützen (1985). Høeg (1987a) produced a fine structure description of male cypris metamorphosis, clearly

showing the penetration of the mobile trichogon into the receptacle duct and its resultant entry into a receptacle, to form the primordial cells which eventually produce sperm. This was followed (Høeg 1987b) by a comparison of cypris ultrastructure and metamorphosis in the short-lived male trichogon and the female kentrogon. Walker (1988) also carried out a detailed study of the derivation of the cypris fourth antennular segment.

This investigation aims to complement the previous studies on *Sacculina carcini* by reporting on the morphology of the four naupliar stages at the light and electron microscope levels.

MATERIALS AND METHODS

Shore crabs, *Carcinus maenas*, parasitised by the rhizocephalan barnacle *Sacculina carcini* Thompson, were supplied from the Plymouth Marine Laboratory. The animals, which had been collected sublittorally by trawl net, were placed in seaweed, or damp newspaper, and packed in large polythene bags containing an ice sac for transport by rail to Bangor.

The crabs were maintained in experimental chambers at 18°C and fed three times a week on mussel tissue (see Walker 1987). Broods of *S. carcini* nauplii were collected (Walker 1985) regularly between October 1987 and February 1988, and in August 1988. The larvae were attracted to a point light source and

pipetted into 500ml. glass beakers full of U.V.-irradiated, fine filtered seawater at room temperature. The mouth of each beaker was covered with a piece of card to avoid air-borne contamination and minimise evaporation. Development of the lecithotrophic larvae took place without any subsequent water changes.

Some nauplii were prepared for scanning electron microscopy (see below). Under the light microscope, *camera lucida* drawings were made of the limbs, together with the outline shape for each larval stage. Photographs were taken using a Leitz Orthoplan photo microscope.

Following full larval development, the carapace lengths of fifty cyprids from each brood, were measured using a calibrated graticule within the ×10 eyepiece. Their sex was determined by antennular morphology.

Scanning electron microscopy

Approximately 100 larvae were attracted to a point light source and pipetted into fresh U.V-irradiated, fine filtered seawater and left for fifteen minutes. Twenty larvae were pipetted into 3ml. screw-top vials. Three vials were used for each larval stage.

Nauplius larvae, at each of their four stages, were relaxed in 0.01% M.S.222 (Sandoz) for twenty minutes. After three washings with seawater they were then fixed with 2.5% glutaraldehyde in seawater for two hours at room temperature. After two further washings with distilled water the larvae were dehydrated by taking them through a graded series of ethanols to

100%.

Having been transferred to acetone for 30 minutes the larvae were critically-point dried, mounted on stubs using double-sided Sellotape and sputter-coated with Pt or Au. They were then viewed in a scanning electron microscope (I.S.I. ML7, Hitachi S520 or Cambridge Stereoscan 120). Relevant photographs were taken and drawings made to illustrate pertinent larval features.

RESULTS

The four naupliar stages bear the standard cirripede larval limbs which comprise one pair of uniramous antennules, one pair of biramous antennae and one pair of biramous mandibles (Figure 1). The segments of each appendage have an array of small spines (Figures 4, 5, 6, & 28), whereas the setae bear fine hair-like setules (Figure 4). No gnathobases are present throughout development (Figures 1, 2, 9, 20 & 28).

The antennules of a stage I nauplius have five setae (Figures 2 & 5), the fifth one being retained in stage II (Figures 1 & 11) but lost in stages III and IV (Figures 1 & 17). This is the only change in the setation formula throughout development, unlike planktotrophic nauplii, where the limbs increase in size and complexity at each stage. The final stage antennae of two planktotrophs and two lecithotrophs are shown in Figure 28. It is at stage IV (*Sacculina carcini*), that segment 3

of the antennules becomes more bulbous (Figures 1, 6, 20 & 21) and the fourth seta has a more complex appearance (Figures 6 & 21). Internally the latter becomes the template for the subterminal sac of the cypris fourth segment, with the cypris terminal sac and three setae developing within the first antennular seta.

Throughout the whole nauplius development the antennae retain their five exopodite and three endopodite setae and the mandibles their four exopodite and two endopodite setae (Figure 1).

The labrum remains a simple structure (Figures 2, 7 & 20), with an opening, from functional labral glands, out through the tip of the labral papilla. No mouth is present beneath the labrum (Figure 7). The frontal horns point dorso-laterally on the stage I nauplius and are closed distally with one dorsal spine (Figure 2). Between the antennules lie a pair of frontal filaments and situated directly anterior to these is a median pore (Figures 2 & 3). Both these features remain throughout development. The ventral thoracic region of the first stage nauplius is smooth and terminates posteriorly in two readily identifiable carapace spines which have smaller epicuticular spines on them (Figures 2, 8 & 27).

In comparison, the three other naupliar stages are far more elongate (Figure 27). This fundamental change in shape occurs almost immediately after hatching, when the larvae moult to stage II. The frontal horns in stage II nauplii point laterally; they are now open distally, with both dorsal and ventral spines at the tip (Figures 9, 20 & 22). The carapace

spines are distinctly longer and areas of aggregated small cuticular spines are present on the ventral thoracic region (Figure 10). Figure 9, shows the moult line which is visible in all stages and the presence of paired spines and setae on the dorsal surface of the carapace. If these are present in stage I nauplius, they were never seen, because after preparation for S.E.M., a wrinkled dorsal carapace always resulted, hindering such observations. Figures 12 and 13, illustrate the smooth dorsal carapace of a stage IV nauplius, with small cuticular spines at the posterior end. The four pairs of dorsal setae and one pair of dorsal spines are clearly visible in Figures 12, 14, 15, and 16. This arrangement also existed in stages II and III.

It is following the emergence of the stage III nauplius that the vestigial ventral thoracic process becomes evident (Figures 17, 18, 19 & 27); this process is even more prominent in the final stage IV nauplius (Figures 23, 24, 25, 26 & 27).

The final nauplius stage (Figures 20 & 24) often has a fold on the ventral surface which is covered with an even distribution of spines. The frontal horns (Figures 20 & 22), display 'shredded' cuticle at the open tip through which frontal horn gland secretion can emerge. This stage IV nauplius 'houses' the developing cyprid with its bivalve carapace and specialised antennules for settlement and attachment.

DISCUSSION

Although the male nauplius of *Sacculina carcini* tends to be larger than the female, no other obvious morphological difference was observed. The slight volume increase between naupliar stages was calculated by Walker (1988) to be 1.03 ≈ 1.09 fold. This range correlates well with that produced by Crisp (1986), who analysed volume changes at each moult for lecithotrophic balanomorphs. Crisp discovered that for planktotrophic nauplii between stages II and VI the volume was approximately doubled for each successive stage. This compared to a mean 1.10 fold increase for the lecithotrophic nauplii examined.

Lecithotrophy has arisen independently in several lines of the Cirripedia (Moyse 1987). It is considered to be advanced in terms of parental investment, but the condition is degenerate and probably irreversible in terms of naupliar morphology.

Rainbow and Walker (1976) carried out a scanning electron microscope study on the planktotrophic nauplius of the thoracican balanomorph *Balanus balanoides* (= *Semibalanus balanoides*). This larva has a setulated tri-lobed labrum and gnathobases on the interior face of the basal segments of all limbs, which bear setulate setae. The ventral thoracic process (also sometimes termed abdominal process) bifurcates terminally and displays six regions corresponding to the cypris thoracic appendages which develop later within the sixth stage nauplius. When the nauplius was feeding, food particles were seen to adhere to the long setae

of the limbs, probably due to frontal horn secretion. However, food would sometimes end up between the caudal process and the ventral thoracic process. Rainbow and Walker (1976) observed that the curling up of this latter process enabled cleaning and also aided the spread of secretion from the labral papilla. The resultant 'bolus' was forced into the mouth by action of the gnathobases. Although there is still controversy concerning particle capture and transfer, the mechanisms for forward transport and ingestion appear to be understood (Walker, Yule & Nott 1987). Consequently, the importance of the rear body region during feeding is evident.

Many other planktotrophic nauplii, which exhibit similar structural characteristics have been described (Knight-Jones & Waugh 1949, Stubbings 1975, Lang 1979, Egan & Anderson 1986 and Moyse 1987, among others). Gnathobases are present in some lecithotrophic larvae such as *Tetraclita pacifica* (Crisp 1986) and *Ibla quadrivalvis* (Anderson 1987), but only as vestigial spines. Also the number of limb setae is greatly reduced compared to planktotrophic larvae found in the same genus.

Lecithotrophic thoracican species such as *Ibla idiotica*, *Ibla quadrivalvis* and *Pollicipes spinosus* (= *Calantica spinosa*) have been studied in detail (Batham 1945a, Anderson 1965 & 1987 and Batham 1945b respectively). In *Ibla idiotica* the free naupliar stages are suppressed and it is the cyprids which are released directly from the adults. Consequently naupliar limbs are greatly reduced and cypris limbs precociously developed. The latter originate in the late embryo as a solid mass. However, as

Batham (1945b) commented, regarding *Calantica spinosa*, the numerous larval moults carried out in close succession for this species seem to represent "a phylogenetic retention of the primitive number of cirripede nauplius moults, rather than a mechanism for permitting increase in size." Although these three lepadomorph species do develop a rudimentary gut, other characteristics of their larval morphology resemble those of *Sacculina carcini*. Their relatively short developmental period, simple setae that change little between stages, their reduced labrum and obvious internal yolk store, all reflect an advanced lecithotrophic condition.

In 1986 Anderson studied *Tetraclita divisa* (Nilsson-Cantell), a circum-tropical balanomorph which releases free-swimming larvae at the cypris stage. The larvae develop through four naupliar stages within the mantle cavity. He cited the convergently similar modifications towards direct development, exhibited by the lepadomorph *Ibla idiotica* (Batham 1945a) and most acrothoracicans (Turquier 1972). Direct development is also the strategy for the akentrogonid rhizocephalans (Høeg 1990) and the thoracican *Anelasma squalicola* (Frost 1928), all of which have abbreviated larval development without free-swimming nauplii.

The caudal papilla of *Tetraclita divisa* (Anderson 1986) is similar in *Ibla quadrivalvis* (Anderson 1987) and termed the abdomen in *Calantica spinosa* (Batham 1945b). This structure would seem to correlate well, in position, with the ventral thoracic process of cirripedes. In *Sacculina carcini*, development of the cypris limbs

occurs in the same region. The basal segments of the limbs develop within the body of the nauplius and the tips of the setae aggregate at the posterior end. *S. carcini* is advanced in the lecithotrophic condition having no mouth or anus and no remnants of a gut, a simple labrum and no gnathobases. Thus it is reasonable to assume that the ventral thoracic process, significantly involved in feeding for planktotrophic larvae, has been greatly reduced to the vestigial structure observed on the stage III and IV nauplii. The carapace spines correspond to the posterior shield spines shown in the diagram of diagnostic morphological features of balanomorph larvae, particularly at stages IV, V and VI published by Lang (1979).

Høeg and Lützen (1985) studied the Rhizocephala from the north east Atlantic area. They observed that *Peltogasterella sulcata* and *Peltogaster paguri*, like *Sacculina carcini*, develop through four naupliar stages. *P. sulcata* and *S. carcini* both bear the prominent pigmented nauplius eye and exhibit strong positive phototaxis to enable them to remain within the plankton. *P. paguri* (Høeg & Lützen 1985), and *Briarosaccus callosus* - a rhizocephalan which parasitises three species of king crab in S. E. Alaska - (Hawkes et al. 1985), have no eye, but the nauplius is encircled by a hollow cuticular annulus used as a flotation device. This would compensate for the absence of phototaxis. In both species the cyprids lack the paired compound eyes of non-parasitic Cirripedia.

Moyse (1987) in considering the lecithotrophic condition in thoracicans, observed a strong correlation between the degree

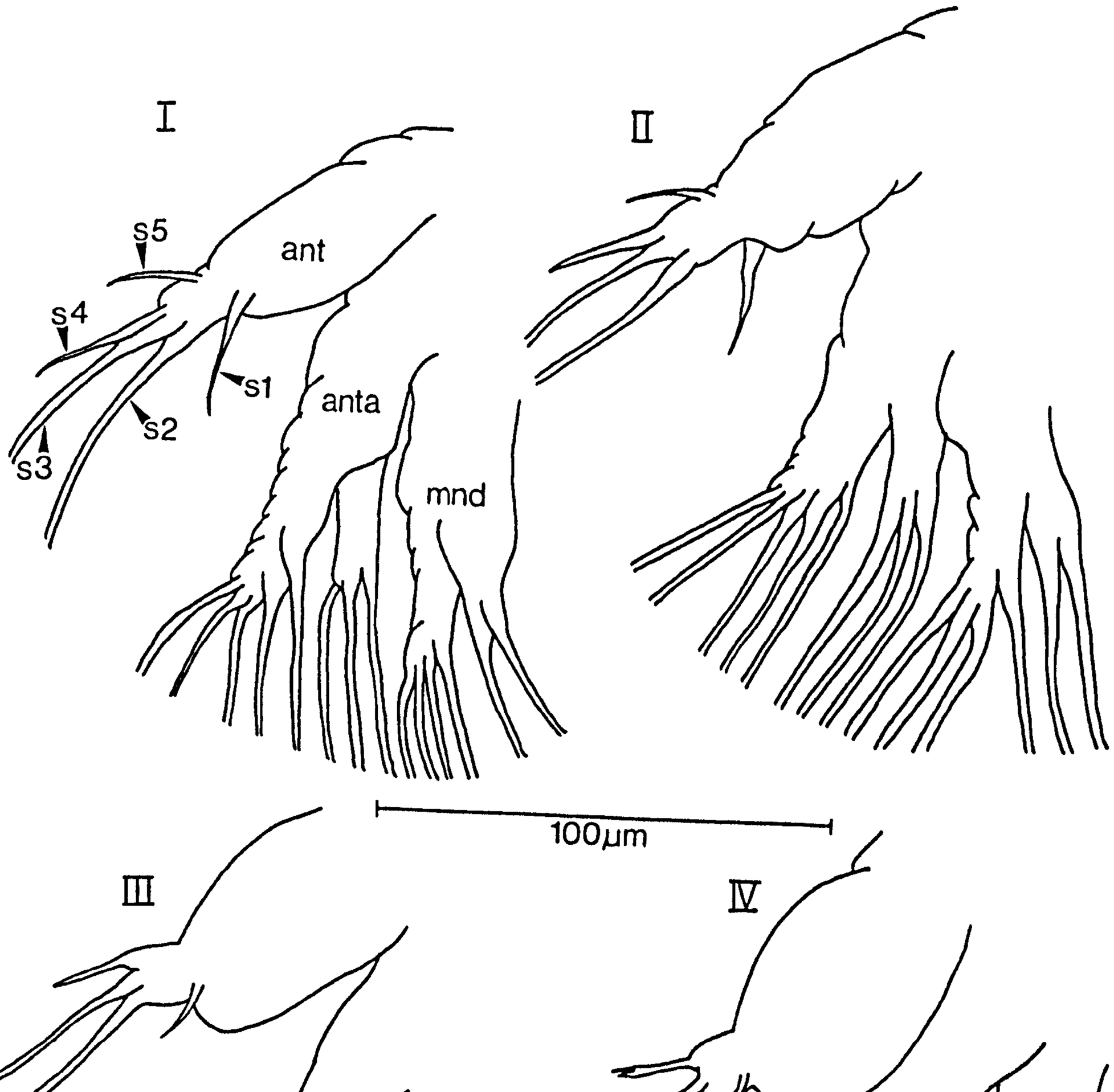
of larval "degeneracy", with bathymetric depth range for the adults. He noted that species of *Arcoscalpellum*, with the exception of *Scalpellum ströemi* which lives between 50 and 1000 metres, occupy depths greater than 2000 metres. They all complete naupliar development within the mantle cavity. As Foster (1978) points out, a planktotrophic larval existence below the photic zone would be "inept". The possession of lecithotrophic larvae is primitive in scalpellids (apart from pollicipids), with suppression of free-living larval stages and release of ambulatory cypris larvae. Thus, colonisation of patchily distributed areas of hard substratum, can be sucessfully achieved in the abyssal habitat (Foster 1978). Moyse (1987) proposed that *Ibla quadrivalvis*, being an intertidal barnacle, may have come to occupy its present niche as a refuge from competition in a previous deep-water habitat. This same strategy could work for the Rhizocephala, enabling the larvae to remain close to members of the host species (Høeg 1990).

During the evolution of thoracican adults, significant morphological changes have been made for adaptation to extreme conditions. However, such changes are only partially reflected in the larvae. Moyse (1987) shows the overall pattern of thoracican nauplius form ranging from the specialised planktotrophic form of *Lepas anatifera*, to the simplified lecithotrophic form of *Ibla quadrivalvis*. The nauplius of the rhizocephalan, *Sacculina carcini* fits well within this suggested pattern and argues for the retention of the Rhizocephala within the Cirripedia *sensu stricto* (*contra* Newman 1987).

Figure 1.

Line drawings of the right side limbs of *Sacculina carcini* nauplii (stages I-IV); ant-antennule; anta-antenna; end-endopodite; ex-exopodite; mnd-mandible.

Fig.1



Figures 2⇒24 inclusive are scanning electron micrographs of *Sacculina carcini* nauplii :-

Figure 2.

A ventral view of a stage I nauplius

Figure 3.

An enlargement from figure 2 showing the median pore.

Figure 4.

A ventral view of the left side limbs of a stage I nauplius.

Labels for figures 2⇒4 inclusive:-

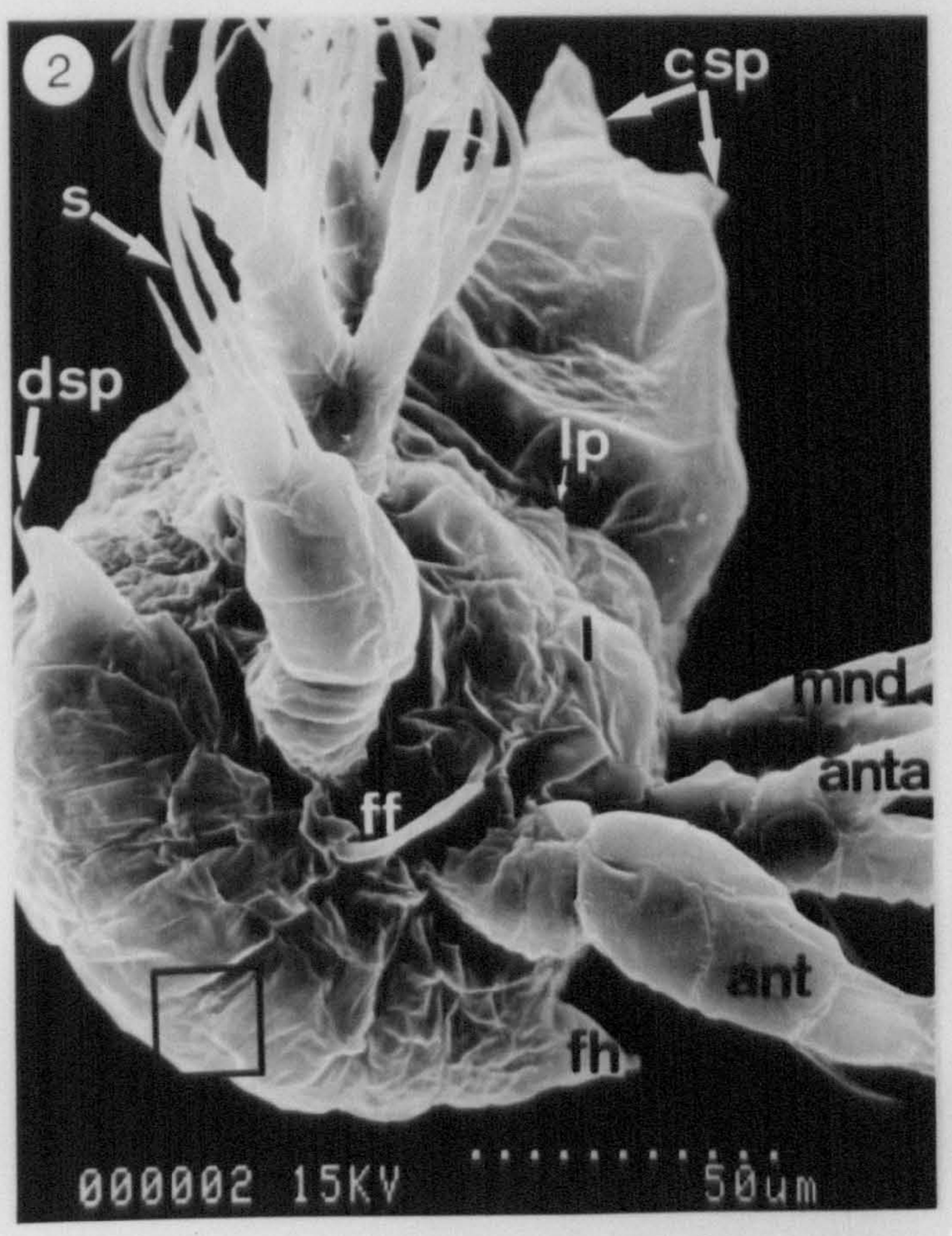
ant-antennule; anta-antenna;

csp-carapace spine; dsp-dorsal spine;

ff-frontal filaments; fh-frontal horn

l-labrum; lp-labral papilla; mp-median pore;

s-seta; sl-setule; sp-spine.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s
dsp
lp
l
mnd
anta
ff
ant
fh
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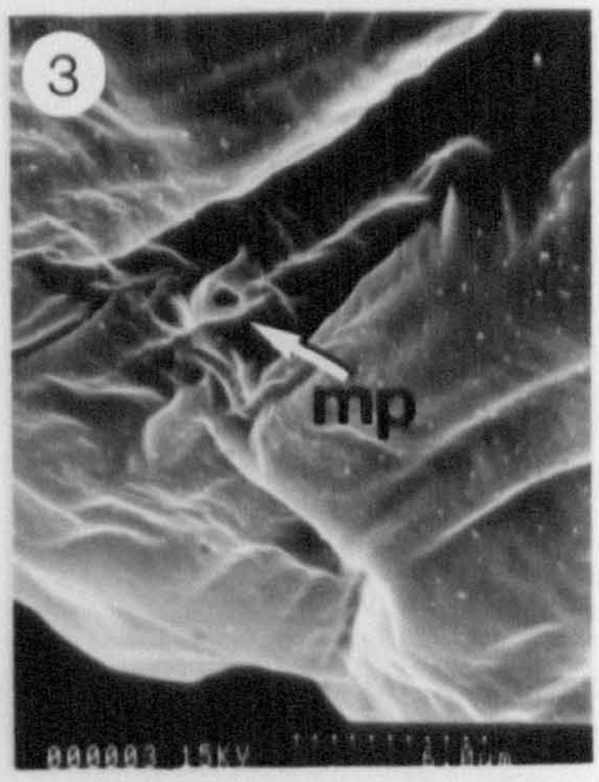
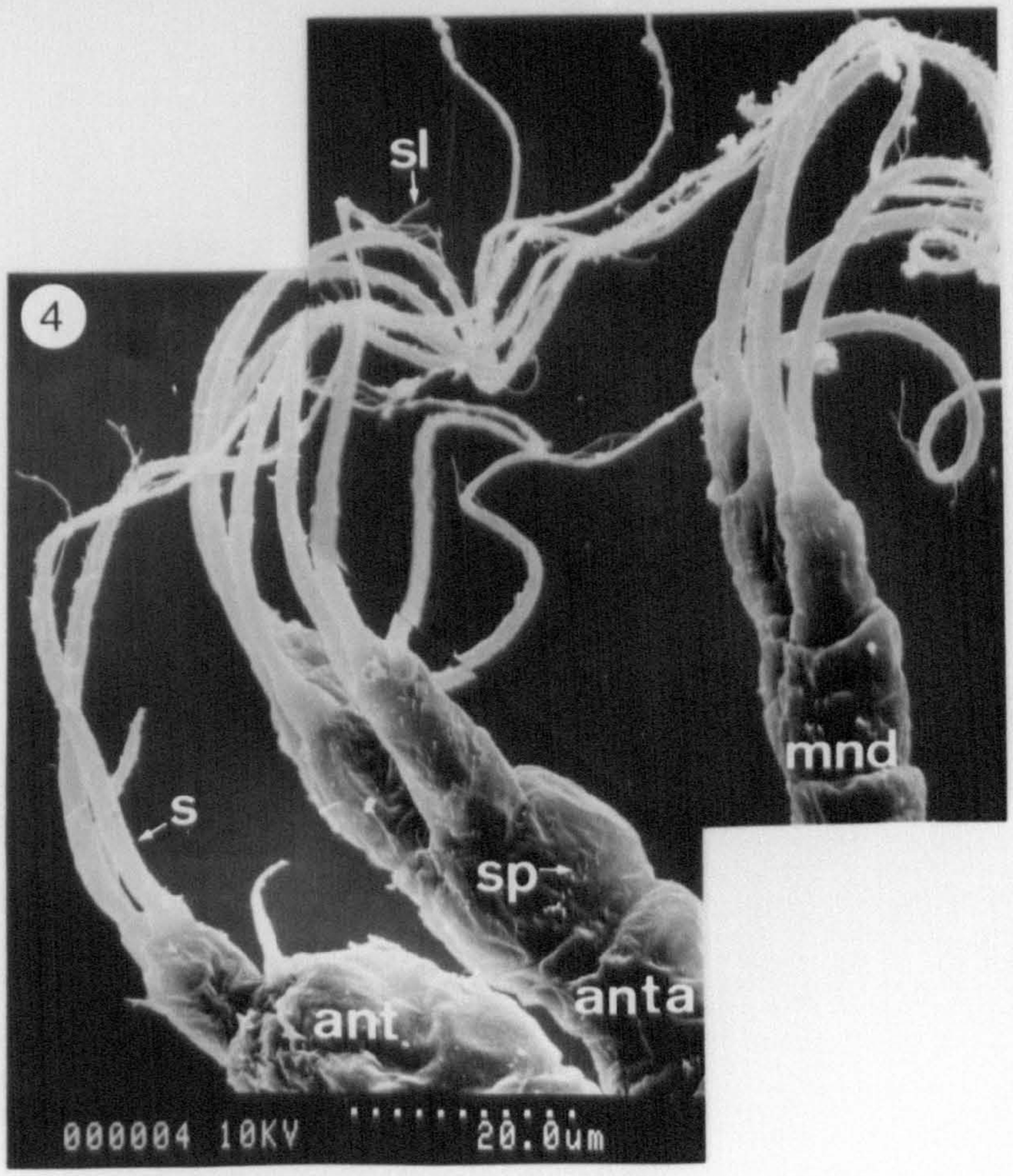
50um


3
mp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4
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000004 10KV
20.0um

Figure 5.

The left antennule and base of the antenna of a stage I nauplius.

Figure 6.

The right antennule and antenna of a stage IV nauplius.

Figure 7.

The ventral thorax and labrum of a stage I nauplius.

Figure 8.

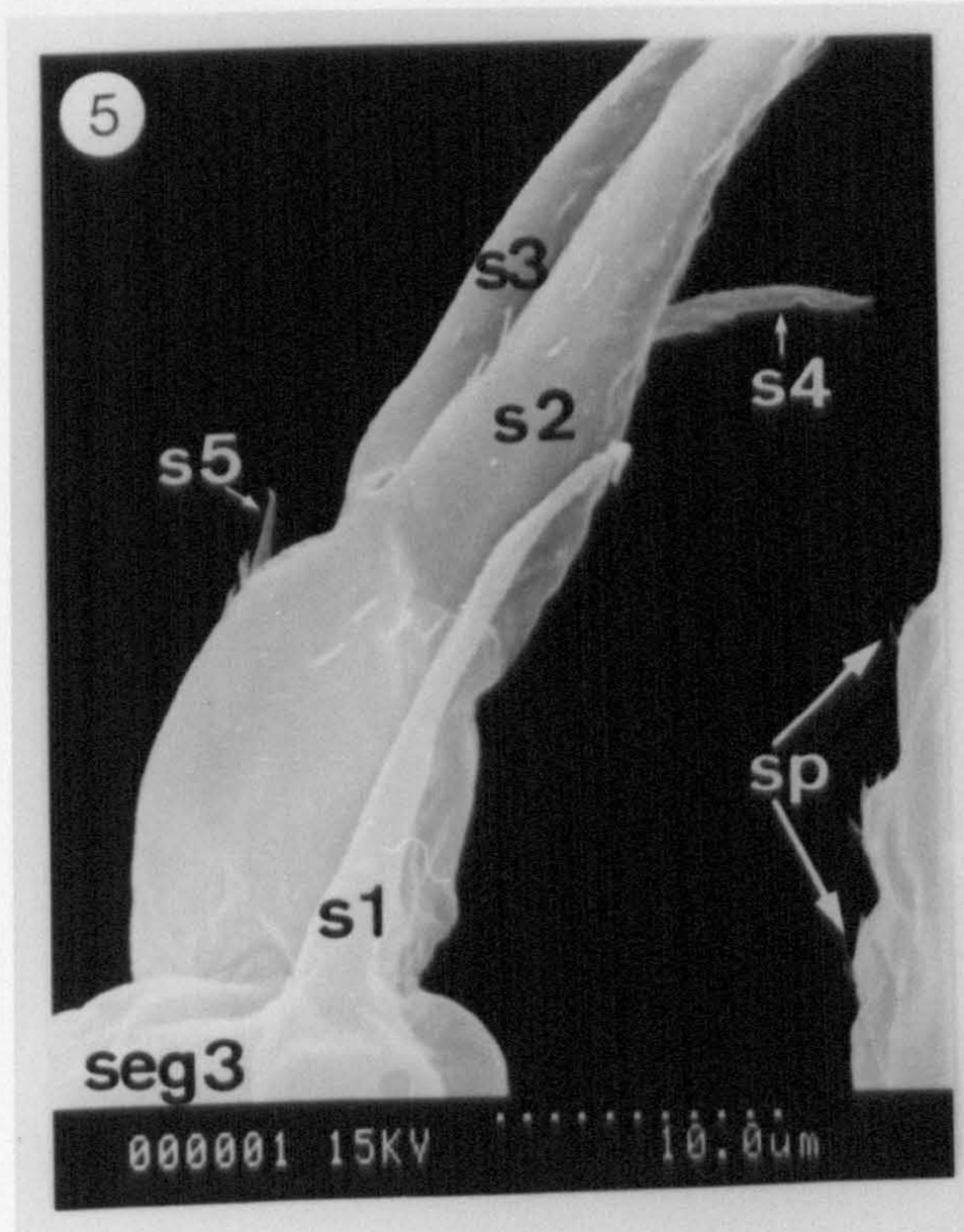
A ventral view of the left carapace spine of a stage I nauplius

Labels for figures 5-8 inclusive:-

csp-carapace spine; l-labrum;

lp-labral papilla; s-seta;

seg-segment; sp-spine.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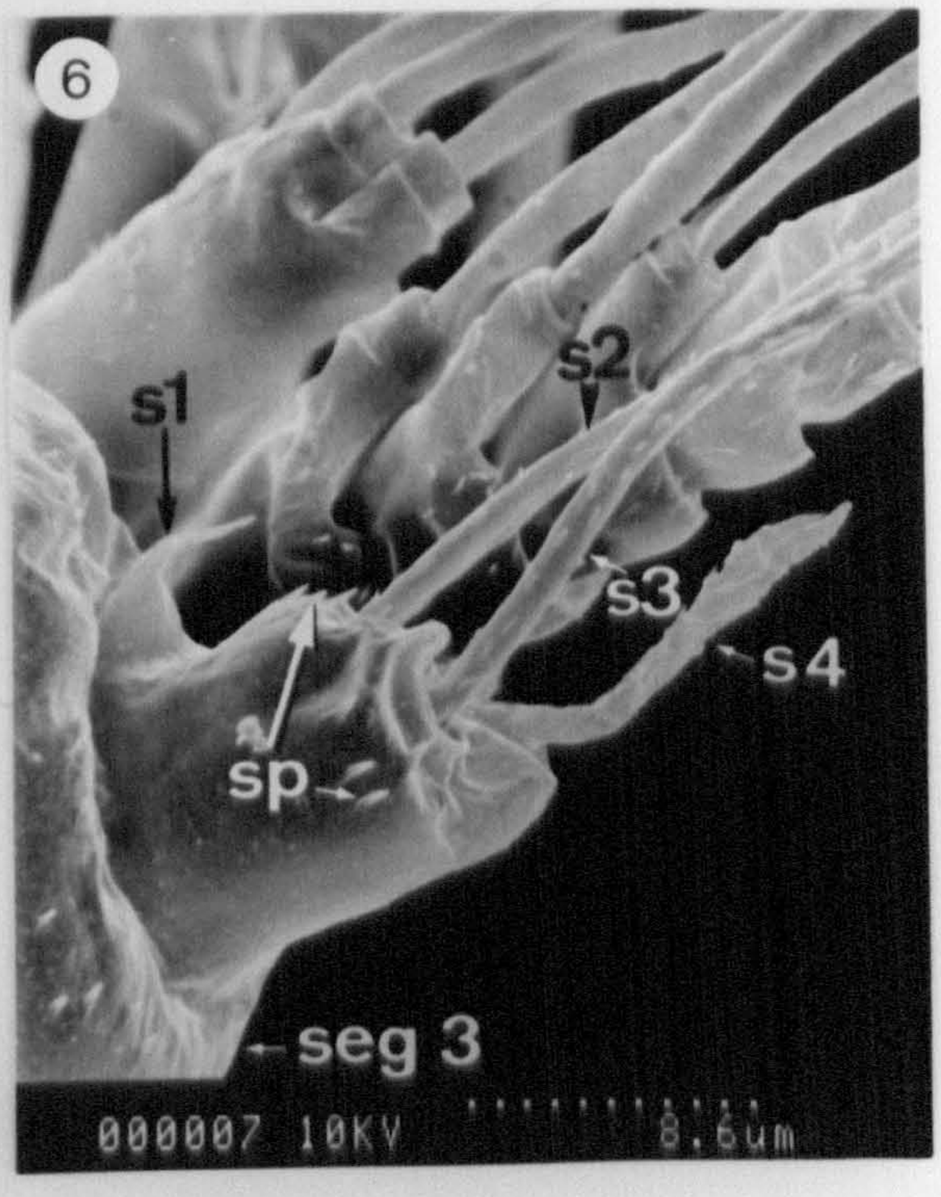
s3
s4
s2
s5
sp
s1
seg3
000001 15KV
10.0um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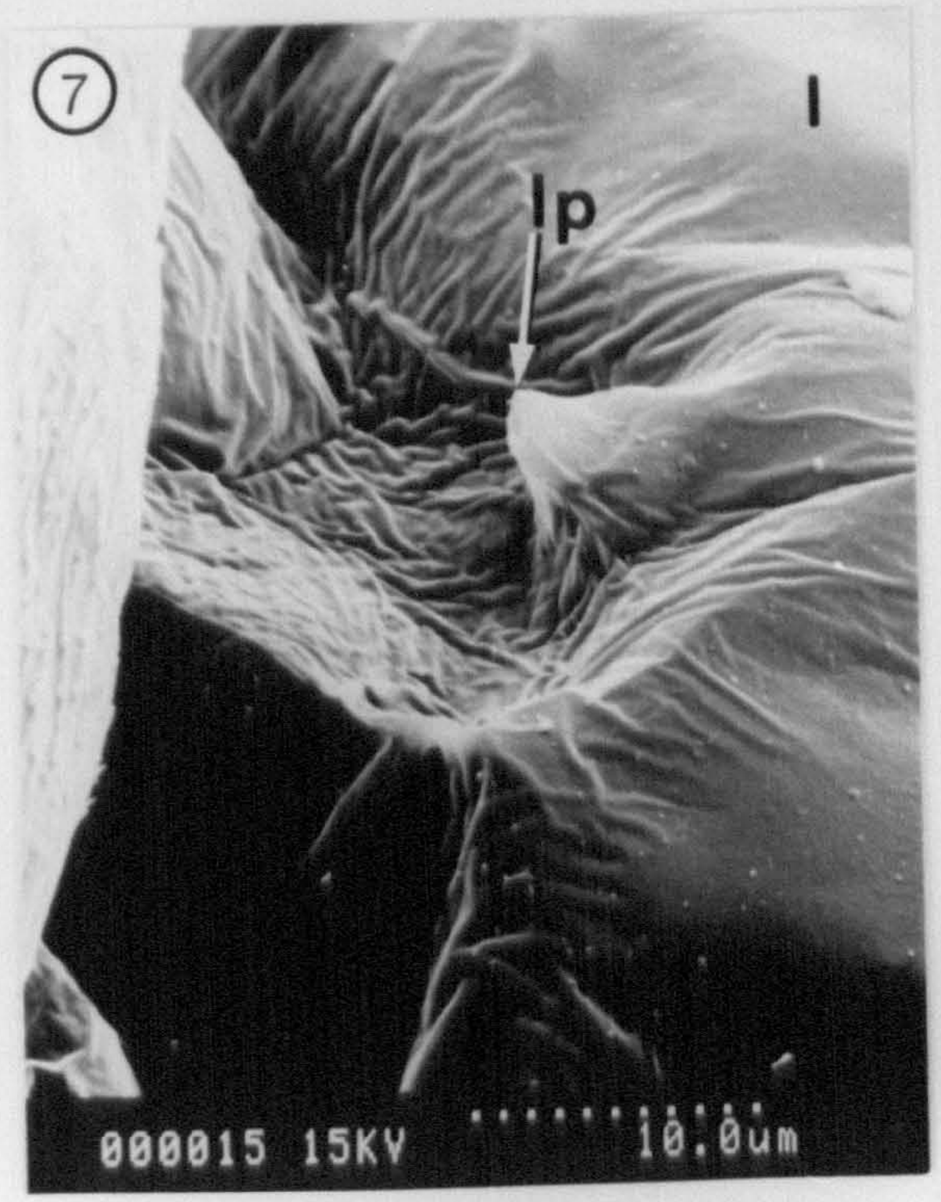
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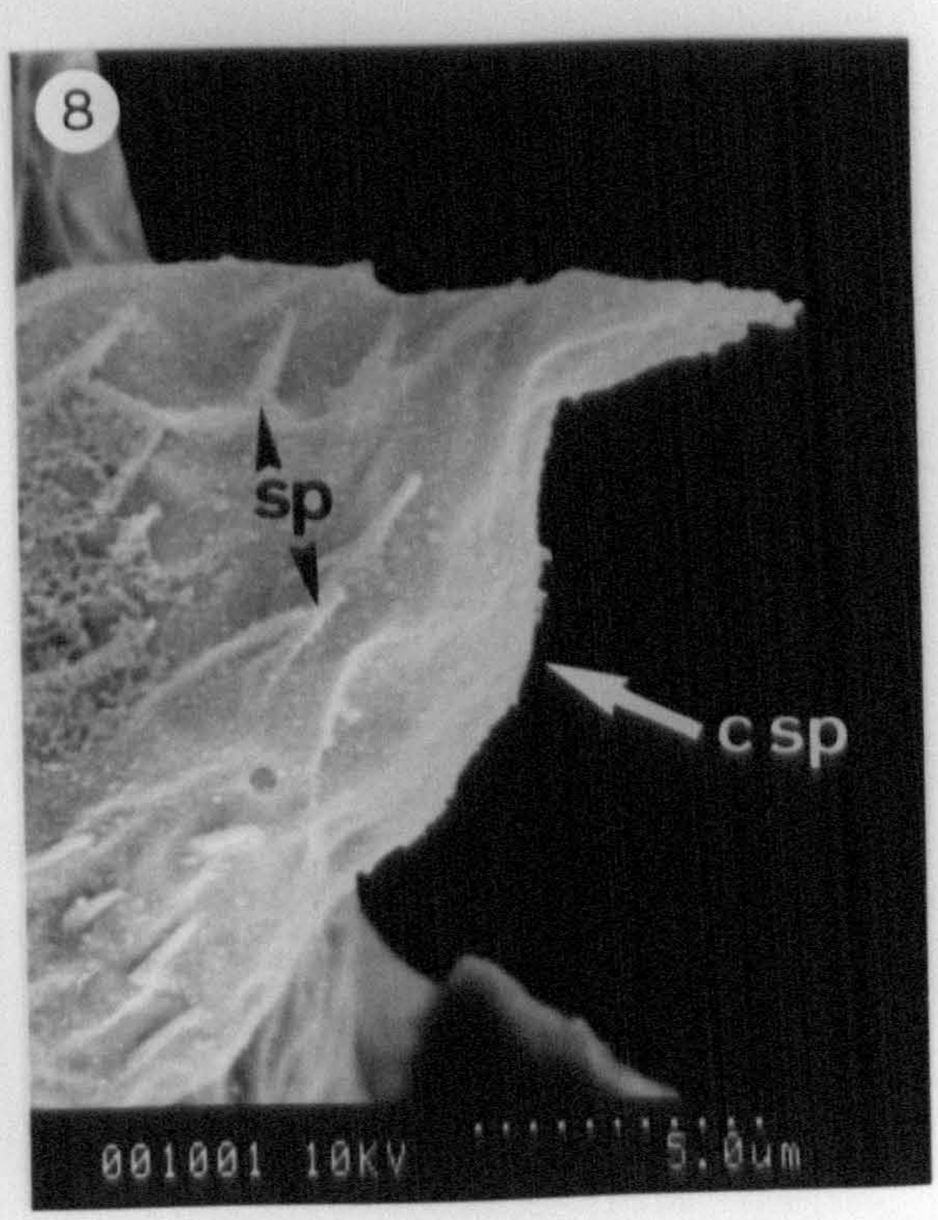
8
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5.0um

Figure 9.

Anterior ventral view of a stage II nauplius.

Figure 10.

Ventral thoracic region of a stage II nauplius.

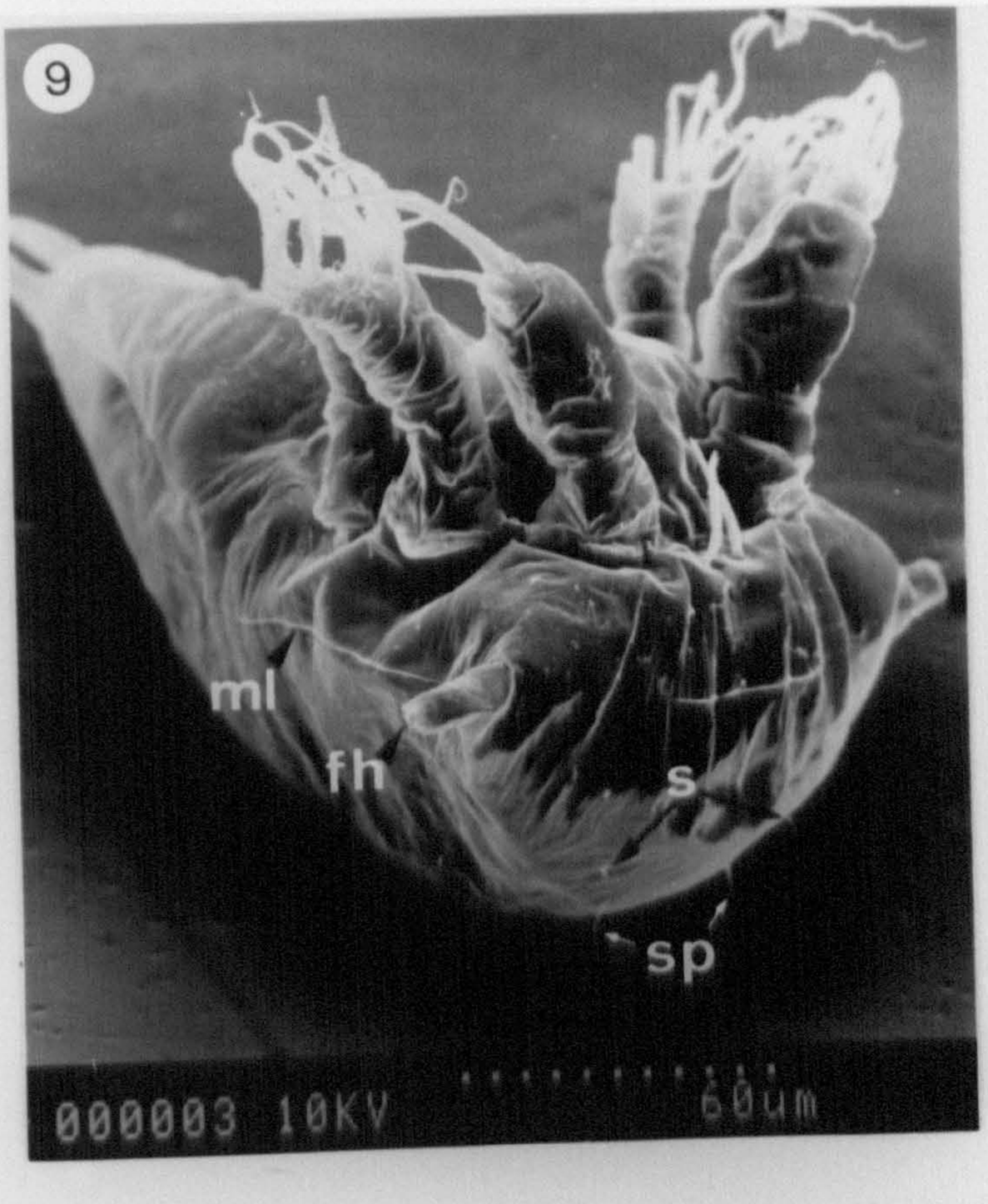
Figure 11.

The left antennule of a stage II nauplius

Labels for figures 9→11 inclusive:-

fh-frontal horn; lp-labral papilla;

s-seta; sp-spine.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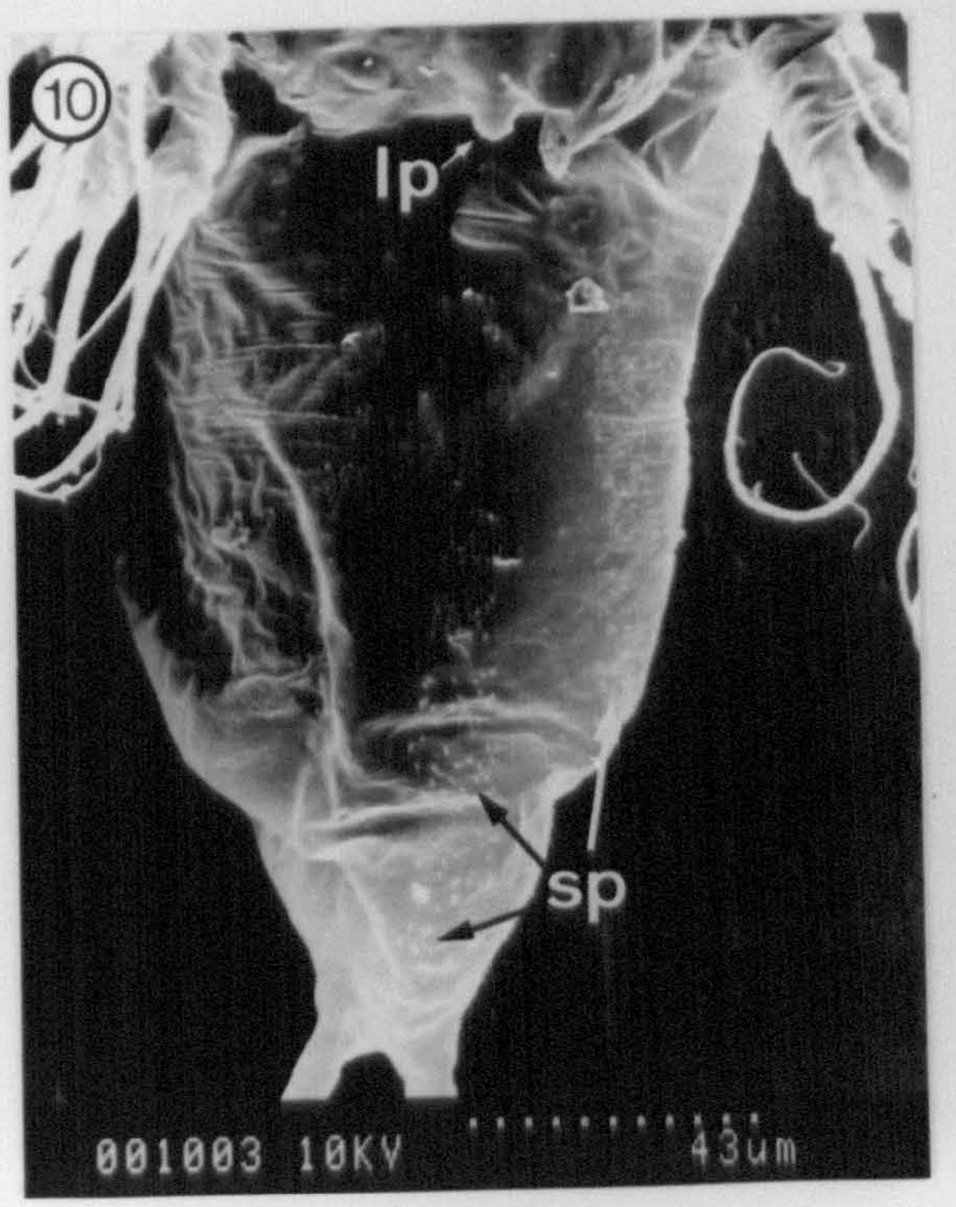
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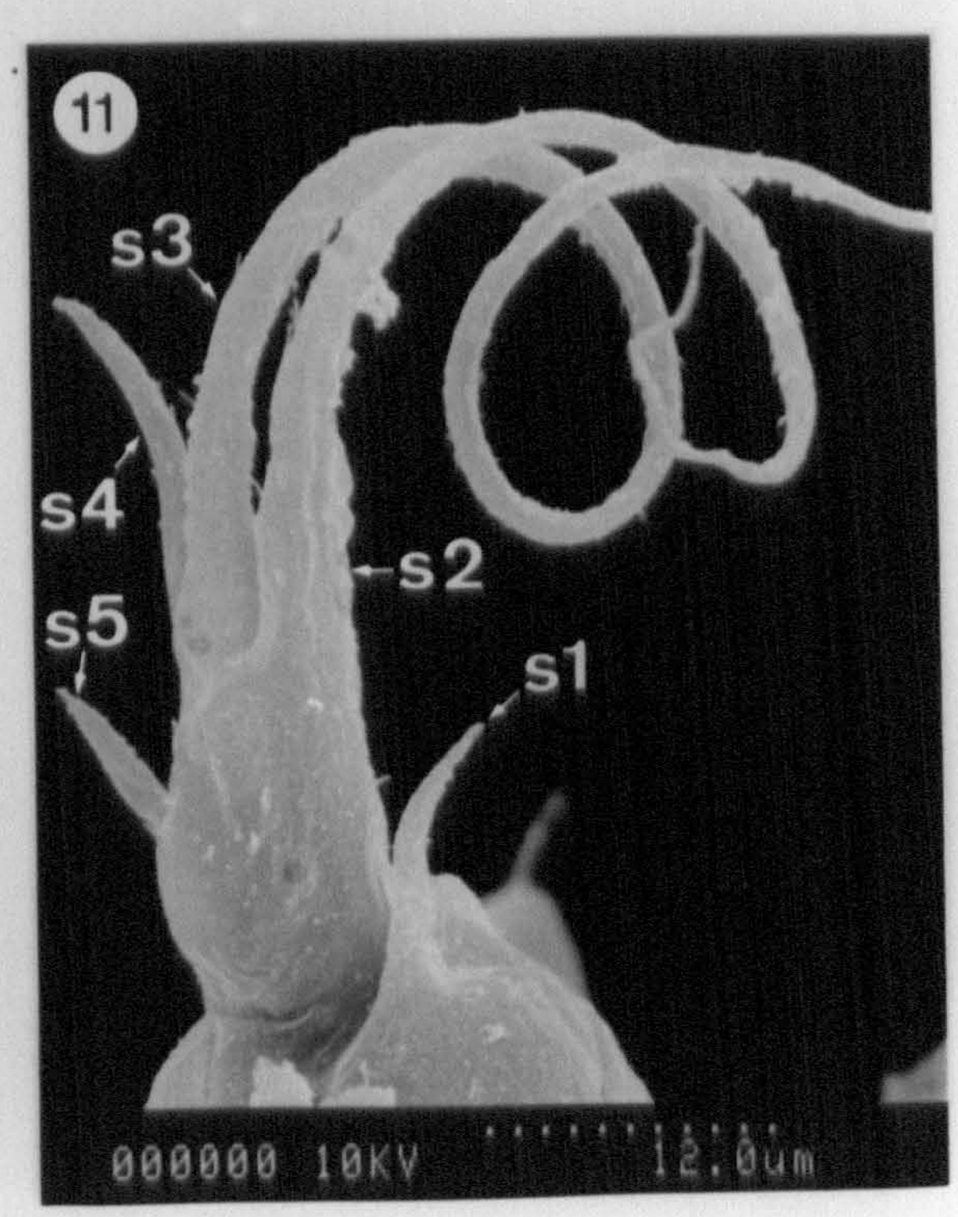
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000000 10KV
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Figure 12.

Dorsal view of a stage IV nauplius

Figure 13.

An enlargement from figure 12 showing the posterior dorsal region.

Figure 14.

An enlargement from figure 12 showing the anterior dorsal region with paired spines and setae.

Figure 15.

A seta on the dorsal surface of a stage IV nauplius.

Figure 16.

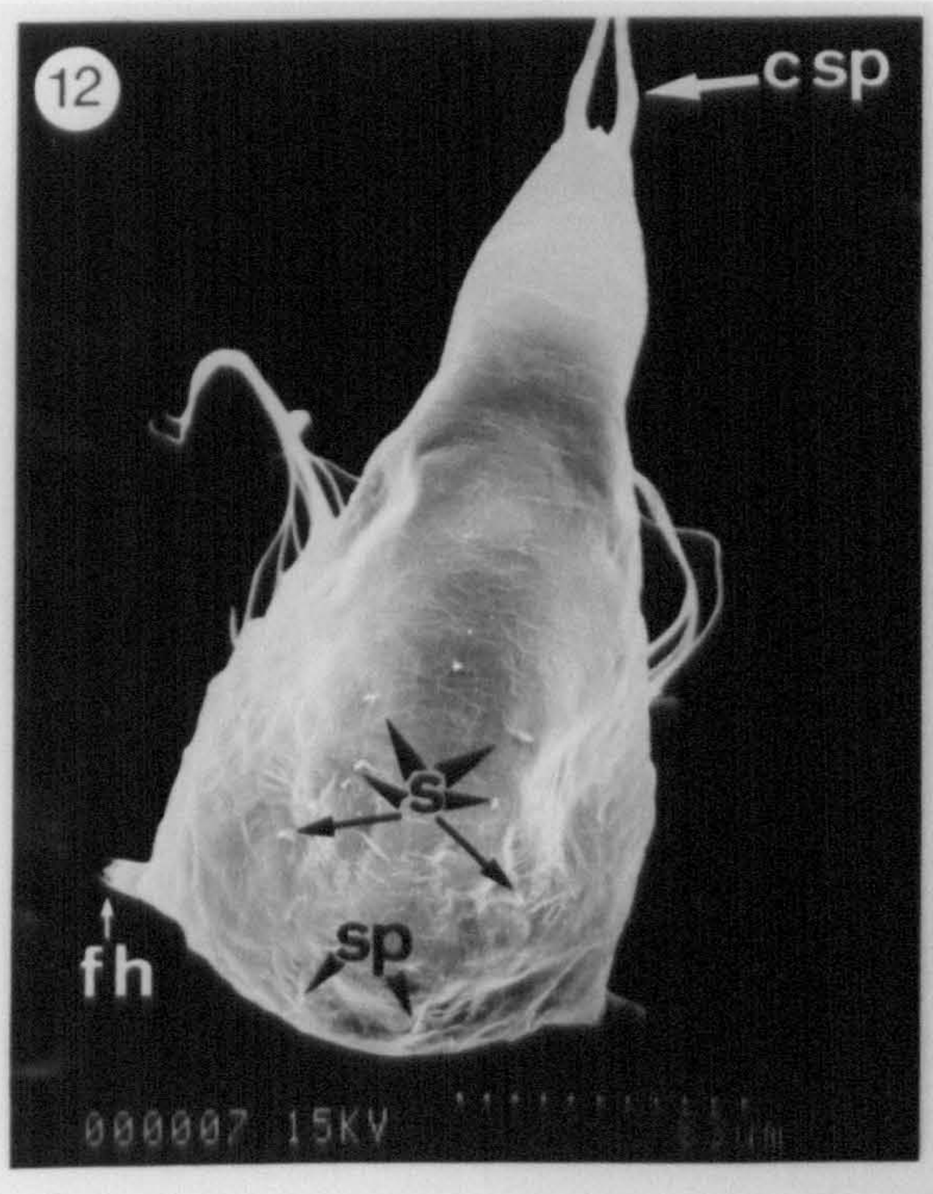
An enlargement from figure 14 showing a dorsal spine and a dorsal seta.

Labels for figures 12-16 inclusive:-

csp-carapace spine; fh-frontal horn;

s-seta; sp-spine;

vtp-ventral thoracic process.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sp
fh
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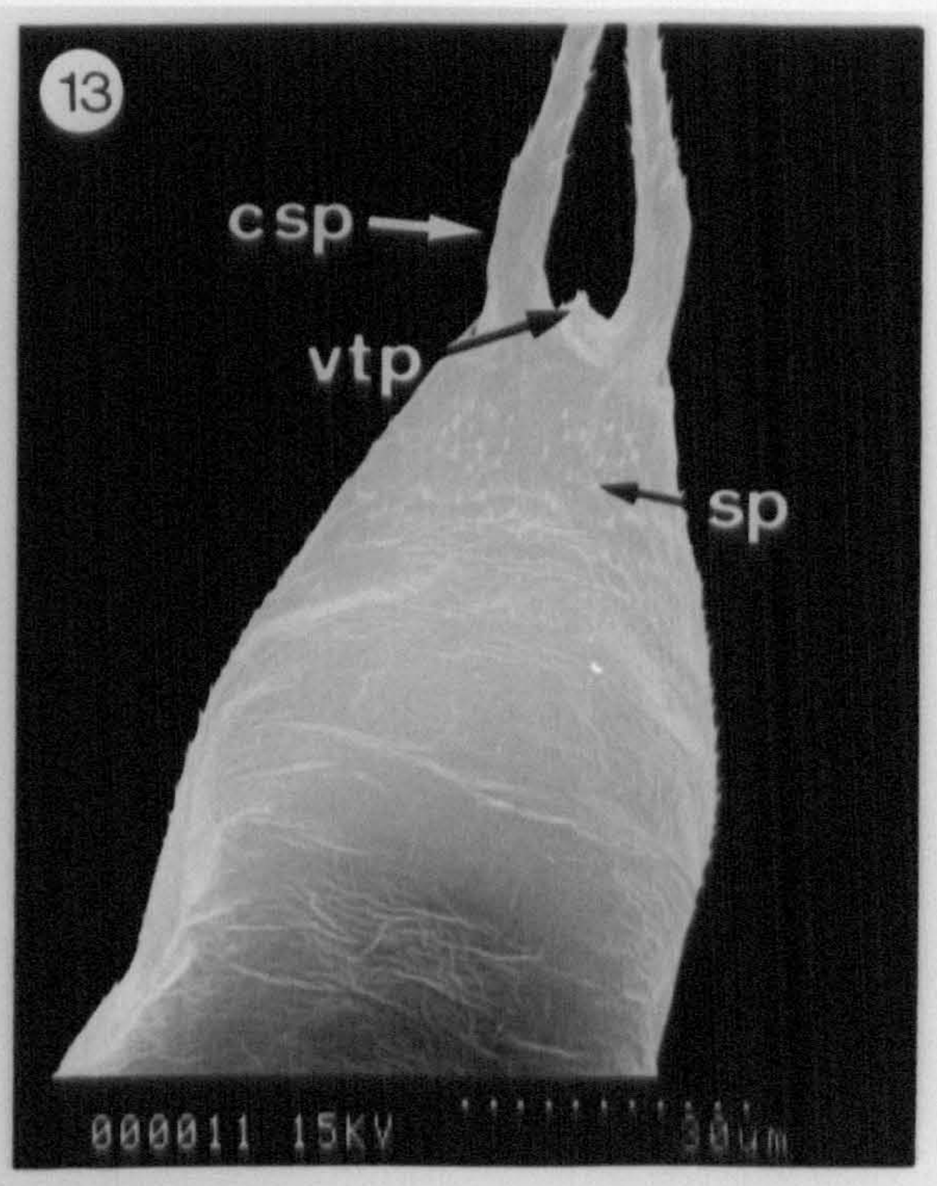
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vtp
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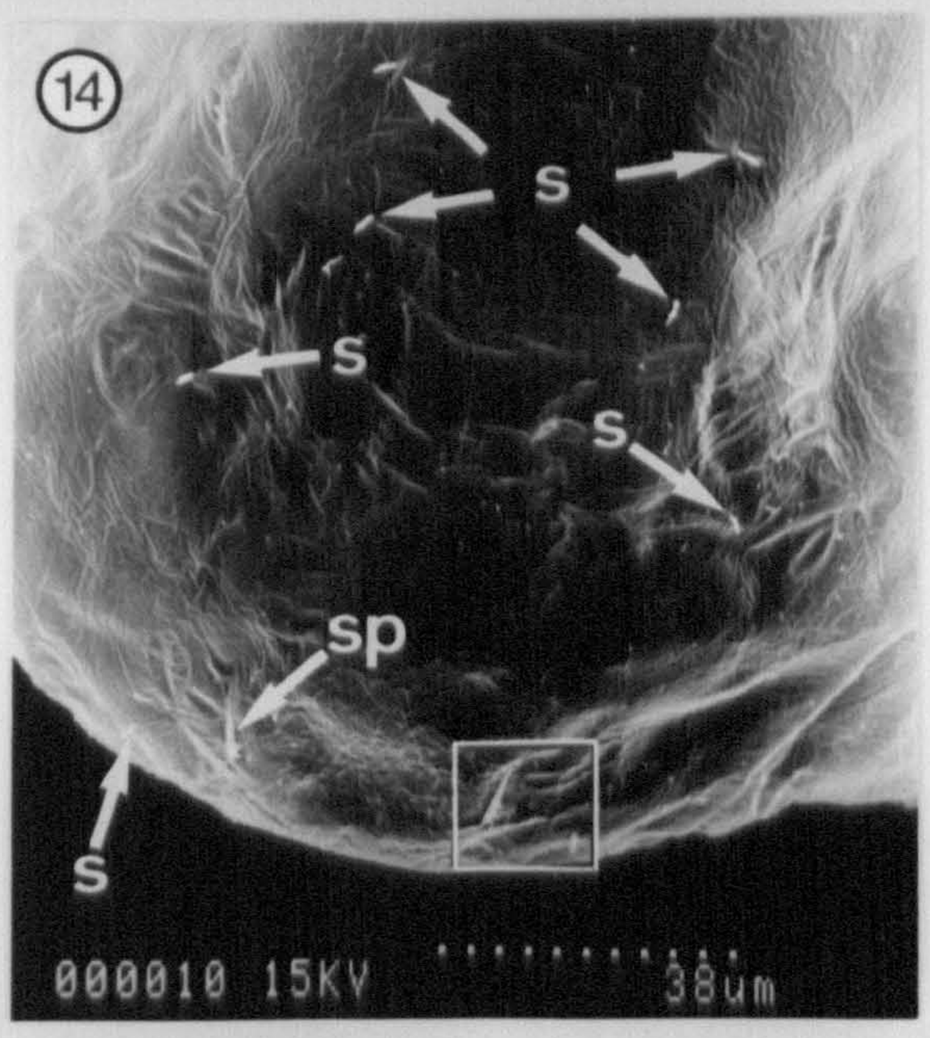
000011 15KV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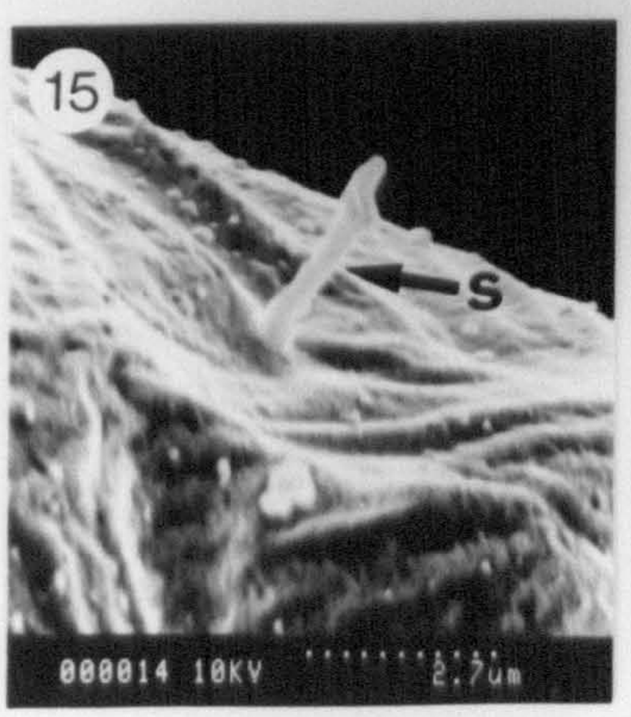
000010 15KV
38um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000014 10KV
2.7um

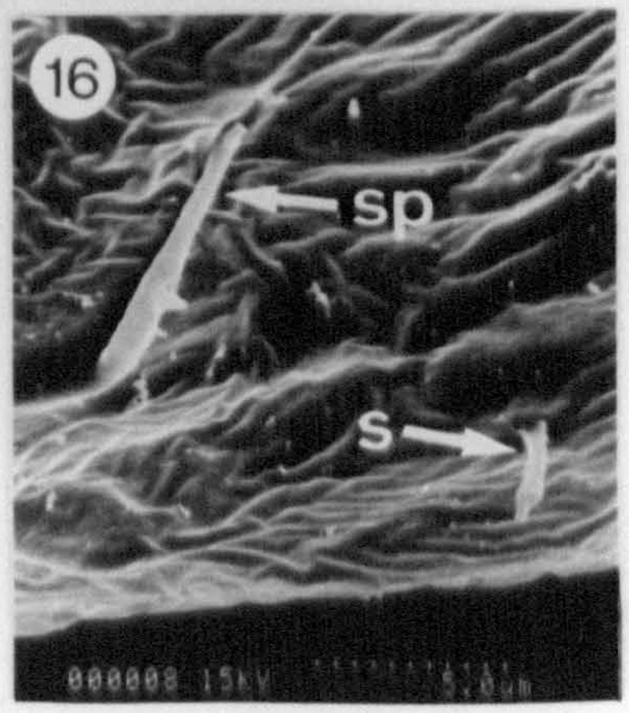

16
sp
S
000008 15KV

Figure 17.

A ventral view of a stage III nauplius.

Figure 18.

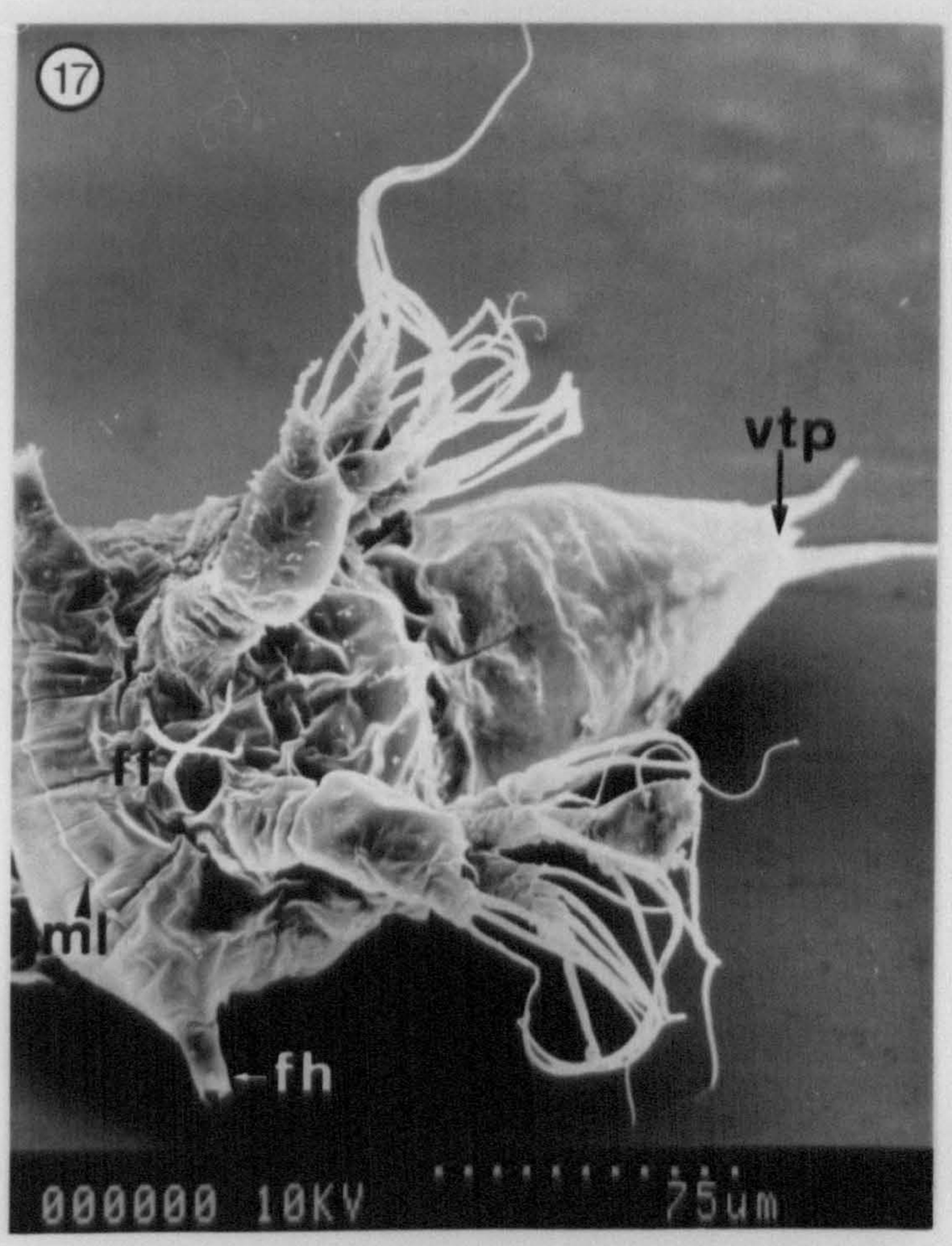
The ventral posterior region of a stage III nauplius.

Figure 19.

The right lateral view of the posterior region of a stage III nauplius.

Labels for figures 17-19 inclusive:-

csp-carapace spine; ff-frontal filament; fh-frontal horn; ml-moult line; vtp-ventral thoracic process.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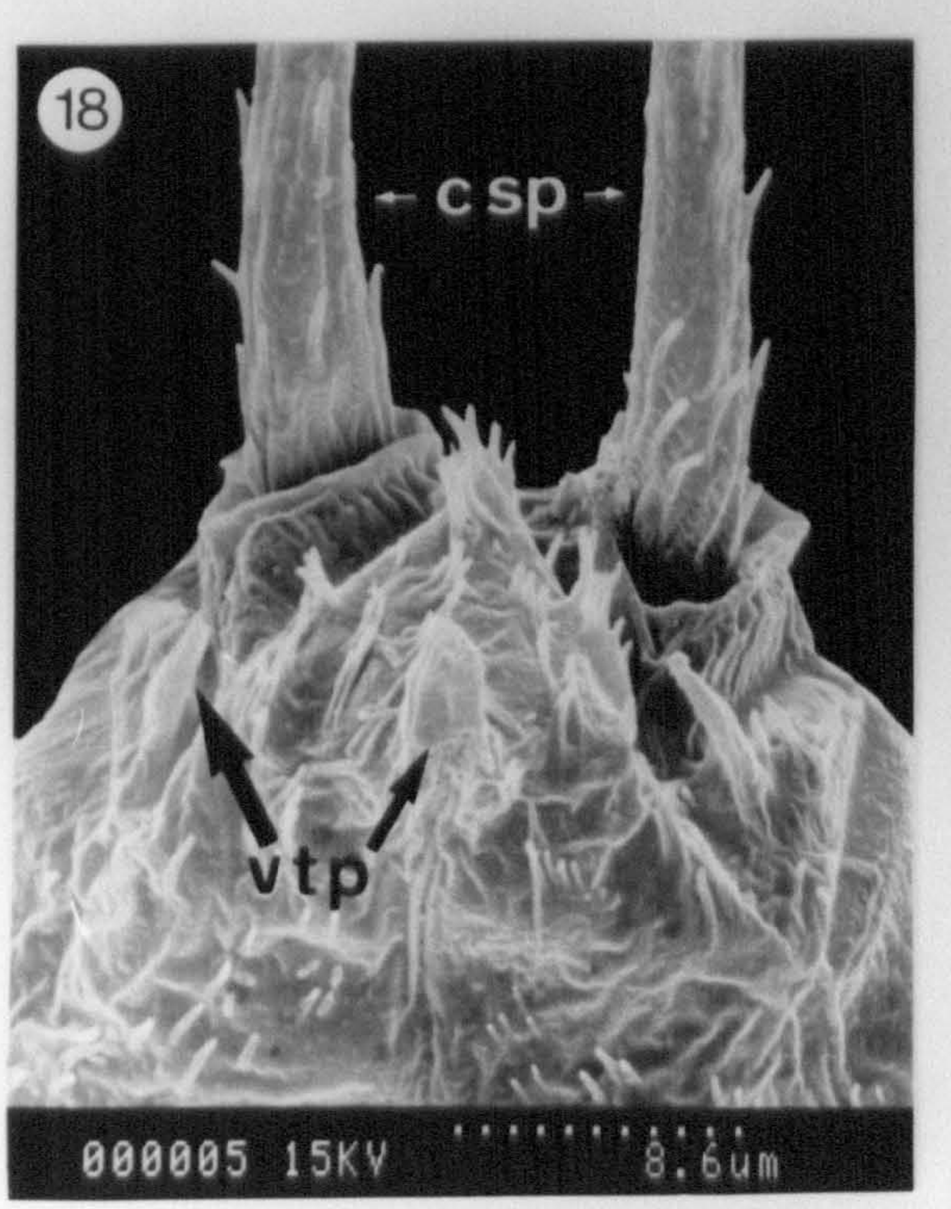
vtp
ff
ml
fh
000000 10KV
25um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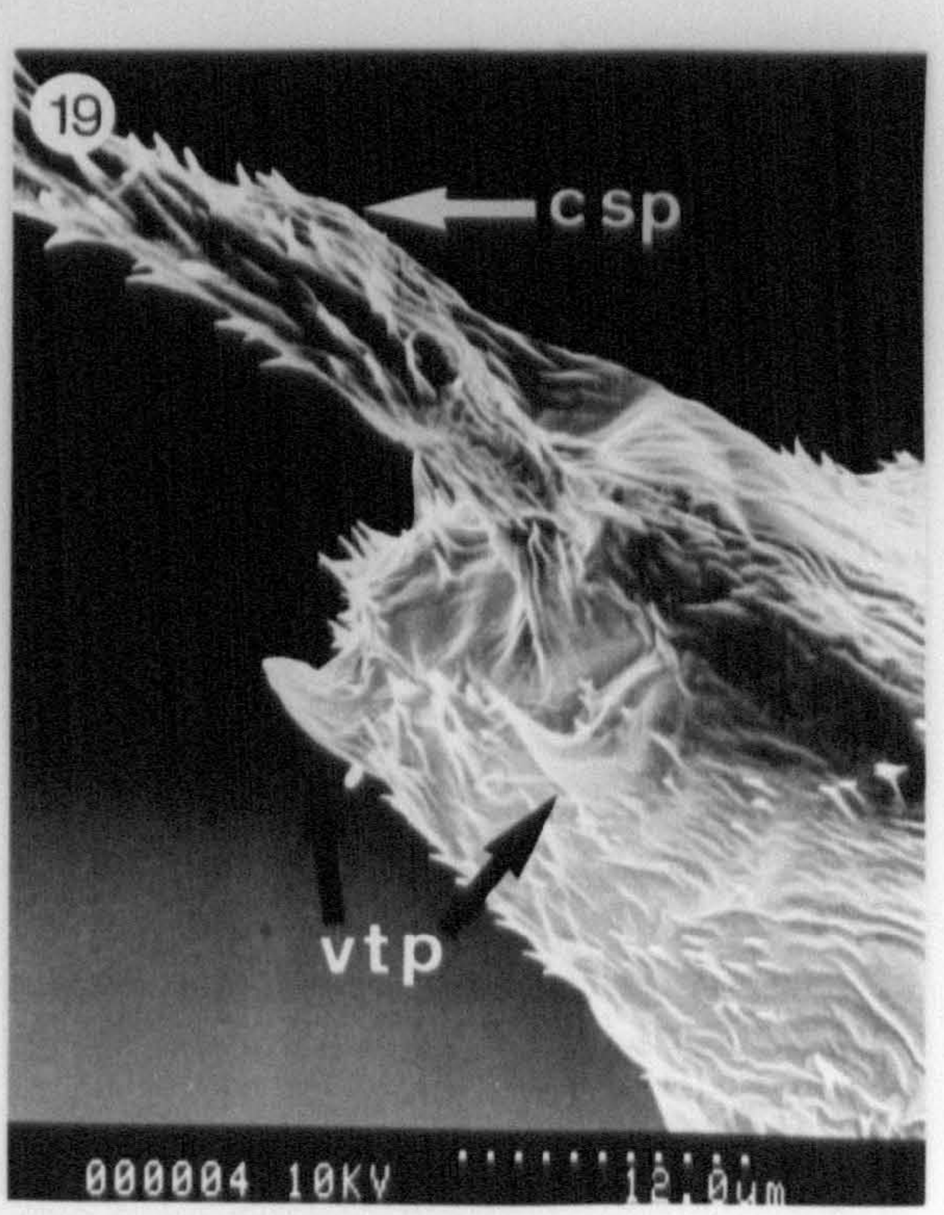

19
csp
vtp
000004 10KV

Figure 20.

The ventral view of a stage IV nauplius.

Figure 21.

An enlargement from figure 20 showing the right antennule.

Figure 22.

An enlargement from figure 20 showing the left frontal horn.

Figure 23.

The ventral posterior region of a stage IV nauplius.

Figure 24.

The ventral thoracic and posterior regions of a stage IV nauplius.

Labels for figures 20-24 inclusive:-

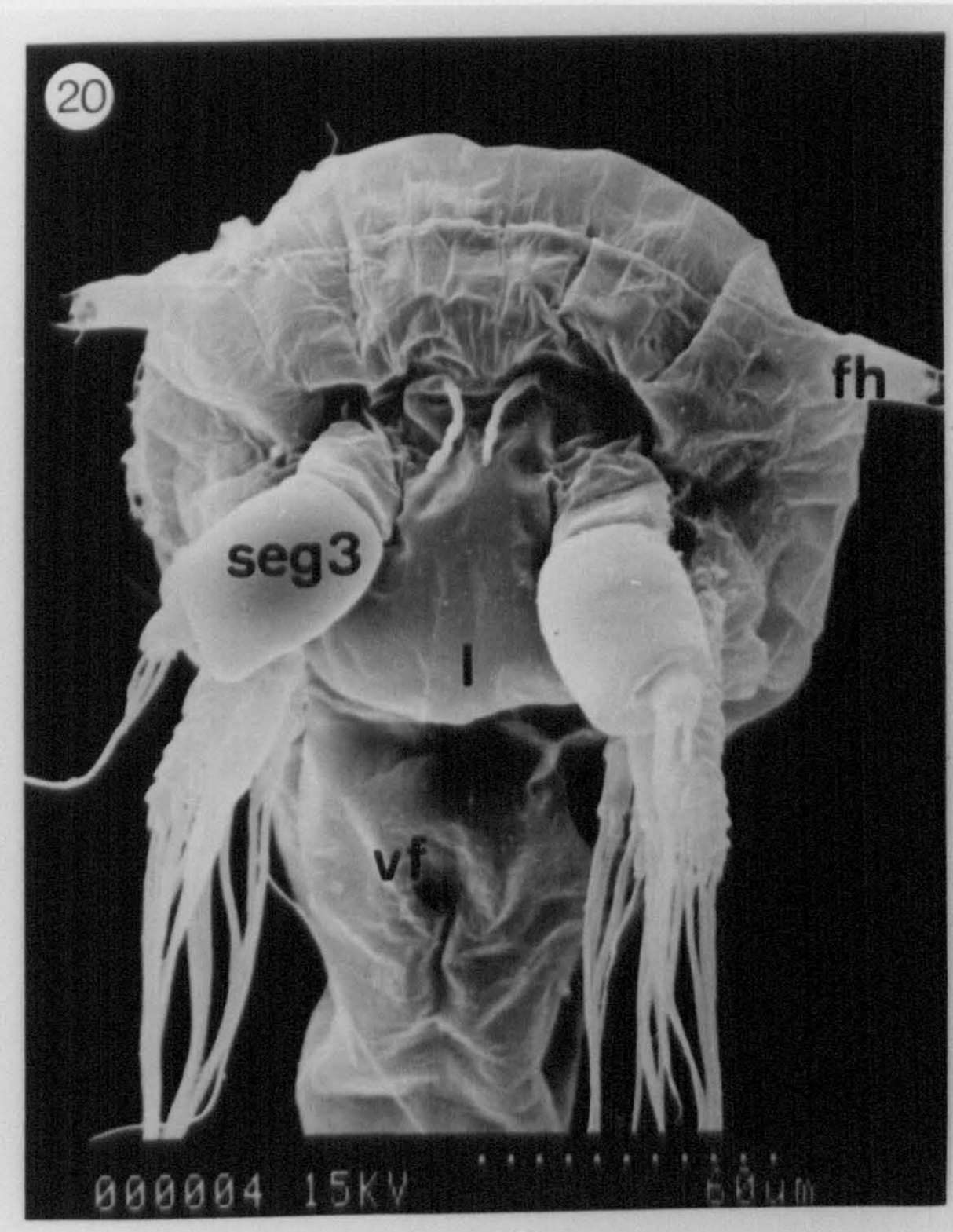
csp-carapace spine; dsp-dorsal spine;

fh-frontal horn; l-labrum;

seg-segment; shc-shredded cuticle;

sp-spine; vsp-ventral spine;

vtp-ventral thoracic process.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vf
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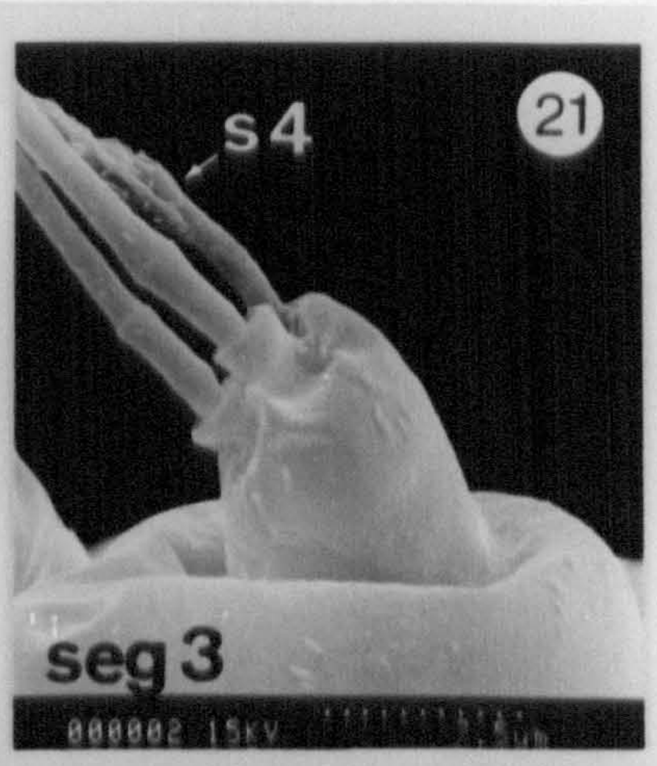
60um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21
seg 3
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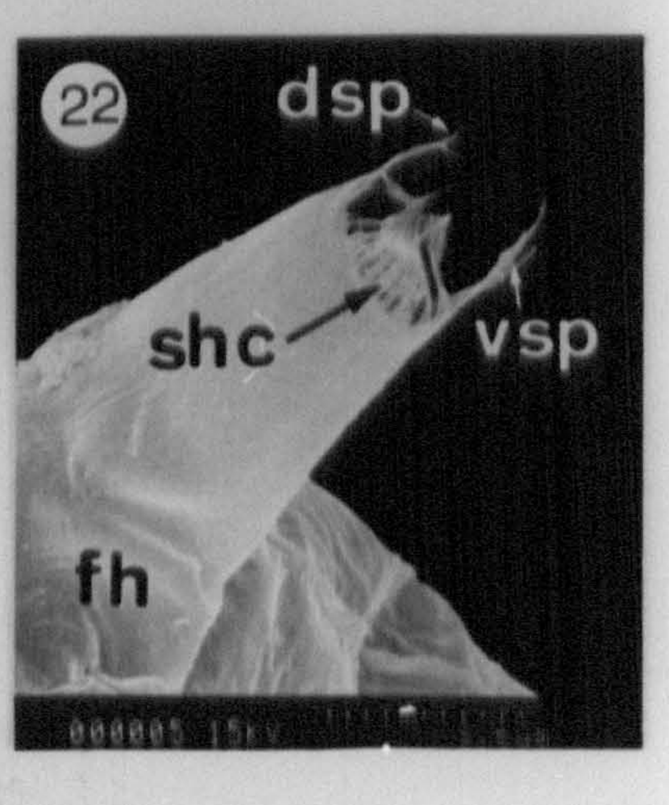
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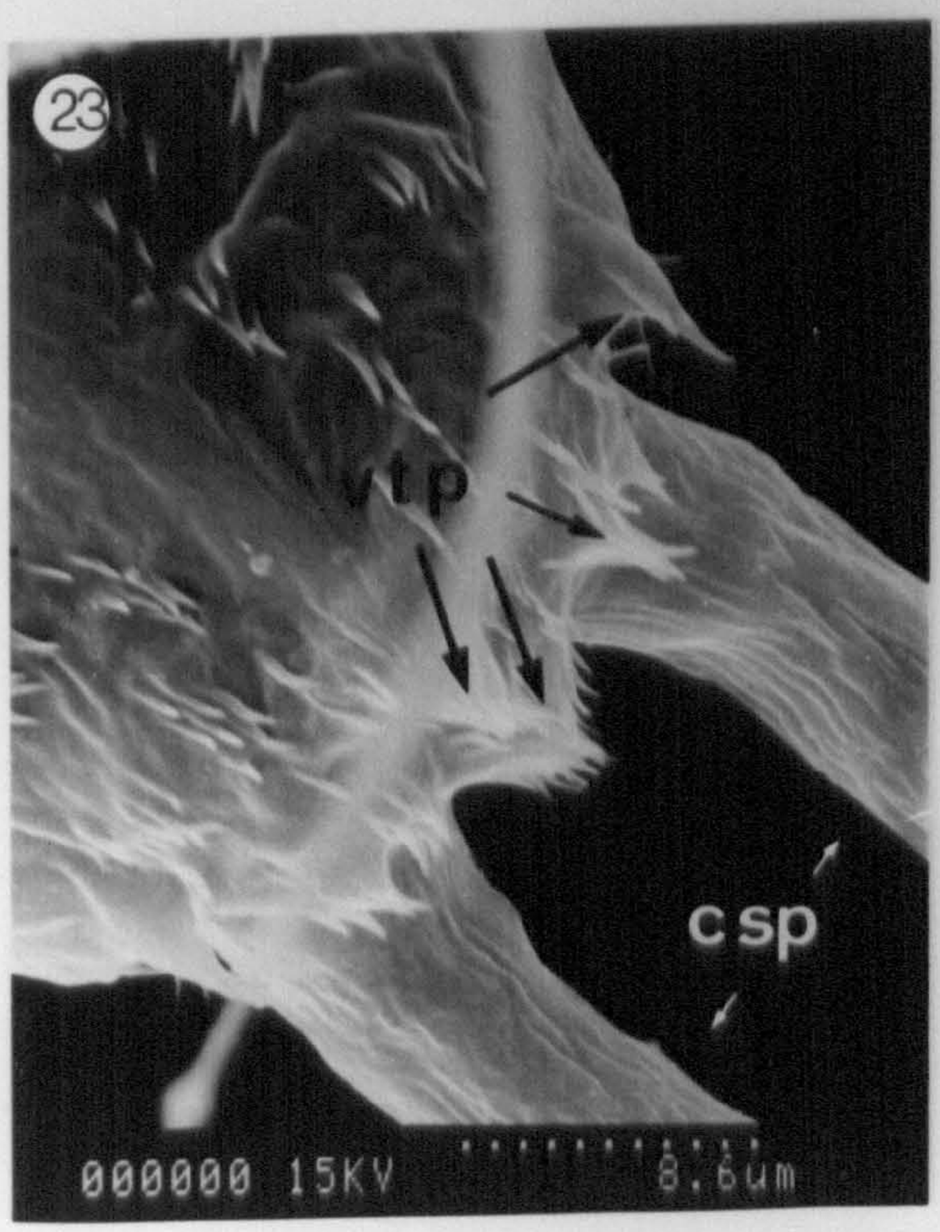
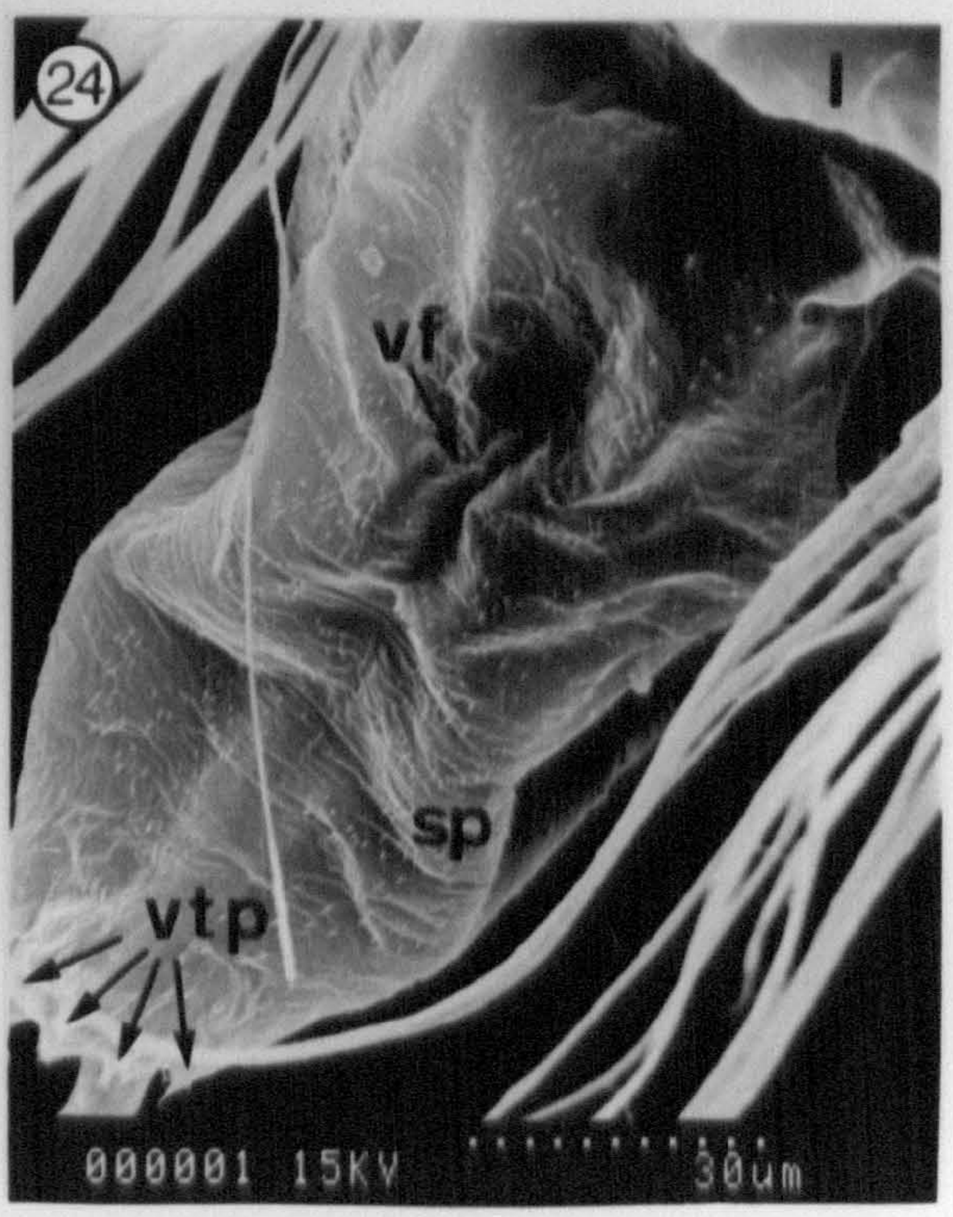
shc
vsp
fh


23
vtp
csp
000000 15KV
8.6um


24
l
vf
sp
vtp
000001 15KV
30um

Figures 25 & 26 are light micrographs of *Sacculina carcini* nauplii.

Figure 25.

A ventral view of two stage IV nauplii

Figure 26.

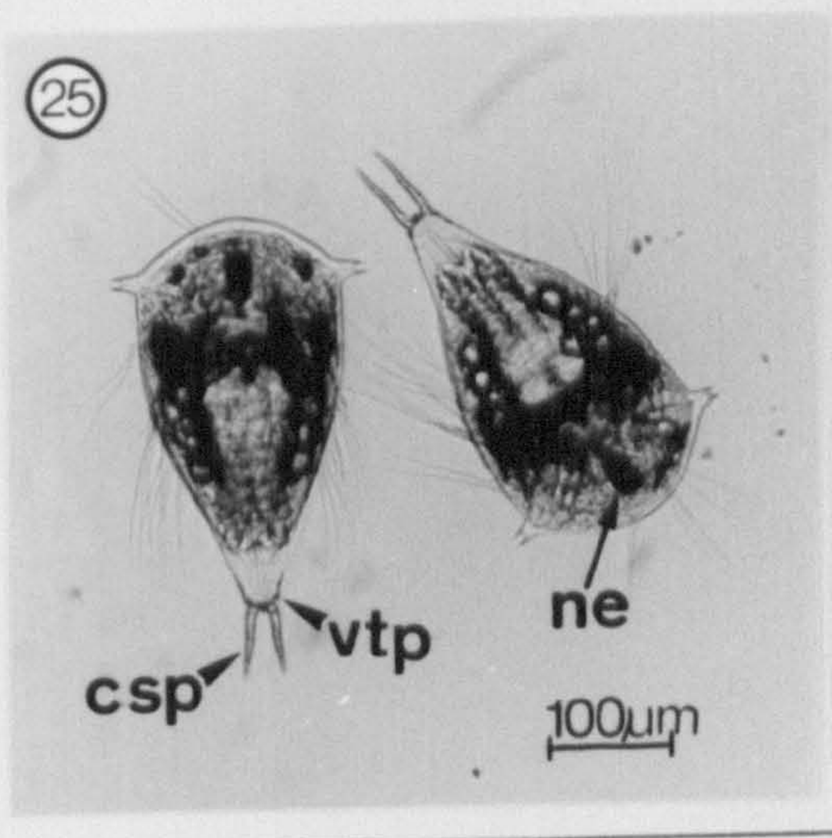
The right lateral view of the thoracic region of a stage IV nauplius

Figure 27.

Line drawings showing the ventral view of *Sacculina carcini* nauplii (stages I-IV). The limbs are omitted.

Labels for figures 25 & 26:-

cl-cypris limbs; csp-carapace spine; ne-nauplius eye; vtp-ventral thoracic process.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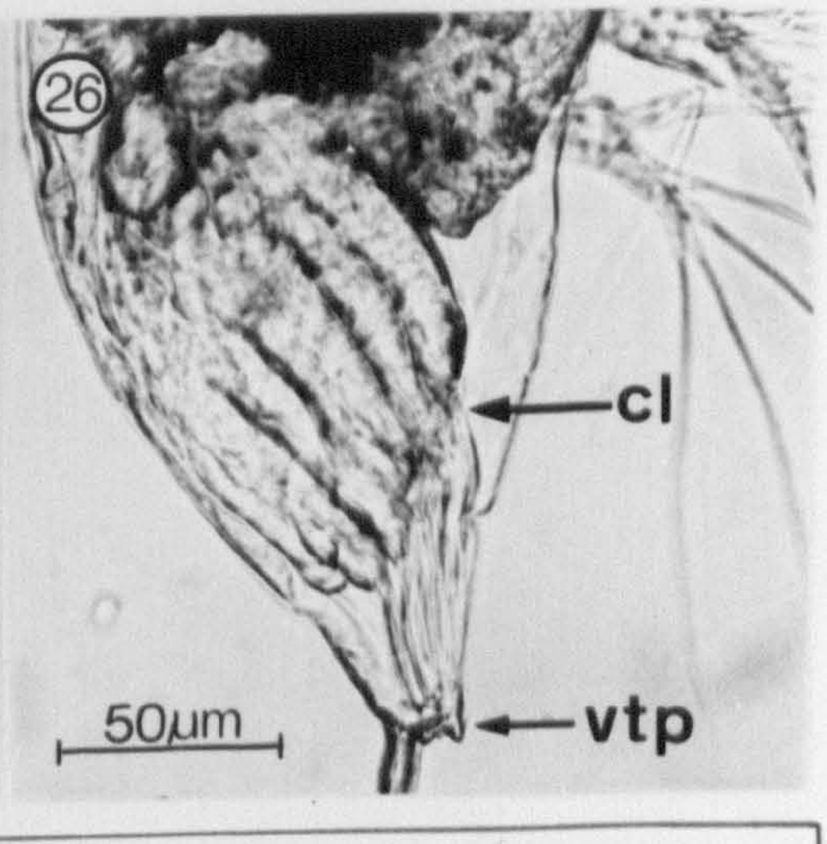
csp
vtp
ne
100µm


26
cl
vtp
50µm

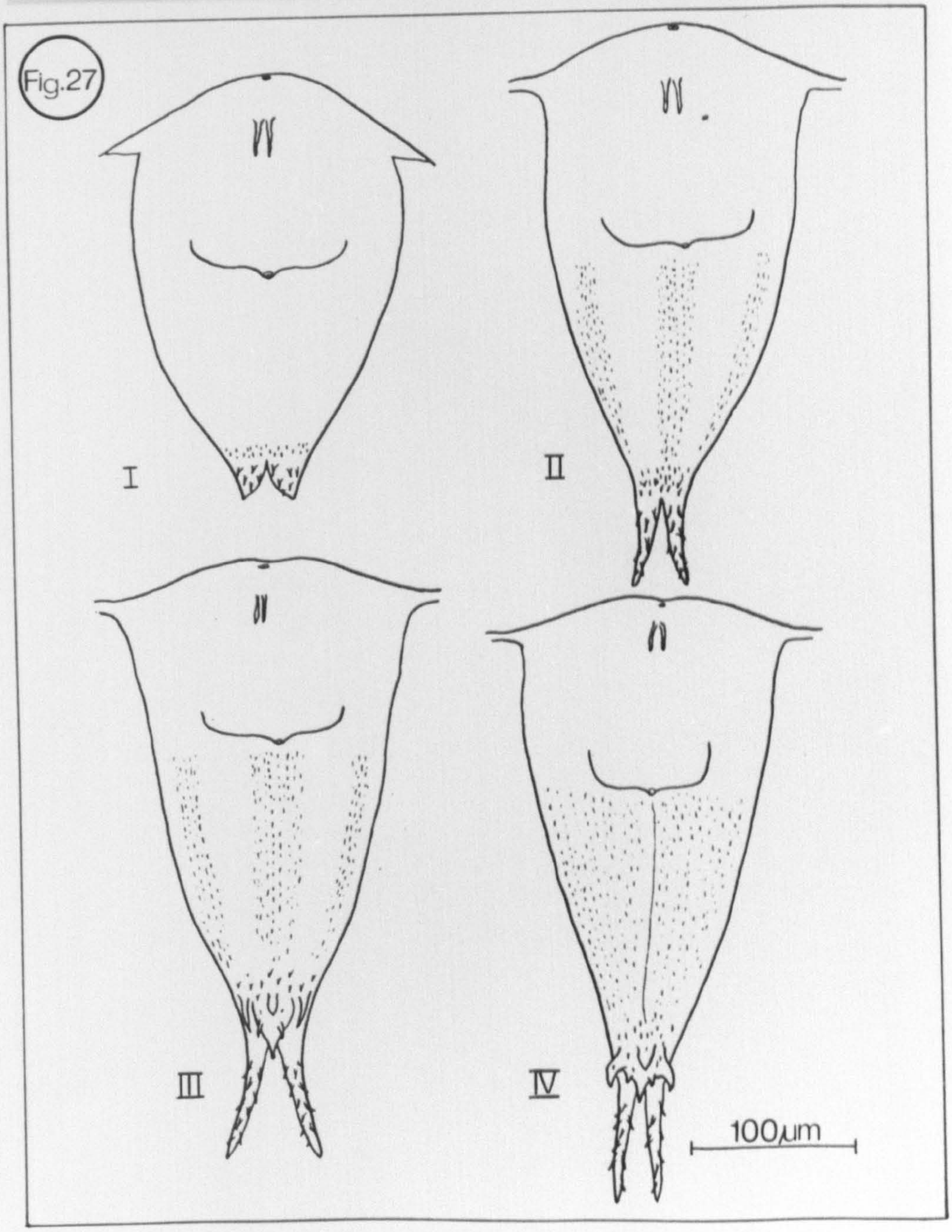


Fig.27
I
II
III
IV
100µm

Figure 28.

Line drawings of the final stage right antennae of four cirripede nauplii :-

A. stage VI *Lepas anatifera* (After Moyse 1987).

B. stage VI *Balanus crenatus* (After Lang 1979).

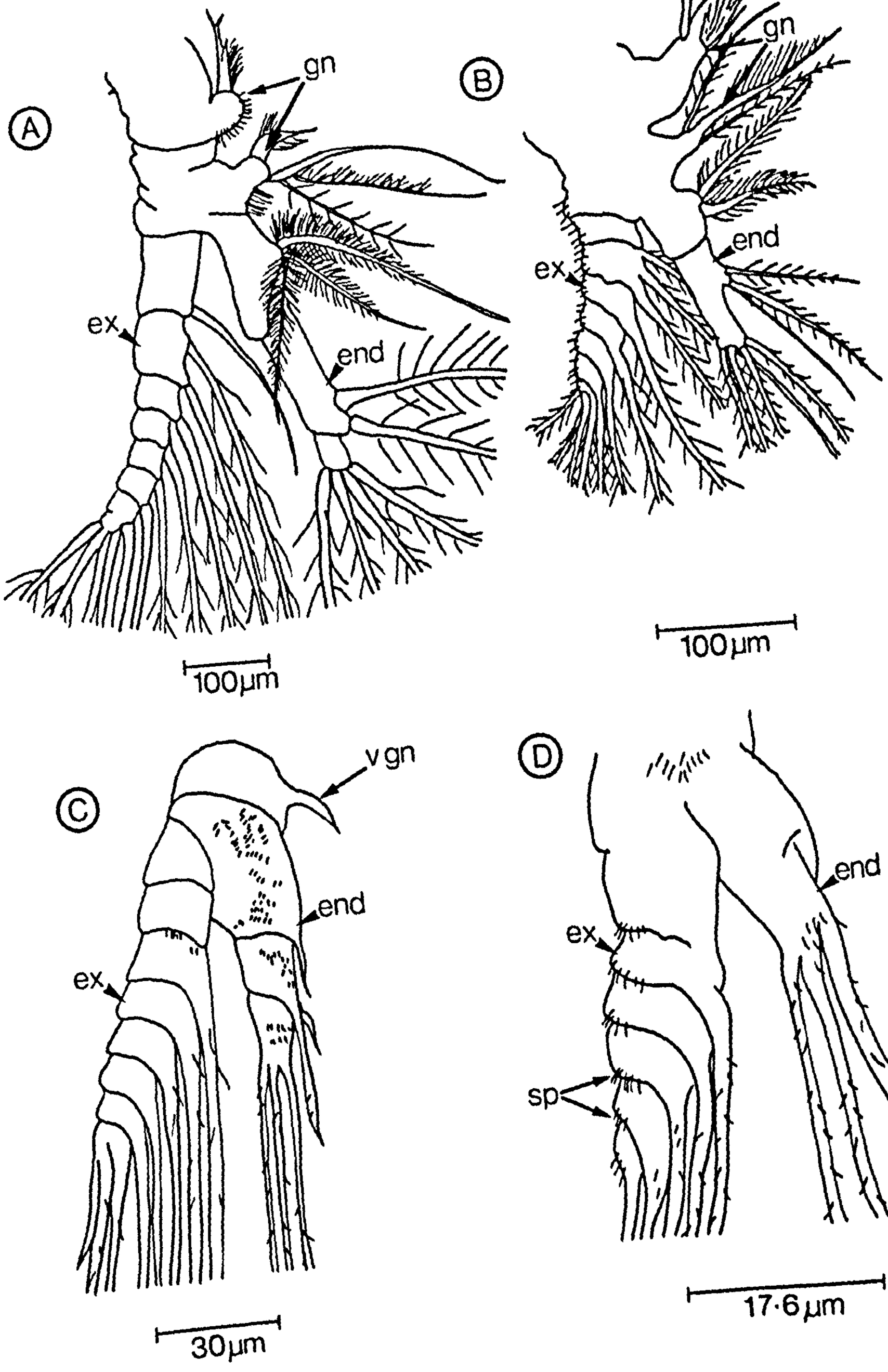
C. stage VI *Ibla quadrivalvis* (After Anderson 1987).

D. stage IV *Sacculina carcini.*

Labels for figure 28:-

end-endopodite; ex-exopodite; gn-gnathobase; sp-spine; vgn-vestigial gnathobase.

Fig.28


A
gn
ex
end
100µm
B
gn
ex
end
100µm
C
vgn
end
ex
30µm
D
end
ex
sp
17·6µm

CHAPTER IV : METHODS TO GAIN THE ASH FREE DRY WEIGHTS OF *SACCULINA CARCINI* LARVAE

In order to compare the energy utilisation of cirripede eggs and larvae already reported with that of *Sacculina carcini*, it was necessary to monitor the weight of *S. carcini* larvae during development.

Different authors have employed different methods for weighing small planktonic animals. Harms (1987), was able to wash *Elminius modestus* larvae in fresh water before placing precounted samples onto Whatman glass fibre filters which were put into silver cartridges for freeze-drying. The larvae were then weighed on a Perkin-Elmer AD 2 electronic autobalance. Lucas & Crisp (1987) washed *Balanus balanoides* eggs with 0·9% aqueous ammonium formate (see Holland & Walker 1975) before pipetting precounted samples into pre-freeze-dried, pre-weighed 1·0 ml. AC60 autoanalyser cups for freeze-drying. Doohan & Rainbow (1971) however, washed samples of 50 rotifers with distilled water and dried them on cavity slides in a $CaCl_2$ desiccator for 48 hours. The samples were then transferred using tungsten needles onto the scale pan of a Cahn Gram Electrobalance. These samples were weighed cumulatively, up to 400 individuals.

In this study it was originally intended to use Teflon boats for weighing, as these would later be used during the gravimetric lipid analysis of the *Sacculina carcini* larvae. However, these boats were found to weigh approximately 60 mg.

each and consequently were too heavy to enable accurate weighing of animals, less than 1 µg. dry weight per individual, in sufficient numbers that would be suitable to count. A method was devised to employ small pieces of plankton netting as seives on which the larvae could be washed quickly, frozen and freeze-dried then weighed and counted.

MATERIALS AND METHODS

Initially, the larvae of *Sacculina carcini* were washed free of seawater with 0·9% aqueous ammonium formate (see Holland & Walker 1975). However, they were seen to quickly burst and release body fluids through their frontal horns. Consequently, an experiment was carried out using a Knauer semi-micro osmometer, with a sample size of 50 µl., to determine the concentration of ammonium formate which would be isotonic with seawater (Figure 1). A glass vial containing this isotonic solution was freeze-dried over 12 hours to assess whether the solution was 100% volatile. The larvae were washed with the solution, monitored under a Leitz Orthoplan photo microscope and relevant photographs were taken. In this way the maximum time available for washing the larvae, without loss of body fluids, was determined.

At each stage of their development, *S. carcini* larvae were attracted to a point light source and pipetted into a glass beaker full of U.V.-irradiated fine filtered seawater. They were, once again, attracted to the light source and approximately 300

larvae were pipetted onto a 1 cm^2 sieve of nylon plankton netting, 45 µm mesh size. Each net had previously been heat-sealed to prevent the edges from fraying. The larvae were then washed six times with 3·9% aqueous ammonium formate and twice with distilled, deionised water. Excess water was pipetted from the net, which was then placed in the deep freeze. Ten replicate nets were prepared in this way for each larval stage. These nets were freeze-dried for 12 hours, placed in a desiccator overnight and weighed the following day using a Cahn C31 microbalance. The nets were weighed sequentially (1 to 10) in the morning and in reverse order (10 to 1), after 4 hours in the desiccator. The average of the two weights for each net was calculated. After weighing, the nets were mounted on graph paper, to act as a grid, and placed under a Wild M3Z binocular microscope. The image was relayed by a Panasonic CCD video camera to a Sony Trinitron KX-14CPI monitor so that the exact number of larvae present on each net could be counted using a click counter. Replicate counts were made. After counting the larvae the nets were soaked in a 2% Decon solution overnight, rinsed in distilled deionised water, oven-dried at 37°C, placed in a desiccator overnight and then reweighed.

In order to assess the absorption of water vapour by the plankton netting on exposure to air, a single net was repeatedly weighed over a 30 minute period. Between weighings the net was replaced in the desiccator and the balance tared (see Figure 2). Another weighing experiment was carried out for ten nets with larvae, and ten nets without larvae over three successive days. The nets were kept in a desiccator and weighed consecutively from

number 1 to 10 in the morning and from number 10 to 1 after a period of 4 hours in the desiccator. A second method of weighing the larvae was also employed. A number of freeze-dried larvae (25 ± 70) were placed on pre-weighed aluminium foil boats, which had been made from circles of aluminium foil pressed onto a foil lined plasticene mould. After weighing, the larvae were rehydrated and counted. The weighings for male and female nauplius stages II, III, IV and cyprids were carried out in this way. In order to assess whether there was any absorption of water vapour by the aluminium foil boats, three boats were weighed at hourly intervals on three occasions during one day. The boats were not kept in a desiccator between weighings, but were covered to prevent any air-borne dust particles settling.

The inorganic fraction of *S. carcini* larvae, at each stage of their development, was then monitored. Aluminium foil boats were prepared and heated to constant weight at 550°C for 18 hours in a muffle furnace. Samples of larvae were collected and washed as previously described. They were poured into glass vials which were frozen at -196°C in liquid nitrogen and placed in a freeze-drier over 24 hours. The freeze-dried larvae were ground up and stored in a desiccator overnight. Three samples of each stage were placed in pre-heated, pre-weighed aluminium foil boats. These boats containing larvae were then weighed, heated at 550°C for 6 hours, placed in a desiccator for one hour and re-weighed. The same procedure was carried out for stage I *Elminius modestus* larvae and for three empty aluminium foil boats. The inorganic

fraction (ash) of the larvae and the percentage error for the boats could then be calculated.

RESULTS

Results from the experiment to determine the concentration of ammonium formate, isotonic with seawater, are shown on Figure 1. The osmolarity of the seawater used, was 1000 milliosmoles and from the linear regression fitted to the data, a 3·9% solution of ammonium formate was calculated to be isotonic. This concentration was also found to be 100% volatile in the freeze-drier. When this solution was used to wash the larvae, the release of body fluids through their frontal horns was delayed for 72 seconds. This compared to a delay of only 20 seconds for distilled water and 30 seconds for 0·9% aqueous ammonium formate.

Larval weights resulting from the method using plankton net seives, were variable. It was difficult to collect sufficient numbers of eggs and stage I nauplii, for the ten replicate nets. Often, a release consisted of a mixture of eggs, stage I and stage II nauplii. Also, many of the eggs were not fully developed. Ten replicate nets were used in order to be statistically viable and on viewing the nets under the microscope, it was evident that salt crystals or glass particles from the pipettes became caught within the mesh. Thus, several of the replicates had to be discounted. Results from the experiments to weigh four separate broods of *S. carcini* larvae, on these nets,

are given in Table 1 (full data in appendix Tables1-4).

Figure 2. shows the change in weight observed when a single net was repeatedly weighed over a 30 minute period and Tables 2 and 3 give the weights obtained when ten nets, with and without larvae, were re-weighed on three separate days.

Larval weights obtained from the method employing aluminium foil boats, are given in Table 4, and Table 5 shows the relatively constant weights of the three boats weighed at hourly intervals. Table 6 gives the percentage ash present in the larvae of *S. carcini*, together with their organic weights, which were calculated from these figures and the weights obtained from the aluminium foil boat method. The percentage ash content of stage I *Elminius modestus* nauplii is also given in Table 6.

DISCUSSION

It is difficult to obtain absolute dry weights for small marine animals as no matter how rigorous the drying process, some water will either be re-absorbed from the atmosphere or retained within the tissues. For this reason it was thought, that to use ten replicate nets for each larval stage, would be sound statistical practice. However, this made the method more complex, due to unavoidable exposure to air during weighing. As the larvae were counted, after drying and weighing, it was possible to observe and eliminate the nets which were "contaminated". Had these nets been included, the mean larval weights would have been

unrealistically high. Nevertheless, the weights obtained using this method were still variable (see female weights, Table 1.). A calculation was undertaken to assess the approximate weight of male and female *S. carcini* eggs from their known volume, assuming they comprised the same constituents as the eggs of *Balanus balanoides* (Lucas & Crisp 1987). The predicted weights came out as 0.50 µg. and 0·38 µg. for male and female eggs, respectively. One brood of *S. carcini* eggs was successfully separated out from the surrounding debris and weighed by the plankton net method. The resultant weight was 0·76 µg. ± 0· 04 µg., using ten nets to gain this mean figure. The mean weight was a lot higher than the calculated figure, but the brood was later found to be a mixture of male and female larvae.

The experiment to repeatedly weigh one plankton net, over a 30 minute period, on two consecutive days, gave a result the first day which was repeatable the next (Figure 2). During the initial 4 minutes the weight increased steeply, and began to level out after approximately 20 minutes. Over the 30 minute period, the weight increase, due to absorption of water vapour, was 100 µg., with an increase of 50 µg. in the first 4 minutes. It was noted that this could lead to a high percentage error when weighing 300 larvae which were less than 1 µg. each, and it would not have been practical to wait 20 minutes for each weighing. The experiment to weigh ten nets with and without larvae on 3 separate days, also showed the variability inherent in the method. Day 2 was noted as a particularly humid day. This observation is reflected in the weights, especially those taken

after 4 hours in the desiccator, when each net was found to be at its greatest recorded weight. It is important to note however, that the initial weights of the day are the most closely repeatable, showing differences of 2·4 µg., 3·5 µg. and 1·1 µg. for the nets with larvae and 5·7 µg., 3·2 µg. and 8·9 µg. for the nets without larvae. Consequently, it was evident that the most consistent results were obtained by taking the first weights of the day, with minimum exposure to air. By taking replicates, the figures diverged greatly.

Conversely, the weights of the the aluminium foil boats, varied by a maximum of 0·2 µg. during a period of three hours exposure to air and, using this method, the larvae were also subjected to minimum air exposure. Thus it was decided that the weights obtained by the aluminium foil boat method were more reliable. On consideration of these weights, together with those from the plankton net method, it appeared that stage II nauplii of *S. carcini* were much heavier than their calculated egg weights. The transport of dissolved organic matter, across the body surface of marine larvae belonging to different phyla, such as molluscs, echinoderms and annelids, has been studied by several authors (Shilling et al 1989, Dimster-Denk & Manahan 1989, Colwell & Manahan 1989, Manahan et al 1989, Manahan 1989 and Manahan 1990). Also, Dawirs (1983) observed a 20% weight gain in the first day for the starved zoea larvae of the decapod crustacean, *Carcinus maenas*. He assumed this to be due to the assimilation of inorganic salts.

A further investigation was carried out to assess whether

the larval weights could be successfully calculated using the method for estimating dry weights of freshwater planktonic crustaceans, from measurements of length and geometric shape, adopted by Lawrence et al (1987). A table was drawn up (see Table 7.), to compare the weights of several cirripede species, obtained by direct weighing, with those values calculated from the formulae used in that study. During the calculations, the assumptions employed for copepod nauplii (Lawrence et al 1987) were adopted for cirripede nauplii. Thus the larvae were considered to be ellipsoidal but with appendages lying outside this shape. To compensate for the apparent volume of the appendages, the depth dimension was modified by a factor of 1·5. This made the formula :-

$$\text{Dry Weight} = 4/3\ \pi\ a.b.1{\cdot}5c$$

Where a, b, and c are half the length, width and depth, respectively.

For *S. carcini*, with the typically reduced limbs of a lecithotrophic larva, the figures do correlate, but nevertheless underestimate the weights obtained in this study. However, for *E. modestus*, typical of the larger planktotrophic larvae which have much bigger more complex limbs, it is only the stage V nauplius which shows close correlation. When considering the eggs of *Balanus balanoides*, the actual loss of weight during development portrayed by the direct weighing method, is confused

using the calculation, due to the increase in size of the egg case with the developing nauplius within.

The experiment to determine the percentage ash present in the larvae of *S. carcini* and stage I *Elminius modestus*, showed a satisfactory percentage error for the empty boats and gave similar results to those figures produced by Holland (1975 unpublished data, in Lucas 1980), when he studied the biochemical composition of *Balanus balanoides* larvae. The inorganic content for the larvae of *S. carcini* barely altered over their development with male cyprids showing the highest percentage ash. A large standard deviation was noted for female *S. carcini* cyprids. This may have been due to the difficulty experienced in collecting sufficient quantities for three replicates. Each replicate, for female cyprids, was less than 1·0 mg. in weight before ashing, whereas, those of other larval stages weighed between 1·0 mg. and 6·5 mg (see data for ash weights in appendix Table 5).

After due consideration of all the methods undertaken, the larval weights to be used in subsequent calculations of the energy budget for *S. carcini* are those obtained using the aluminium foil boat method. The dry organic weights of *S. carcini* larvae were calculated from these figures, together with their percentage ash weights (Table 6 - full data in appendix Table 5).

Table 1. The mean weights (± S.D.) of *Sacculina carcini* larvae during their development, using the plankton net method.

	Male Larvae (2 broods)		Female larvae (2 broods)	
Stage	Weight ± S.D. (µg.)		Weight ± S.D. (µg.)	
I	0·47 ± 0·15 (9)		n.d.	
II	0·84 ± 0·14 (3)	0.81 ± 0.06 (10)	0·85 ± 0·10 (9)	0·59 ± 0.06 (9)
III	0.72 ± 0·13 (8)	0·84 ± 0·03 (8)	0·82 ± 0·07 (6)	n.d.
IV	0·87 ± 0·04 (3)	0.64 ± 0·03 (9)	0·81 ± 0·05 (3)	0·54 ± 0·10 (10)
Cyp.	0·52 ± 0·19 (6)	0·65 ± 0·06 (5)	0·35 ± 0·22 (6)	0·45 ± 0·07 (9)

The figures in brackets show the number of clean replicate nets. It is from these nets that the mean larval weights and standard deviations were calculated.

The full data is in the appendix (Tables 1-4).

Table 2. The weights of ten nets (with larvae) weighed twice daily, morning and afternoon, on three separate days.

a.m. order	Day 1 (mg.)	Day 2 (mg.)	Day 3 (mg.)	p.m. order
1st	9·3977	9·3953	9·3942	
	9·4610	9·4846	9·4794	10th
2nd	8·6995	8·6926	8·6714	
	8·7324	8·7644	8·7421	9th
3rd	9·0378	9·0270	9·0067	
	9·0638	9·0913	9·0764	8th
4th	7·2753	7·2691	7·2549	
	7·2848	7·3072	7·2915	7th
5th	8·8678	8·8670	8·8464	
	8·8730	8·8954	8·8833	6th
6th	9·0842	9·0829	9·0558	
	9·0764	9·1048	9·0861	5th
7th	8·9228	8·9195	8·8923	
	8·9026	8·9264	8·9112	4th
8th	8·5959	8·6049	8·5794	
	8·5748	8.6020	8.5842	3rd
9th	8·4976	8.5059	8.4820	
	8·4595	8·4838	8·4731	2nd
10th	8·3610	8·3721	8·3462	
	8·3035	8·3218	8·3014	1st

Table 3. The weights of ten nets (without larvae) weighed twice daily, morning and afternoon, on three separate days.

a.m. order	Day 1 (mg.)	Day 2 (mg.)	Day 3 (mg.)	p.m. order
1st	6·8455 6·8699	6·8512 6·8881	6·8423 6·8855	10th
2nd	6·3805 6·3911	6·3854 6·3953	6·3704 6·4000	9th
3rd	6·7261 6·7274	6·7326 6·7378	6·7211 6·7377	8th
4th	5·9764 5·9690	5·9807 5·9814	5·9717 5·9744	7th
5th	6·2653 6·2473	6·2692 6·2645	6·2624 6·2554	6th
6th	6·4160 6·3867	6·4216 6·4038	6·4088 6·3916	5th
7th	9·4920 9·4523	9·5184 9·4811	9·4975 9·4641	4th
8th	6·7377 6·6942	6·7537 6·7167	6·7385 6·7006	3rd
9th	6·9943 6·9363	7·0084 6·9589	6·9990 6·9441	2nd
10th	7·2816 7·2016	7·2947 7·2206	7·2836 7·1989	1st

Table 4. The weights of *S. carcini* larvae during their development, using the aluminium foil boat method.

	Male Larvae	Female Larvae
Stage	weight per larva (µg.)	weight per larva (µg.)
II	0·85 (65)	0·58 (67)
III	0·79 (28)	0·50 (32)
IV	0·74 (74)	0·54 (26)
Cyp.	0·41 (49)	0.29 (27)

The figures in brackets denote the number of larvae in each boat.
The full data is in appendix Table 5.

Table 5. The weights of three aluminium foil boats at hourly intervals, on one day.

Boat 1 (mg.)	Boat 2 (mg.)	Boat 3 (mg.)
22·8509	20·9139	17·9819
22·8511	20·9141	17·9820
22·8511	20·9140	17·9821

Table 6. Percentage ash (± S.D.) present in the eggs and larvae of *Sacculina carcini*, and their dry organic weights.
The percentage ash without standard deviations are from an average of two values.

	Male Larvae		Female Larvae	
Stage	% Ash	Organic Weight (µg.)	% Ash	Organic Weight (µg.)
Egg	n.d.	n.d.	6·16	n.d
I	5·70 ± 0·09	n.d.	6·97 ± 0·18	n.d.
II	5·18 ± 0·08	0·81	5·98 ± 0·24	0·55
III	6·97 ± 0·58	0·74	7·01	0·47
IV	5·99 ± 0·20	0·70	7·70 ± 0·79	0·50
Cyp.	8·86	0·37	6·30 ± 1·92	0·27

N.B.

For total dry weights of the larvae (including ash), see Table 4.

The percentage error using three empty boats was, 0·02% ± 0·01%

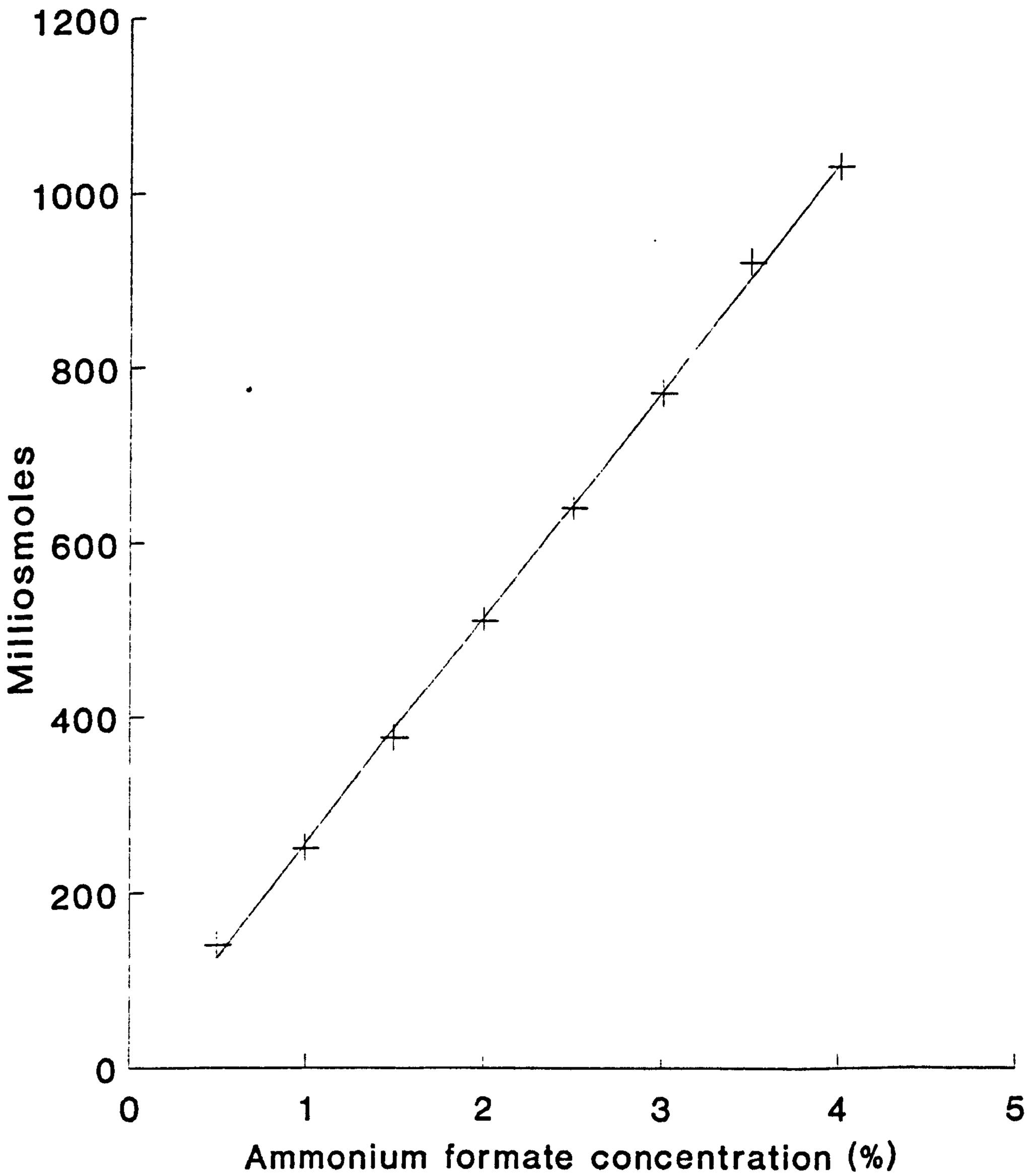
The percentage ash in Stage I *Elminius modestus* was 8·25% ± 0·05%.

n.d. = not done.

Table 7. Dry weights per individual, obtained by direct drying and weighing, compared to those weights calculated using the formula $4/3\pi\ ab1{\cdot}5c$ for two species of cirripede larvae, and $4/3\pi\ abc$ (Lawrence et al 1987), for the eggs of one of these species.

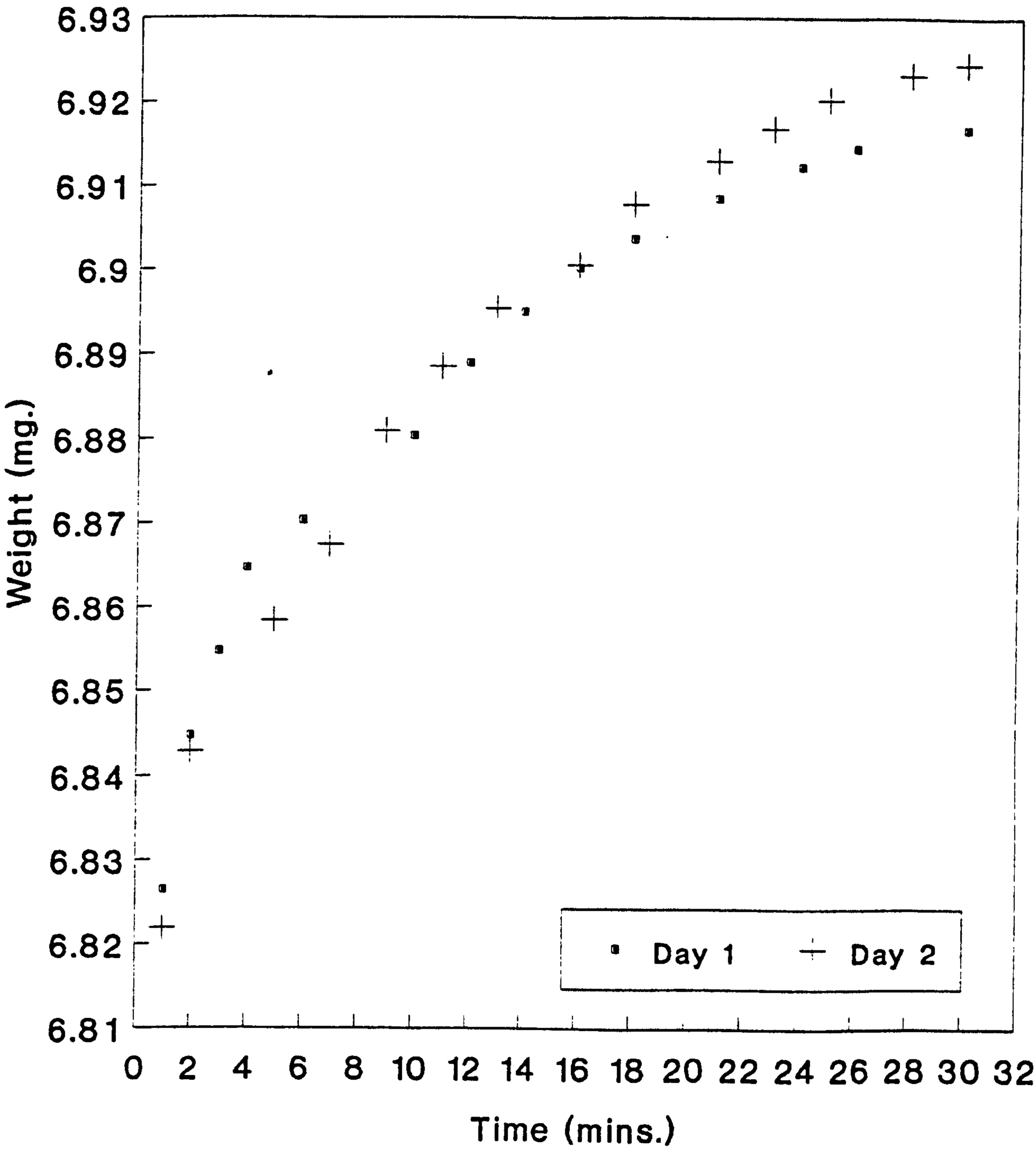
Species	Stage	Direct weight ($\mu g.\ larva^{-1}$)	Calculated weight ($\mu g.\ larva^{-1}$)	Author
S. carcini (male larvae)	II	0·85	0·47	This study (weights and dimensions)
	III	0·79	0·50	
	IV	0·74	0·53	
	Cyp.	0·41	0·33	
S. carcini (female larvae)	II	0·58	0·30	This study (weights and dimensions)
	III	0·50	0·33	
	IV	0·54	0·33	
	Cyp.	0·29	0·21	
E. modestus (larvae)	II	0·41	1·19	Harms (1987) (weights) Knight-Jones(1949) (dimensions)
	III	0·75	1·39	
	IV	1·47	2·00	
	V	2·62	2·78	
	VI	5·19	3·10	
	Cyp.	5·81	2·33	
B. balanoides (eggs)	1	1·32	0·26	Lucas & Crisp (1987) (weights and dimensions)
	2	1·30	0·25	
	3	1·29	0·26	
	4	1·33	0·26	
	5	1·25	0·28	
	6	1·21	0·29	
	7	1·20	0·29	
	8	1·15	0·29	
	9	1·17	0·32	
	10	1·10	0·34	
	11	1·04	0. 39	
	12	1·07	0·45	

Figure 1. **To show ammonium formate osmolarity at different concentrations**



The correlation coefficient r = 0.999

Figure 2. **Weights of a plankton net over 30 minutes on two separate days.**



The balance was tared between weighings.

CHAPTER V *SACCULINA CARCINI*: LARVAL RESPIROMETRY

Respiration rates for small free-swimming organisms, such as the larvae of *Sacculina carcini*, can be accurately measured under conditions approaching those of their natural environment, using a method based upon the Clarke-type oxygen electrode system (Davenport 1976). With a microrespiration cell, oxygen-uptake levels are generally found to be a good indirect measurement of the rate of energy utilisation assuming that respiration is fully aerobic (Crisp 1971). In the case of non-feeding or starved larvae, these respiration rates may be compared with biochemically determined losses (Crisp 1984). This investigation was carried out to monitor the metabolic rates of male and female *S. carcini* larvae throughout their development and under different temperature conditions.

MATERIALS AND METHODS

Two Strathkelvin R.C.200 microrespiration cells, connected to a J.J.Lloyd graphic 1002 two-pen chart recorder were used to measure larval respiration rates. The two respiration chambers and plungers were always cleaned in sequence. Firstly they were filled with a 1% Chloros (sodium hypochlorite) solution and thoroughly rinsed with deionised distilled water before calibration to 50 µl. volume, using a Precision micropipette. Any

seawater used was fine filtered and U.V.-irradiated. The chart recorder was switched on and the respirometers were calibrated for 0% and 100% oxygen concentration levels, with sodium dithionate dissolved in seawater and fresh seawater respectively. At this point, fresh seawater was pipetted into the chambers, the plungers inserted and the respirometers left to run as blanks for one hour. The chambers were then rinsed with seawater before 10 - 100 larvae were introduced into each one. The swan necks of a Volpi intralux 250HL cold light were directed at the chambers to keep the larvae active and oxygen uptake allowed to proceed for 40 minutes. This was the optimum time that a constant slope could be achieved on the chart recorder, making sure that the oxygen concentration level did not fall below 80% saturation (Belman & Childress 1973).

The larvae were carefully pipetted out of the chambers into two Bogorov trays and counted under a Wild M3Z binocular microscope, while another blank run was carried out in the respirometers. Thus the average consumption rate for each oxygen electrode was calculated each day and subtracted from that obtained for the larvae.

Initially, the respirometry was carried out at 18·0°C ± 0·1°C, for different larval stages from the same broods, measured on consecutive days. This was the temperature at which the parasitised crabs were maintained. Subsequently, the respiration rates at 18·0°C ± 0·1°C and 10·0°C ± 0·1°C were measured each day. *Sacculina carcini* nauplii remain as stage IV for two days, so it was at this point, that the respiration rates for one brood

were measured at 6·0°C, 8·0°C, 12·0°C, 14·0°C, 16·0°C, 20·0°C, 22·0°C and 24·0°C (all ± 0·1°C), with no acclimation.

RESULTS & DISCUSSION

Respiration rates measured in $\mu l. O_2 h.^{-1} ind.^{-1}$ at 18·0°C and 10·0°C for male and female *S. carcini* larvae, at each stage of their development, are shown in Tables 1, 2, 3, and 4. Table 5 shows the data measured at 18·0°C converted to $ml. O_2 h.^{-1} g.^{-1}$ dry weight, for each larval stage. The results from the experiments to measure the rates of oxygen consumption for male stage IV nauplii at different temperatures, are given in Table 6 and Figure 1, with the rates at 10·0°C and 18·0°C taken from previous stage IV measurements.

To minimise handling time, an approximate number of larvae was pipetted into the respiration chambers and accurate counts taken after each experimental run was completed. No stirrer was necessary as the larvae were active. The number of larvae within each chamber was low, to prevent any contact interaction in the 50 µl. volume and to facilitate counting afterwards. Depending on the time available, two or three blank runs were carried out each day and the mean oxygen consumption was calculated. A consistent rate of between 0·01 and 0·02 $\mu l. O_2 h.^{-1}$ was observed for the electrodes. However, due to the small numbers of larvae and their relatively low metabolic rates, the electrode consumption often constituted up to 50% of the

total oxygen consumed. Nevertheless, it was evident that the respiration rates obtained were clearly repeatable, as seen in Tables 1 ⇒ 5, thus endorsing the accuracy of the method.

Observations of *S. carcini* cyprids showed that when they were photopositive, they were very active, swimming regularly in short bursts. However, at another time, often on the same day, this activity level would drop and they were no longer photopositive. From the oxygen consumption rates obtained, it was clear that when the mean rates were calculated for male and female cyprids at 18·0°C ± 1·0°C, the standard deviations from the mean were high (i.e. 0·76 ± 0·38 $\mu l.O_2h.^{-1}ind.^{-1}$ and 0·64 ± 0·32 $\mu l.O_2h.^{-1}ind.^{-1}$, respectively.). It was considered that the two observed levels of activity were reflected in these results. The data was then separated into two sets, with those rates greater than and less than 0·75 $\mu l.O_2h.^{-1}ind.^{-1}$. The means and standard deviations of these two data sets were calculated. An analysis of variance carried out on these two sets of data for male cyprid respiration at 18·0°C, showed the rates to be significantly different at the 5% level ($F = 5·63$; $P = 0·05$). Therefore it is acceptable to separate the data in this way (see Table 5).

The largest standard deviation from the mean, for a particular larval stage, was found at stage II, for both males and females. When a brood is released, it comprises a mixture of eggs, stage I and stage II nauplii. Eggs hatch to stage I's which in turn, rapidly moult to stage II's within a few minutes. Hagerman (1976), when studying the brackish water shrimp, *Crangon*

vulgaris, noted an increase in oxygen uptake just before and just after a moult. He proposed that more energy was required to regulate the physiological processes within the animal at this stage. The rate recorded for stage I *S. carcini* nauplii, was the highest rate per individual at 1·17 $\mu l.O_2 h.^{-1} ind.^{-1}$ (see Table 5) and those recorded for stage II varied considerably. At this time, the larvae are very active and photopositive, presumably increasing their chances of dispersal in surface waters.

In the normal temperature range of a poikilotherm, respiration increases with increasing temperature (Barnes, Calow & Olive 1989). As with most chemical reactions responding to temperature, this is not a linear relationship. Taking this into account, the Q_{10} index is widely used to indicate the effect of temperature on metabolism. The respiration results at 18·0°C and 10·0°C gave Q_{10} values ranging from 1·65 ⇒ 2·60 (see Table 8) over the full larval development of *Sacculina carcini*. Barnes and Barnes (1958) reported a Q_{10} of 2·2 for the duration of stage I *Balanus balanoides* and Tighe-Ford et al (1970) found a temperature coefficient of 2 for total larval development of *Elminius modestus*. The Q_{10} values calculated for male stage IV *S. carcini* nauplii (shown in Table 7) vary from 1·21 ⇒ 2·86, demonstrating a relatively temperature-independent range, often found when animals are readily adapted to that particular temperature range (Wieser 1973). There were two notable exceptions however (Table 7). Between 6·0°C and 8·0°C a Q_{10} of 5·06 was calculated. From an earlier experiment (Chapter II, this study) it was observed that below 7·0°C male nauplii were unable

to complete larval development and were not able to progress beyond stage II, whereas between 7·0°C and 8·0°C the nauplii were capable of metamorphosis to the cyprid within 16 days. The fact that *S. carcini* nauplii are not well adapted to low temperatures is reflected in this high Q_{10} value and was demonstrated by those observations (this study), when larvae were incapable of moulting successfully, possibly due to the inability of certain enzymes to work at these temperatures. A similar situation occurs with *Elminius modestus* (Harms 1987). Between 12·0°C ⇒ 18·0°C the Q_{10}'s for stages II and IV were 4·50 and 4·95 respectively, whereas between 18·0°C ⇒ 24·0°C the same stages showed Q_{10} values of 1·41 and 1·71. Harms (1987) commented that this may reflect the original subtropical habitat of the species (Luckens 1976, Foster 1978). Another high Q_{10} value was noted for stage IV *S. carcini* nauplii between 12·0°C and 14·0°C. The respiration rates measured at 12·0°C were particularly low and the mean figure does appear to deviate from the general trend (see Figure 1). This may be a spurious result.

The relationship between metabolism and body size for the animal kingdom is based on the fact that less oxygen is used on a weight specific basis as body size increases (Hemmingsen 1960). This is not the case for *Balanus eburneus* larvae (see Table 9) where the weight specific oxygen uptake increases by 60% from stage I to stage VI nauplius (Jorgensen & Vernberg 1982). Anderson (1975), when studying the larval stages of the parasitic isopod *Probopyrus pandalicola*, noted a similar situation. He proposed that the metabolic rate correlated more closely to the

"mode of existence". The low values for the free-living stage and higher values for the parasitic stage reflected the latter's need to synthesise relatively large amounts of protein required during development. Other workers have studied the oxygen consumption of marine invertebrate planktotrophic larvae including *Ostrea edulis* veliger 1, *Mytilus edulis* veliger 2, *Littorina littorea* veliger 3, with resulting values of 1·60-4·80, 2·26-2·77 and 1·95-4·63 ml.O_2h.$^{-1}$g.$^{-1}$dry weight, respectively (see Zeuthen 1947 and Walne 1966). These rates are close to those of the cirripede larvae listed on Table 9, except for *Balanus eburneus* nauplii. Lucas & Crisp (1987) measured the weight specific oxygen consumption rate for *Balanus balanoides* eggs during embryogenesis. Although a decrease in dry weight was observed during developoment, an increase in egg size was noted and weight specific oxygen consumption rates increased from 0·05 to 0·45 ml.O_2h.$^{-1}$g.$^{-1}$dry weight, measured at 10°C. Shilling & Manahan (pers. comm.) measured the metabolic rate of one-day old embryos of the echinoderm *Strongylocentrotus purpuratus* at 17°C. From two spawnings, maintained in natural seawater, weight specific respiration rates were recalculated as 2·43 and 2·57 ml.O_2h.$^{-1}$g.$^{-1}$. The higher metabolic rates of these echinoderm embryos may be due to their active uptake of dissolved organic material from the seawater, as proposed by Shilling & Manahan (pers. comm.). Although there appears to be a paucity of data on the respirationm rates of lecithotrophic larvae, Jaeckle & Manahan (1989b), when studying the energy budget for the non-feeding larvae of the gastropod mollusc, *Haliotis rufescens*, at

17°C, measured the respiration rate. Their data gave an equivalent larval respiration rate of 1·26 ml.O_2h.$^{-1}$g.$^{-1}$dry weight, which is in accord with the rates reported for *Sacculina carcini* larvae (this study). The modified cyphonautes larva of the bryozoan *Bugula neritina* however, which was reported by Crisp (1976, from unpublished data by Crisp & Vernberg) to have a respiration rate of 10·0 ml.O_2h.$^{-1}$g.$^{-1}$dry weight, does appear to have a very high respiratory rate for a lecithotrophic larva (see Table 10). This larva is short-lived and can settle within 12 hours from release.

Oxygen consumption is dependent on many different physiological processes, including basal metabolism and swimming activity for lecithotrophic larvae. For planktotrophic larvae this includes contributions for feeding and growth (Clarke & Morris 1983). Basal metabolism comprises protein catabolism, membrane lipid turnover, ion pump activity and nervous activity (Clarke 1983). These processes serve to keep the animal alive. When considering the respiration rates measured for the larvae of several cirripede species (Table 9), it is evident that *Balanus balanoides* cyprids, at the exploring stage, exhibit an exceptionally low respiratory rate of 0·63 ml.O_2h.$^{-1}$g.$^{-1}$dry weight (Lucas 1980). The weight specific oxygen consumption rates for cyprids from all the species listed in Table 9 are lower than those for their corresponding earlier naupliar stages and was admirably demonstrated for *Balanus eburneus* with an eight-fold drop in the rate from the stage VI nauplius to the cyprid (Jorgensen & Vernberg 1982). These weight specific oxygen

consumption rates for the non-feeding cypris stage, correlate closely with the figures observed throughout the lecithotrophic larval development of *S. carcini* (Tables 9 & 10). The values in these tables will be used, in conjunction with the biochemical constituents determined, to calculate an energy budget for the larvae of *Sacculina carcini* (see Chapter VI).

Table 1. The respiration rates of male *Sacculina carcini* larvae at each stage, measured in $\mu l.O_2\ h.^{-1} ind.^{-1} \times 10^{-3}$, at 18·0°C ± 0·1°C. CH = chamber of respirometer; the small figures denote the number of larvae in the chamber; I, II, III, IV = nauplius stage; Cyp = cypris stage; different larval batches are separated by a line.

CH	Day 1 I	Day 1 II	Day 2 III	Day 3 IV	Day 4 IV	Day 5 Cyp.	Day 6 Cyp.	Day 7 Cyp.	Day 12 Cyp.	Day 15 Cyp.	Day16 Cyp.
①	1·16 57	1·04 25	0·61 38	0·89 39	0·82 36	0·52 97	0. 39 23		0. 48 12		
②	1·17 55	1·04 22	0·90 34	1·00 40	1·12 57	0·90 41	1·12 57		0·42 12		
①		0·89 36									
②		1·19 40									
①			0. 82 20		1·07 30	0·43 72	0·37 65	0·48 29			
②			0·89 25		0·96 33	1·12 23	1·10 38	1·22 35			
①			0·86 59		1·05 28	0·99 52	0·33 44	0·39 20			
②			0·95 52		0·93 41	1·08 42	0·98 36	1·26 17			
①		0·63 34	0·86 14	0·93 23	1·04 48						
②		0·87 24	0·87 20	0·82 37	0·96 46						
①		0·65 42				1·12 10					
②		0·66 45				1·38 13					
①		0·49 35				1·14 14					
②		0·62 50				1·30 17					
①				0·82 25							
②				0·83 34							
①										0·34 27	0·38 30
②										0·34 30	0·43 20
①					0·86 31		0·47 19				

Table 2. The respiration rates of female *Sacculina carcini* larvae at each stage, measured in $\mu l.O_2\ h.^{-1} ind.^{-1} \times 10^{-3}$, at 18·0°C ± 0·1°C. CH = chamber of respirometer; the small figures denote the number of larvae in the chamber; I, II, III, IV = nauplius stage; Cyp = cypris stage; different larval batches are separated by a line.

CH	Day 1 II	Day 2 III	Day 3 IV	Day 4 IV	Day 5 Cyp.	Day 6 Cyp.	Day 7 Cyp.	Day 8 Cyp.	Day 12 Cyp.	Day 13 Cyp.	Day 14 Cyp
①	0·45 31	0·62 27	0·63 38	0·81 39	0·48 28						
②	0·50 31	0·65 25	0·80 34	0·82 42	0·44 15						
①		0·88 46	0·65 25	0·55 41	0·55 31						
②	0·11* 26	0·83 30	0·65 33	0·64 35	0·45 27						
①	1·34 27	0·78 30	0. 83 40	0·86 19	1·40 13			0·43 39		0·43 23	0·51 10
②	1·00 35	0·83 33	0·90 46	0·76 35	1·21 17			0·63 24		0·46 10	0·57 15
①	0·67 66				1·10 18						
②	0·69 39				0·45 12						
①	0·89 46				0·29 10						
②	0·89 52				0·48 43						
①	1·18 23									0·35 53	
②	0·90 60									1·03 13	
①	0·80 36										
②	0·94 65									0·38 82	
①				0·76 40	1·00 21						
②				0·74 32	0·89 20						

* The electrolyte was changed in the respiration chamber after this reading, which was not included in the calculations.

Table 3. The respiration rates of male *Sacculina carcini* larvae at each stage, measured in $\mu l. O_2\ h.^{-1} ind.^{-1} \times 10^{-3}$, at 10·0°C ± 0·1°C. CH = chamber of respirometer; the small figures denote the number of larvae in the chamber; I, II, III, IV = nauplius stage; Cyp = cypris stage; different larval batches are separated by a line.

CH	Day 1 II	Day 2 III	Day 3 IV	Day 4 IV	Day 5 Cyp.	Day 6 Cyp.	Day 7 Cyp.	Day 12 Cyp.	Day 15 Cyp.
①	0·56 (39)	0·45 (32)	0·39 (58)	0·42 (17)	0·22 (46)	0·22 (51)			
②	0·56 (49)	0·51 (38)	0·49 (40)	0·59 (38)	0·58 (48)	0·51 (34)		0·58 (33)	
④	0·51 (20)								
⑧	0·53 (40)								
①		0·41 (40)	0·51 (30)	0·50 (20)	0·43 (43)	0·49 (12)			0·23 (29)
②		0·47 (44)	0·56 (31)	0·50 (45)	0·45 (16)	0·60 (7)			0·48 (14)

Table 4. The respiration rates of female *Sacculina carcini* larvae at each stage, measured in $\mu l. O_2\ h.^{-1} ind.^{-1} \times 10^{-3}$, at 10·0°C ± 0·1°C. CH = chamber of respirometer; the small figures denote the number of larvae in the chamber; I, II, III, IV = nauplius stage; Cyp = cypris stage; different larval batches are separated by a line.

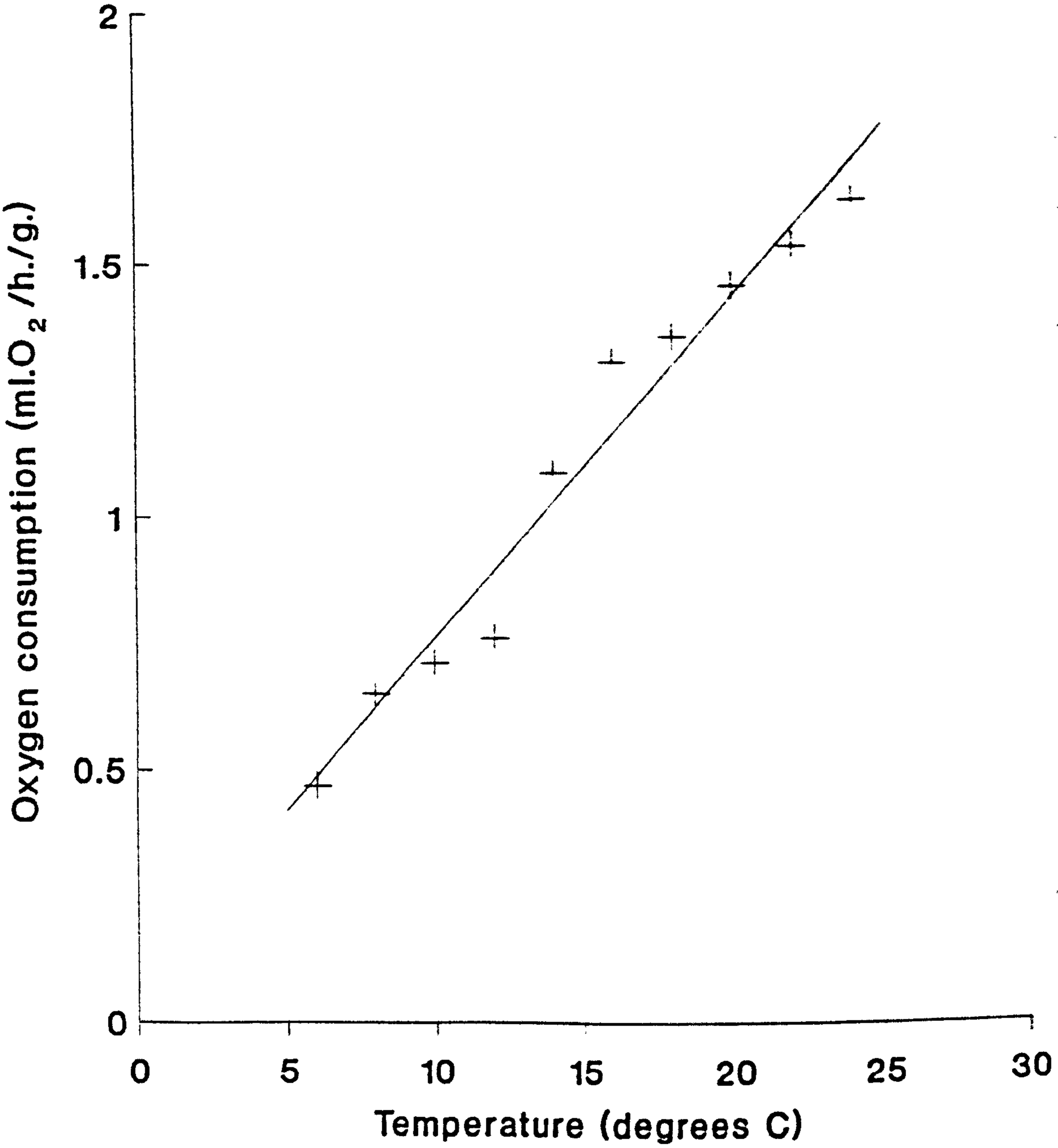
CH	Day 1 II	Day 2 III	Day 3 IV	Day 4 IV	Day 5 Cyp.	Day 6 Cyp.	Day 7 Cyp.	Day 8 Cyp.	Day 12 Cyp.	Day 13 Cyp.	Day 14 Cyp.
①	0·41 (33)	0·39 (35)	0·35 (28)	0·31 (30)	0·47 (27)			0·28 (22)			0·26 (15)
②	0·57 (33)	0·39 (36)	0·46 (22)	0·25 (35)	0·38 (28)			0·28 (27)			0·31 (20)
①										0·48 (12)	
										0·48 (20)	

Table 5. The mean (± S.D.) respiration rates of male and female *S. carcini* larvae at each stage, taken from all data (see Tables 1 & 2), measured in $\mu l.O_2h.^{-1}ind.^{-1}$ at 18·0°C ± 0·1°C and converted to $ml.O_2h.^{-1}g.^{-1}$ dry weight.

Stage.	Mean individual organic wt. (µg.)	Average rate using all data. ($\mu l.O_2h.^{-1}ind.^{-1}$) x$10^{-3}$	Respiration rate. ($ml.O_2h.^{-1}g.^{-1}$ dry weight)
Male larvae			
I	n.d.	1·17 ± 0·01	-
II	0·81	0·81 ± 0·23	1·00
III	0·74	0·85 ± 0·10	1·15
IV	0·70	0·95 ± 0·10	1·36
Cyp.	0·37	0·41 ± 0·06 1·13 ± 0·16 [0·76 ± 0·38*	1·11 3·05 2·05*]
Female larvae			
II	0·54	0·85 ± 0·26	1·57
III	0·47	0·77 ± 0·11	1·64
IV	0·50	0·74 ± 0·10	1·48
Cyp.	0·27	0·45 ± 0·10 1·11 ± 0·18 [0·64 ± 0·32*	1·67 4·11 2·37*]

* = Cyprid respiration rates, using all the data (i.e. without separation into those rates greater than, or less than 0·75 $\mu l.O_2h.^{-1}ind.^{-1}$)

Figure 1. **Oxygen consumption of Stage IV *S.carcini*, at different temperatures.**



Correlation coefficient r = 0.982

Table 6. The mean rates of oxygen consumption by male Stage IV *S. carcini* nauplii, at different temperatures. [The mean is from two results, except for 10°C and 18°C when many results were taken and therefore ± S.D. is given for these values.]

Temp.	Rates of oxygen consumption.	
(°C) (± 0·1)	($\mu l. O_2 h.^{-1} ind.^{-1}$) $x10^{-3}$	($ml. O_2 h.^{-1} g.^{-1}$ dry weight)
6·0	0·33	0·47
8·0	0·46	0·65
10·0	0·50 ± 0·07	0·71
12·0	0·53	0·76
14·0	0·76	1·09
16·0	0·92	1·31
18·0	0·94 ± 0·10	1·36
20·0	1·02	1·46
22·0	1·08	1·54
24·0	1·14	1·63

N.B. The mean organic weight of a Stage IV male nauplius was 0·70 µg. [see Table 5.].

The rates used were an average of all the data collected for male Stage IV *Sacculina carcini* nauplii.

Table 7. Q_{10} values, calculated for male stage IV *Sacculina carcini* larvae, using the mean respiration rates for all the data measured between 6·0°C and 24·0°C inclusive.

Temperature range. (°C)	Q_{10}
6·0 - 8·0	5·06
8·0 - 10·0	1·56
10·0 - 12·0	1·41
12·0 - 14·0	6·07
14·0 - 16·0	2·51
16·0 - 18·0	1·21
18·0 - 20·0	1·43
20·0 - 22·0	1·31
22·0 - 24·0	1·33
6·0 - 8·0	5·06
6·0 - 10·0	2·81
6·0 - 12·0	2·23
6·0 - 14·0	2·86
6·0 - 16·0	2·79
6·0 - 18·0	2·42
6·0 - 20·0	2·25
6·0 - 22·0	2·10
6·0 - 24·0	2·00

Table 8. Q_{10} values, calculated for male and female *Sacculina carcini* larvae, using the mean respiration rates from all the data measured at 18·0°C ± 1·0°C and 10·0°C ± 1·0°C.

Stage	Mean respiration rates (ml. O_2 h. $^{-1}$ g. $^{-1}$ dry weight)		Q_{10}
	18·0°C ± 1·0°C	10·0°C ± 1·0°C	
Male larvae			
II	1·00	0·67	1·65
III	1·15	0·62	2·17
IV	1·36	0·71	2·25
Cyp.	2·05	1·19	1·97
Female larvae			
II	1·57	0·91	1·99
III	1·64	0·83	2·34
IV	1·48	0·69	2·60
Cyp.	2·37	1·36	2·00

Key: Cyp. = cyprid

The equation used to calculate Q_{10} values was:-

$$\ln Q_{10} = \frac{10(\ln R_2 - \ln R_1)}{(T_2 - T_1)}$$

where R_1 & R_2 are the respiration rates measured at temperatures T_1 & T_2.

Table 9. The respiration rates of several cirripede species. The temperatures at which these rates were measured, by different authors are also included.

Species	Temp. (°C)	Stage	Dry wt. larva^{-1} (µg.)	O_2 consumption (µl. O_2h.$^{-1}$ind.$^{-1}$) x10^{-3}	O_2 consumption (ml. O_2h.$^{-1}$g.$^{-1}$ dry weight)	Reference
B. bal.	10·0	I	0·63	3·31	5·26	Lucas (1980)
		II	1·24	3·96	3·19	
		III	2·16	3·56	1·65	
		IV	5·12	9·83	1·92	
		V	8·41	19·81	2·35	
		VI	14·81	32·73	2·21	
		Cyp	33·22	36·57(s)	1·10	
				21·01(e)	0·63	
B. bal.	10·0	I	n. d.	3·90	-	Davenport (1976)
		VI	n. d.	32·20	-	
B. ebu.	25·0	I	0·27	4·88	18·08	Jorgensen & Vernberg (1981)
		IV	0·68	16·52	24·29	
		VI	1·50	50·17	33·45	
		Cyp.	2·18	9·38	4·30	
E. mod.	16·0	II	0·3	1·47 to 2·17	4·92 to 7·24	Bhatnagar & Crisp (1965)
E. mod.	12·0	II	0·39	0·72 & 0·95	1·85 & 2·43	Harms (1987)
		III	0·71	1·67	2·35	
		IV	1·20	2·94	2·45	
		V	2·33	5·34	2·29	
		VI	4·27	7·28	1·70	
		Cyp.	4·56	6·80	1·49	
	18·0	II	0·41	1·32 & 2·32	3·22 & 5·67	
		III	0·75	3·62	4·83	
		IV	1·47	7·69	5·23	
		V	2·62	9·95	3·80	
		VI	5·19	12·10	2·33	
		Cyp.	5·81	10·01	1·73	
	24·0	II	0·39	2·27 & 2·86	5·83 & 7·33	
		III	0·70	5·39	7·69	
		IV	1·06	10·59	10·01	
		V	2·45	16·35	6·68	
		VI	4·39	18·33	4·18	
		Cyp.	4·38	13·89	3·18	

Key: *B. bal.* - *Balanus balanoides*; *B. ebu.* - *Balanus eburneus*; *E. mod.* - *Elminius modestus*; s- swimming cyprid; e- exploring cyprid; The first rates shown (Harms 1987) for Stage II nauplii, are for newly hatched larvae.

Table 10. Comparative table of the respiration rates of several lecithotrophic larvae from different phyla.

Species & Stage.	Temp. (°C)	Dry wt. larva^{-1} (µg.)	O_2 consumption. (µl. O_2h.$^{-1}$ind.$^{-1}$) x10^{-3}	O_2 consumption. (ml. O_2h.$^{-1}$g.$^{-1}$) x10^{-3}	Reference.
S. c. (male)	18·0				This study
I		n. d.	1·17	-	
II		0·81	0·81	1·00	
III		0·74	0·85	1·15	
IV		0·70	0·95	1·36	
Cyp.		0·37	0·41 (l. a.)	1·11	
			1·13 (h. a.)	3·05	
(female)					
II		0·54	0·85	1·57	
III		0·47	0·77	1·64	
IV		0·50	0·74	1·48	
Cyp.		0·27	0·41 (l. a.)	1·67	
			1·11 (h. a.)	4·11	
B. n. Cyph.	28·0	1·5	15·00	10·00	Crisp (1976) (Lucas 1980)
H. r. Vel.	17·0	1·5	1·88	1·26	Jaeckle & Manahan (1989)

Key: *S. c.* = *Sacculina carcini* (cirripede); *B. n.* = *Bugula neritina* (bryozoan); *H. r.* = *Haliotis rufescens* (gastropod); I, I, III, IV = nauplius stage; Cyp. = cypris stage; Cyph. = cyphonautes; Vel. = veliger; (h. a.) = high activity; (l. a.) = low activity.

CHAPTER VI : BIOCHEMICAL COMPOSITION AND ENERGY BUDGET OF *SACCULINA CARCINI*

The biochemical constituents contained within the body of a lecithotrophic larva, when it has hatched, must contain all the essential components for development through to the primary feeding stage of the animal's life. Crisp (1976) observed that lecithotrophs rarely survive more than 12 hours, with the urge to settle generally becoming irresistable after approximately 6 hours, and many settling between one and three hours of release. These observations were based on the pelagic phase of the bryozoan, *Bugula neritina*, which has been noted to have a relatively high metabolic rate for a non-feeding larva (see Chapter V this study).

However, for most larvae whose pelagic phase is planktotrophic, there are two critical periods when they are unable to obtain nutrients from the external environment and have to rely on their internal resources; i.e. throughout embryonic development and at metamorphosis (Lucas et al 1979 and Lucas & Crisp 1987). Manahan & Crisp (1983) were able to show that some dissolved organic compounds are taken up by the fertilised eggs and larvae of the bivalve *Crassostrea gigas*. Nevertheless it is believed that this capacity is limited to living surfaces bearing microvilli and has not been demonstrated in crustaceans, which are surrounded by cuticle (Lucas 1980). Shilling & Manahan (pers. comm.) carried out a study on the energetics of early development

for the sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus* and the branchiopod crustacean *Artemia salina*. Unlike the two echinoderms, *A. salina* was able to account for all its metabolic requirements from the use of endogenous reserves. Jaeckle & Manahan (1989b) also observed an energy imbalance during the development of the lecithotrophic molluscan larva, *Haliotis rufescens*, and deduced that, as long as the external nutrient pool was in a dissolved form this so called non-feeding larva can take in organics and supplement existing stores. This is unlikely to be the case for nauplius larvae of *Sacculina carcini* which are encased in a cuticle and develop through to the cypris stage within five days at 18°C. The cyprids require another two days before becoming competent to settle and subesquently metamorphose to the trichogon or kentrogon stage (see Høeg 1991). Consequently, those nutrients which are required for development, swimming activity, settlement and subsequent successful metamorphosis must be contained within the fertilised egg. This investigation was carried out to monitor the utilisation of energy reserves throughout the free larval development of *S. carcini*.

MATERIALS AND METHODS

Broods of *S. carcini* larvae were collected and maintained at 18°C (see Chapter III). Male and female larvae were sampled at each stage of their development and prepared for biochemical

analysis. The larvae were attracted to a point light source, pipetted onto 45 µm. mesh plankton netting sieves and rinsed 6 times with 3·9% aqueous ammonium formate (see Chapter IV) and twice with distilled deionised water. They were then washed with distilled deionised water, into glass vials, which were frozen at -196°C in liquid nitrogen. There was some doubt about the efficiency of the freeze-drier at this point, so the extra washings with distilled deionised water were a further precaution to avoid contamination with ammonium formate residue. After freeze-drying for 24 hours, the larvae were kept in a desiccator over $CaCl_2$. Before analysis, the freeze-dried larvae were broken up with a clean spatula, to increase their surface area. They were then stored in the desiccator overnight. Fractions from the same sample were taken for protein, lipid and carbohydrate analysis using colorimetric techniques. A gravimetric technique (see Folch et al 1957) was also used to assess the total lipid content of male and female stage II *S. carcini* nauplii. After extraction (Folch et al 1957), the fatty acids were methylated using a 14% boron fluoride-methanol complex (Morrison & Smith 1964) and analysed on a Carlo Erba Vega 6180 capillary gas chromatograph, fitted with an Alltech carbowax 20M capillary column. A flame ionisation detector and a Hewlett Packard 3390A integrator were used to measure the peaks and calculate the underlying areas, which are proportional to the amounts of respective fraction. These lipid profiles for male and female nauplii were compared.

The gravimetric lipid extraction (Folch et al 1957) was

used on further samples of male and female stage II *S. carcini* nauplii. Thin layer chromatography, of the resulting neutral lipid and phospholipid extracts, was carried out on pre-coated silica gel plates in a continuous solvent system. The phospholipid solvent was chloroform ; methanol ; acetic acid ; water, in the ratio of 65:50:1:4, stained with iodine vapour and run in a closed tank for 90 minutes. The neutral lipid solvent was petroleum spirit (boiling range 40-60°C) ; diethyl ether ; glacial acetic acid, in the ratio of 85:15:1, left for 90 minutes at room temperature (20-25°C) then stained with iodine vapour and run in a closed tank for one hour. The R.f. values of the separated fractions were calculated.

Colorimetric analyses

An Eppendorf Varipette 4710 was used to dispense volumes in the range of 100 µl. ⇒ 1000 µl. and a Precision micropipette was used for 50 µl. volumes. Between 1·0 mg. and 11·0 mg. of dried, powdered larvae, at each stage of their development, were accurately weighed in aluminium foil boats on a Cahn C31 microbalance. Each sample was homogenised with 500 µl. of distilled deionised water in a 1·0 ml. ground glass homogeniser, set in ice. The homogenate was then poured into a glass vial and 50 µl. were pipetted into a 1·5 ml. Eppendorf polyethylene reaction tube, into which 150 µl. of distilled deionised water were added and the solution vortexed for 5 seconds. The remaining 450 µl. of homogenate in the vial were covered and retained for lipid analysis, later the same day. Protein precipitation was then carried out on the solution in the Eppendorf tube, following

Figure 1. FLOW DIAGRAM

Preparation of larvae for colorometric analyses.

Homogenate in 500 µl. of water
(1·0 ⇒ 11·0 mg. dried larvae)

3 x 100 µl.

Lipid Analysis

Boehringer Mannheim GmbH
Diagnostica, Test-Combination
for total lipids, with the
standard provided.

50 µl.

Add 150 µl. water & vortex.
Add 100 µl. cold 15% T.C.A. &
shake for 5 mins. Stand at 4°C
overnight. Centrifuge at 9980g.
for 10 mins. Collect
supernatant & wash precipitate
with 200 µl. cold 5% T.C.A.
Centrifuge at 9980g. for 10 mins.
Combine supernatant & washings.

Supernatant
500 µl.

Carbohydrate Analysis

Initial conversion of
glycogen to glucose with
amyloglucosidase (see text).

Boehringer Mannheim GmbH
Diagnostica, Test-Combination
for glucose.

Precipitate

Add 500 µl. 1·0N NaOH & heat
for 90 mins. at 56°C.
Add 500 µl. 1·0N HCl to
neutralise the solution.

Protein Analysis

Bio-Rad Microassay with
B.S.A. as standard.

the scheme adopted by Holland and Gabbott (1971). 100 µl. of 15% trichloracetic acid (T.C.A.) were added to the reaction tube, which was shaken for 5 minutes and left overnight at 4°C (see flow diagram, Figure 1).

The lipid analysis was carried out using a modification of the Boehringer Mannheim GmbH Diagnostica, Test-Combination for total lipids. Three, 100 µl. replicates of homogenate were dissolved in 2·0 ml. of concentrated H_2SO_4 instead of the 50 µl. replicate suggested. Consequently 50 µl. of conc. H_2SO_4 were also added to the 50 µl. of standard, which was provided in the analysis kit, to compensate for this volume change. The reason for doubling the test volumes was to increase the lipid content and therefore improve the accuracy of the readings, taken at a wavelength of 530 nm., on the Cecil instruments C.E.303 Grating Spectrophotometer. The absorbances were read and the percentage lipid in each sample was calculated and compared with the standard.

The following day, the samples for protein analysis were centrifuged for 10 minutes at 9980 g. in an Eppendorf 5412 centrifuge. The supernatant from each tube was pipetted out and retained. At this point 200 µl. of 5% T.C.A. were added to the precipitate and the tubes centrifuged for another 10 minutes at 9980 g. The supernatant was pipetted into that already collected and maintained at -20°C, for carbohydrate analysis at a later date. The precipitated protein was then dissolved in 500 µl. of 1·0 N NaOH and heated for 90 minutes at 56°C, using an Eppendorf Thermostat 3401 heating block, to completely solubilise the

protein. The solution was then neutralised with 500 µl. of 1·0 N HCl, before protein analysis was carried out following the Bio-Rad Microassay procedure using Bovine Serum Albumin standard. The same spectrophotometer was used to read the absorbances at a wavelength of 595 nm. and the percentage protein was calculated from the standard curve.

Carbohydrate content was estimated following the method of glycogen determination described by Keppler and Decker (1974) and modified by Peek (1987). The 500 µl. sample of deproteinised supernatant was added to 500 µl. of amyloglucosidase enzyme (1mg. ml^{-1} in 0·2 M sodium acetate, pH 4·8), to convert the free sugars to glucose. The solution was heated in an Eppendorf tube for 2 hours at 37°C using the heating block, and was mixed every 15 minutes. Analar D+ glucose was made up into six solutions of known concentration. Following the Boehringer Mannheim GmbH Diagnostica Test-Combination for glucose, absorbances were read for these six solutions, the kit standard and for the sample, on the same spectrophotometer at a wavelength of 610nm. A standard curve was drawn, using the absorbances for the six solutions of known concentration, from which the glucose concentration of the kit standard was read. The expected glucose concentration of the latter was 18·2 $\mu g.ml.^{-1}$ and the reading from the standard curve was 18·0 $\mu g.ml.^{-1}$. Unfortunately, absorbances for the sample were so low that they were not readable on the scale. Although a low carbohydrate content was expected, it was assumed that there had been insufficient enzyme to convert all the free sugars present in the 500 µl. sample, to glucose during the enzyme reaction. A

second experiment was carried out using a smaller aliquot of the deproteinised supernatant (i.e. 100 µl. of supernatant with 500 µl. of enzyme). Absorbances for the sample were once again, barely detectable. Consequently an experiment was carried out to check that there had been complete conversion of glycogen to glucose. Fresh enzyme, amyloglucosidase (1 mg. ml^{-1} in 0·2 M sodium acetate buffer, ph 4·5) was prepared. Samples of male and female stage II *S. carcini* larvae were homogenised with distilled deionised water, at concentrations of 10·872 mg. $ml.^{-1}$ and 10·808 mg. $ml.^{-1}$, respectively. A sample of glycogen from *Mytilus edulis* Type VII (Sigma), was also prepared in a 0·1 M sodium acetate buffer (pH 4·5) at a concentration of 1·106 mg. $ml.^{-1}$. To each 200 µl. aliquot of sample, 100 µl. of enzyme were added. Once again samples of the larvae gave zero readings on the spectrophotometer.

RESULTS

The results from the gravimetric analysis for the extraction of total lipid and the separation into neutral lipid and phospholipid are given on Table 1, with tracings from the thin-layer chromatography (T.L.C.) plates for neutral lipid and phospholipid on Figure 2. Table 2 gives the R.f values calculated for the T.L.C. standards and experimental samples. The fatty acid composition of the triacylglycerols of male and female stage II *S. carcini* nauplii are in Table 3.

Results from the colorimetric analyses are given in Table 4 and Figures 1 & 2, with the exception of carbohydrate. For the single carbohydrate analysis that produced a detectable reading, the percentage present in a stage II nauplius was calculated as 0·07% of the ash free dry weight. It was noted however, that glycogen from the *Mytilus edulis* sample was successfully converted to glucose (the expected conversion factor was 0·96 - Peek 1987) when the observed conversion factor was 0·97. Consequently carbohydrate figures are not included in Table 4, but it was considered that any estimation of carbohydrate, present in *S. carcini* larvae could be calculated from the data obtained by Holland (1978, from unpublished data - Holland 1976), which gave the carbohydrate content as 3·3% of the total dry weight for the '*S. carcini* nauplius'. For *Balanus balanoides* cyprids, Holland & Walker (1975) measured the carbohydrate content as 3·5% of the cypris total dry weight. This level was recorded as 6·1% of the ash free dry weight, by Lucas et al (1979) but barely altered during the 8 week period that the cyprids were studied.

The loss of biochemical constituents observed between larval stages was converted into a predicted respiration rate for that larval stage, to determine whether lipid or protein was the main energy source for metabolism. The calorific equivalents used were those given by Crisp (1971).

Calculation e.g.

If X µg. = lipid loss over 24 hours per larva.

$$\frac{X \times 9{\cdot}45}{1000} = Y \times 10^{-3} \text{ cals.}$$

$$\frac{Y \times 10^{-3}}{4{\cdot}8} = Z \times 10^{-5} \text{ ml.} O_2 \quad \text{i.e.} \quad Z \times 10^{-2} \text{ µl.} O_2$$

$$\frac{Z \times 10^{-2}}{24} = A \times 10^{-3} \text{ µl.} O_2 h.^{-1} ind.^{-1}$$

A back calculation was also carried out, using the measured respiration rates, to predict the amount of biochemical component lost over that period, due to metabolism. The results of these calculations are given in Table 5, together with the estimates of biochemical losses due to excretion and the loss of exuviae. Both these factors were estimated relative to larval size. Figures for excretion rates, as total nitrogen, were taken from Lucas (1980) and converted to µg. of protein for the larval weight and duration of each stage of *Sacculina carcini* larvae. As the exuviae of *S. carcini* larvae were not weighed, an estimation was made, based on data from Harms' (1987) study on *Elminius modestus*. The exuviae (Harms 1987) accounted for 5·5% - 8·7% of the total dry larval weight and although the total dry weight of the nauplius only increased 1·07 fold to that of the cyprid, the

exuvia weight increased 1·30 fold. Consequently, an average figure of 7·5% of the total dry larval weight of a stage II *S. carcini* nauplius was adopted for the exuvia weight of each of the naupliar stages and this figure was multiplied by 1·30 for the cypris exuvia.

The lipid and protein content of female stage III and IV *S. carcini* nauplii appear to be the same. This cannot be the case in reality as the stage III nauplius is active for 24 hours at 18·0°C before moulting to the stage IV nauplius. This discrepancy occurred as a result of the calculation, using the percentage biochemical constituent present in the total dry weight of the larva. The dry weights were seen to rise from stage III to IV which is very unlikely to be the case in life. In order to calculate the energy budget for the female nauplii, the stage III and IV data were therefore amalgamated to give an overall larval period of 72 hours.

DISCUSSION

The total lipid extraction and subsequent gravimetric analysis for the stage II nauplii, gave notably differing figures for male and female larvae. The freeze-dried male nauplii, which contained 26·9% total lipid, had been retained in a desiccator for 6 months at room temperature prior to analysis. The females containing 14·5% total lipid had spent one month in a desiccator prior to analysis and the other female sample, with 9·1% total

lipid, was analysed immediately following the freeze-drying. Thus, low lipid content did not correlate with a long period in the desiccator at room temperature. However, it is possible that the unsaturated fatty acids present in the lipid fraction may have become oxidised, particularly as the desiccators were not flushed out with nitrogen when the freshly freeze-dried larvae were stored in them. The host crabs, bearing the mature externae from which the *S. carcini* larvae were released, were all males of similar size exhibiting a similar degree of feminisation. It was noted that they had been maintained in the holding chambers for different lengths of time. The male *S. carcini* larvae had been released from the mature externa on a crab that had been held for 2 months, compared to 7 and 8 months for the other two crabs. Whether these observations relate to the energetic content of the parasite larvae is not known, but may be worth taking into consideration and certainly needs further study.

The analysis of neutral lipids and phospholipids by thin-layer chromatography (T.L.C.) was carried out on two separate occasions, a month apart. This may be the reason for slight differences in some of the R.f. values for the identification standards (see Table 2). The neutral lipid fractions for male and female larvae were very similar (see Figure 2 & Table 2), with the triacylglycerols being the dominant group, particularly in the female larvae. The methyl ester, which is man-made, was incorporated to increase the volatility of the standards and samples. Consequently, the spots corresponding to those of methyl ester for male and female larvae are probably due to glycerol

ether. There was no spot to correspond with the free fatty acid standard on the female neutral lipid plate, although possibly this group, of which one may expect a presence of more than 1% or 2%, may have run on to the 24% R.f. value. The phospholipid T.L.C. plates also gave similar results for male and female *S. carcini* nauplii. In this case it was only the phosphatidyl serine standards which had different R.f. values. Phosphatidyl ethanolamine, for both male and female nauplii showed a small difference in R.f. value from the standard. This phospholipid together with phosphatidyl choline is a classic component of animal tissue. Some iodine staining was observed close to the solvent front on both male and female phospholipid T.L.C. plates. Neutral lipid would be expected in this region but, having already been separated out, the staining may be due to glycolipids.

The fatty acid compositions of male and female stage II *S. carcini* nauplii, analysed by gas liquid chromatography (G.L.C.) are not strictly quantitatively comparable as the results for the females cover the total lipids present, whereas the males are separated into neutral lipid and phospholipid fractions. However, as the only other main component of the lipid complement is made up of the membrane glycolipids, which represent a small fraction quantitatively, relative comparisons for male and female nauplii may be made. The fatty acids present in the larvae of *S. carcini* must result from endogenous *de novo* synthesis, together with uptake from the host crab haemolymph via the rootlets of the adult parasite. Palmitic acid (16:0) which is synthesised *de novo*

as the normal end product of fatty acid synthesis in animals (Holland 1987), is present in the male and female nauplii at 21·18% & 19·49 and 14·18 respectively. By chain elongation, palmitic acid is converted to stearic acid (18:0), which occurs in both male and female larvae at between 6% and 11%. Although stearic is the most common saturated fatty acid to be found in animal tissue there is less stearic than palmitic found in *S. carcini*, which is also the case for *Balanus balanoides* adults (Holland 1987) and is often a feature of lipids in marine organisms. The mono-unsaturated fatty acids, palmitoleic (16:1ω7), oleic (18:1ω9) and cis-vaccenic (18:1ω7) constitute more than 55% of the triacylglycerol fatty acid complement for both male and female *S. carcini* nauplii. These fatty acids could be readily synthesised *de novo* by desaturation of 16:0 and 18:0 fatty acids. Certain poly-unsaturated fatty acids, termed essential fatty acids, are important for optimum growth, development and other physiological processes at the cellular level. Arachidonic acid (20:4ω6), was present at approximately 1% to 2·5% in male and female *S. carcini* larvae respectively. Eicosapentanoic (20:5ω3) and docosahexanoic (22:6ω3) acids, which are prominent marine lipids, found in large amounts in *Balanus balanoides* adults (Holland 1987) and *Elminius modestus* (Took 1978), were only present in percentage terms of 3·30 & 3·19 and 1·24 & 3·01 respectively in male *S. carcini* larvae compared to 10·34 and 7·44 in the females and was the one striking difference in fatty acid composition between the male and female nauplii. This however, may be an artefact due to the storage of the

freeze-dried male larvae. The female larvae used for this analysis were taken straight from the freeze-drier. These essential fatty acids cannot be synthesised *de novo* by animals (Moreno et al 1979) and a precursor, principally linolenic acid (18:3ω3) must be provided from previous food sources. For adult *Balanus balanoides* feeding on a wide range of planktonic organisms, a deficiency in essential fatty acids should not occur Even when starved over the winter, they are able to conserve essential fatty acids and utilise non-essential fatty acids as their energy reserve. The adult *S. carcini*, situated within the host crab is unlikely to experience starvation, although the nutrient levels in the haemolymph may vary seasonally. Any variation in nutrient acquisition by the adult parasite may be reflected ultimately in the amount of stored energy in its larvae.

From the colorimetric analyses carried out on the larvae of *S. carcini*, it is evident that the percentages of the biochemical components accounted for were variable for the male larvae, but more consistent for the females (see Table 4). It is likely that the total protein content of the larvae is not readily accessible and may include residual proteins bound up in the cuticle. Crustacean cuticle is made up of a protein-chitin mixture (Neville 1975). Chitin has a chemical similarity to cellulose but with an acetamido group on the C-2 atom compared to the hydroxyl group in cellulose. However, Neville (1975) states that proteins are the main component of cuticle. Attwood & Zola (1967), when studying the chitin-protein ratio in the horny pen

of the cephalopod species *Loligo*, noted that some of this protein can be extracted by mild methods, whereas some may be covalently bound to chitin and therefore not readily extracted. After urea-extraction on *Loligo* pen, to solubilise the readily accessible protein, this material was subjected to a further extraction to determine the chitin-protein ratio in the remaining residue (Attwood & Zola 1967). The first alkaline extraction was for 5 hours at 20°C in 0·01N NaOH and the proportion of chitin to protein remaining was 0·6 : 1·0. Thus, most of the protein in the chitin-protein complex was still bound up. After the second alkaline extraction for 5 hours at 50°C in 1·0N NaOH, a mean figure of 57% of the material was solubilised. A further alkaline extraction gave another 4% into solution. These solubilised fractions together constitute the protein liberated from the chitin-protein complex. The *Loligo* pen was made up of some readily accessible water-soluble protein, together with a chitin-protein complex, 61% of which was also solubilised after the three alkaline extractions. Unfortunately, the initial water-soluble protein, released by urea extraction, was not presented as a percentage of the original material (Attwood & Zola 1967).

From the information available on crustacean cuticle, Welinder (1974) observed 36·5% chitin was present in the unhardened cuticle of *Astacus fluviatilis* compared to 71·0% in the hardened cuticle. These results correlated well with those reported by Drach & Lafon (1942) for the pre-exuvial and postexuvial cuticle of *Cancer pagurus* and *Maia squinado*. However, with the paucity of information, in percentage biochemical terms,

for cirripede cuticle, and following the arguments above, it was decided to assume a total protein figure for *S. carcini* exuvia to approach 66%. The protein extraction employed on the larvae of *S. carcini* (this study) is comparable to the alkaline extractions carried out by Attwood & Zola. Therefore it is evident that there is always protein bound up in the chitin-protein complex, which is inaccessible to analysis and may be termed residual protein. Thus, 66% of the *S. carcini* exuvia weight was regarded as protein. There is also lipid in the cuticle, but some biochemical components may be resorbed before the exuvia is shed.

The other biochemical component - carbohydrate - was barely detectable in this study and from reports on the biochemical composition of the cypris larvae of *Balanus balanoides*, with time (Holland & Walker 1975 and Lucas et al 1979), it was noted that the carbohydrate level remained relatively stable throughout. Consequently, when considering the carbohydrate content of 'the *S. carcini* nauplius', as determined by Holland (1978 from unpublished data 1976), it was not considered pertinent to incorporate this value as a further estimation in Table 4.

As the colorimetric analyses were carried out in the summer months, the male larvae had been collected, freeze-dried and stored in a desiccator at room temperature for more than 6 months, whereas the females were prepared 1 month prior to analyses. It is not known whether a prolonged period in the desiccator was detrimental to the biochemical content of the larvae other than to the fatty acid composition.

From the respiration rates recorded, over larval development and the calculated oxygen consumption rates due to protein loss alone (see Tables 4 & 5), the metabolism of *S. carcini* larvae is not protein driven. However, there are anomalies in the figures for the calculated oxygen consumption rates due to lipid loss alone, compared to the observed rates, particularly for male stage II nauplii. From colorimetric analysis, 23·5% lipid was recorded to be present in the male stage II nauplius and 16.0% for the female stage II nauplius. These results correlate well with those determined by gravimetric analysis (see Table 1). Nevertheless the lipid levels for other male larval stages are all relatively low for some reason. Thus, a considerable lipid loss is noted from stage II to stage III for the male larvae, which results in a very high predicted oxygen consumption rate over that period. The other predicted respiration rates due to lipid loss are also slightly higher than observed, in some cases two-fold. This may reflect the true condition in life. The respirometry was only measured over a 40 minute period, within a 24 or 48 hour larval stage. During this overall larval stage time, larval activity may vary tremendously, particularly when the animal moults. Such activity was shown by Lucas et al (1979) for *Balanus balanoides* cyprids, where the respiration rate was seen to increase four fold, prior to the moult.

When the utilisation of protein is considered, there are several factors which are unquantifiable. These are mainly due to the proteinaceous secretions released from the active frontal

horn glands and labral glands. The moulting fluid also contains proteinaceous materials. When considering the biochemical constituent losses over larval development it is essential to remember that there are basic cell constituents which are not available as energy sources. After maintaining the non-feeding *Balanus balanoides* cyprids over a period of eight weeks, Lucas et al (1979) measured their final biochemical composition which constituted 28·6% of the dry organic weight as protein and 6·0% as lipid. To project these proportions as the basic cell constituents in the male and female cyprids of *Sacculina carcini*, predictions can be made on the length of time they could survive utilising the remaining lipid and protein reserves at the lower oxygen consumption rate measured for these cyprids. These calculations result in a figure of 11 days for the male and only 4 days for the females. The longer survival time for the males agrees with the fact that the males are larger than the females and have to search out the ephemeral settlement site of a virgin externa. As one brood of female cyprids was seen to survive in the laboratory for 28 days (pers. obs.), the reverse calculation was carried out to predict the amount of lipid necessary for 28 days maintainence at the low respiration rate. A figure of 0·14 µg. of lipid per larva results for both male and female cyprids, whereas only 0·04 µg. and 0·03 µg. were measured by colorimetric analysis. However if the total calories available from the measured lipid content of the male and female stage II nauplii are utilised for respiration during each stage of their naupliar development, the amount of lipid left in the cyprid would be 0·15

µg. for the male and 0·05 µg. for the female.

The protein losses over larval development were calculated from the previous estimations for excretion and the exuviae, together with the lipid loss due to the measured respiration rates at each larval stage. These biochemical losses were converted to their calorific equivalents and compared to the total calorific content measured in the stage II *S. carcini* nauplius of the males and females (see Table 6). The time taken from release of the larval brood to the moult to the stage III nauplius, lasts 24 hours in total. These calculations incorporate the stage I & II nauplii within this larval period (see Table 6), and as a respiration rate was measured for the stage I male nauplius, it was possible to include lipid loss due to respiration for the first 6 hours for this stage.

The total calorific content, measured for the stage II nauplius was $4{\cdot}68 \times 10^{-3}$ cals. for the male and $2{\cdot}55 \times 10^{-3}$ cals. for the female. The total amount of calories utilised during respiration and lost with the exuviae was calculated as $2{\cdot}04 \times 10^{-3}$ cals. and $1{\cdot}50 \times 10^{-3}$ cals. for the males and females respectively (Table 6). Thus it would appear from these figures that the larvae are well in budget. However, several other factors have to be incorporated. These include [1] the calorific content due to the basic cell constituents [2] the calorific losses due to respiration and excretion during embryogenesis [3] secretions released throughout larval development [4] the energetic cost of the two metamorphoses, from stage IV nauplius to the cyprid and from the cyprid to the kentrogon or trichogon

(see Figure 5). It is only after these calorific quantities have been accounted for that the energy available to the cyprid to search for the relevant settlement target may be assessed.

Table 1. Results from the total lipid extraction, for male and female stage II *S. carcini* nauplii, using gravimetric analysis.

	Male larvae	Female larvae	
Freeze-dried larvae (mg.)	20·85	17·64	31·11
Total lipid extracted (mg.)	5·60 (26·86)	2·56 (14·50)	2·83 (9·10)
Neutral lipid (mg.)	3·00 (53·57)	n.d.	2·00 (70·67)
Phospholipid (mg.)	2·40 (42·86)	n.d.	0·36 (12·72)

The figures in parentheses are:-
1. percentages of total lipid in the complete sample of freeze-dried larvae,
2. percentages of neutral lipid and phospholipid in the total lipid fraction.

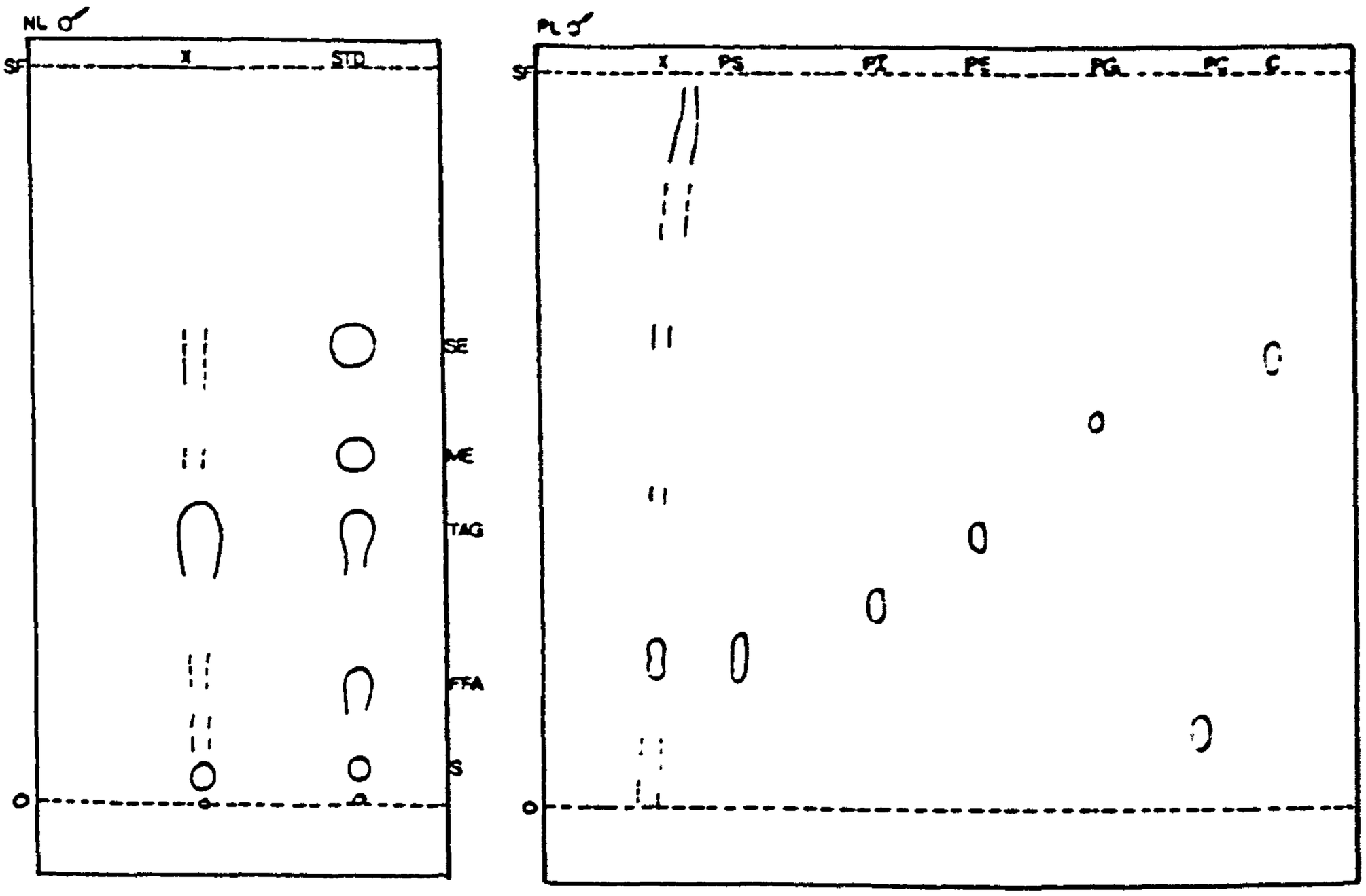


Figure 2. Drawings from the thin-layer chromatography plates for the neutral lipid (NL) and phospholipid (PL) fractions from male and female *S. carcini* stage II nauplii. For the R.f. values and an explanation of abbreviations used, see Table 2 (opposite page).

Table 2. The R.f. values for the neutral lipids and phospholipids of male and female stage II *S. carcini* nauplii from thin-layer chromatography, calculated from the drawings shown in Figure 2; O = origin; SF = solvent front.

NEUTRAL LIPIDS			PHOSPHOLIPIDS		
Identification standards	STD %	X %	Identification standards	STD %	X %
MALE LARVAE					
(SE) sterol ester	60	62	(C) cardiolipin	64	61
(ME) methyl ester*	47	(47)	(PC) phosphatidyl choline	9	10
(TAG) triacylglycerol	36	36	(PE) phosphatidyl ethanolamine	42	38
(FFA) free fatty acids	17	15	(PI) phosphatidyl inositol	-	28
(S) sterol	3	5	(PS) phosphatidyl serine	20	20
			(PG) phosphatidyl glycerol	-	53
FEMALE LARVAE					
(SE) sterol ester	67	70	(C) cardiolipin	64	61
(ME) methyl ester*	53	(52)	(PC) phosphatidyl choline	9	10
(TAG) triacylglycerol	47	47	(PE) phosphatidyl ethanolamine	42	38
(FFA) free fatty acids	-	17	(PI) phosphatidyl inositol	-	28
(S) sterol	6	6	(PS) phoshatidyl serine	27	27
			(PG) phosphatidyl glycerol	-	53

Key: * = this is man-made and is incorporated to increase volatility.
The figures in parentheses are possibly glycerol ether.
The letters in parentheses are the abbreviations shown on Figure 2 (opposite page).

Table 3. Fatty acid composition of the male and female stage II *S. carcini* nauplii, analysed by gas liquid chromatography. The percentages represent the relative amounts of triacylglycerols (T. A. G.) present in each fraction.

T. A. G.	Trivial name	P. L. Male (%)	N. L. Male (%)	Total lipid Female (%)
14: 0	myristic	0·79	0·93	0·38
15: 0				0·33
16: 0	palmitic	19·49	21·18	14·18
16: 1ω7	palmitoleic	5·30	9·20	3·62
17: 0		0·44	0·18	0·78
18: 0	stearic	11·70	6·27	8·04
18: 1ω9	oleic	42·29	41·14	44·85
18: 1ω7	cis-vaccenic	8·64	12·67	4·76
18: 2ω6	linoleic	0·38	1·35	0·98
18: 3ω3	linolenic	0·32	0·30	0·46
18: 4ω3		1·55	1·06	0·18
20: 1ω9	gondoic	1·90	0·44	0·28
20: 2ω6				0·39
20: 4ω6	arachidonic	1·00	0·74	2·46
20: 5ω3	eicosapentanoic	3·19	3·30	10·34
22: ω11				0·07
22: 4ω6				0·26
22: 5ω3				0·20
22: 6ω3	docosahexanoic	3·01	1·24	7·44
		100·00	100·00	100·00

Key: P. L. = phospholipid; N. L. = neutral lipid.
The traces for the lipid profiles are in the appendix (Figure 1.).

TABLE 4. OXYGEN CONSUMPTION RATE AND BIOCHEMICAL COMPOSITION EXPRESSED AS µg. COMPONENT PER INDIVIDUAL OF S.CARCINI LARVAE

STAGE	TIME	RESPIRATION RATE ($\mu l.O_2 h.^{-1}$ ind.$^{-1} \times 10^{-3}$)		DRY WT.	ASH	ASH FREE DRY WT.	TOTAL LIPID	TOTAL PROTEIN	CALORIFIC EQUIVALENTS (cals. x 10^{-3})		% BIOCHEMICAL COMPONENT
	(hrs.)	High-Low range	Mean	(µg.)	%	(µg.)	(µg.)	(µg.)	Lipid	Protein	ACCOUNTED FOR
(MALE LARVAE)											
I	0-6	1.17-1.16	1.17	n.d.	5.70	0.87*	n.d.	n.d.	-	-	-
II	24	1.19-0.49	0.81	0.85	5.18	0.81	0.19(23.5)	0.51(63.2)	1.80	2.88	86.7
III	24	0.95-0.61	0.85	0.79	6.97	0.74	0.08(10.1)	0.34(45.3)	0.76	1.92	55.4
IV	48	1.12-0.82	0.95	0.74	5.99	0.70	0.06(8.0)	0.26(37.5)	0.57	1.47	45.5
CYP.		1.38-0.34	0.41 & 1.13	0.41	8.86	0.37	0.04(11.8)	0.13(34.0)	0.38	0.74	45.8
(FEMALE LARVAE)											
I	0-6	n.d.	n.d.	n.d.	6.97	0.58*	n.d.	n.d.	-	-	-
II	24	1.34-0.45	0.85	0.57	5.98	0.54	0.09(16.0)	0.30(56.3)	0.85	1.70	72.3
III	24	0.88-0.62	0.77	0.50	7.01	0.47	0.07(14.1)	0.27(57.3)	0.66	1.53	71.4
IV	48	0.90-0.55	0.74	0.54	7.70	0.50	0.07(14.7)	0.27(54.3)	0.66	1.53	69.0
CYP.		1.40-0.29	0.45 & 1.11	0.29	6.30	0.27	0.03(10.0)	0.09(33.9)	0.28	0.51	43.9

KEY: * = estimated stage I weight; n.d. = not done; I,II,III,IV = nauplius stage; CYP. = cypris stage.

The figures in parentheses are the amounts of total lipid and total protein obtained using colorimetric analysis, as a percentage of the ash free dry weight. Calorific equivalents were from Crisp (1971); lipid = 9.45 cal. mg.$^{-1}$, protein = 5.65 cal. mg.$^{-1}$

TABLE 5. ENERGY LOSSES DURING LARVAL DEVELOPMENT OF S.CARCINI. ACTUAL BIOCHEMICAL LOSSES, CALCULATED BIOCHEMICAL LOSSES USING RESPIROMETRY DATA AND ESTIMATED LOSSES DUE TO MOULTING AND EXCRETION

STAGE	TIME (hrs.)	OBSERVED LIPID LOSS (µg.)	OBSERVED PROTEIN LOSS (µg.)	P* O_2 CONSUMPTION FROM LIPID LOSS ($\mu l.O_2 h.^{-1} ind.^{-1} \times 10^{-3}$)	P* O_2 CONSUMPTION FROM PROTEIN LOSS ($\mu l.O_2 h.^{-1} ind.^{-1} \times 10^{-3}$)	P* LIPID REQUIRED TO COMPLETE STAGE AT MEASURED RESPIRATION RATE (µg.)	P* PROTEIN REQUIRED TO COMPLETE STAGE AT MEASURED RESPIRATION RATE (µg.)	ESTIMATED EXUVIA WEIGHT (µg.)	MAXIMUM ESTIMATED EXCRETION (µg. protein)
(MALE LARVAE)									
II	24	0.11	0.17	9.03	8.34	0.01	0.02	0.06	0.014
III	24	0.02	0.08	1.64	3.91	0.01	0.02	0.06	0.013
IV	48	0.02	0.13	0.82	3.17	0.02	0.04	0.06	0.023
CYP.								0.07	
(FEMALE LARVAE)									
II	24	0.02	0.03	1.64	1.47	0.01	0.02	0.04	0.009
III	72	0.04	0.18	1.10	2.95	0.03	0.05	0.08	0.025
IV									
CYP.								0.05	

KEY: Calorific equivalents were from Crisp (1971); lipid = 9.45 cal. $mg.^{-1}$, protein = 5.65 cal. $mg.^{-1}$ and 1 ml. O_2 at N.T.P. = 4.8 cals; P* = predicted.

Calculations for the predicted values in this table are given in the results section of this chapter.

Table 6. The complete larval development from the stage I nauplius to the inoculation stage for the larvae of *S. carcini* in terms of the energetic losses which are quantifiable.

Stage	Time (hrs)	Lipid loss due to resp.	Protein loss due to excr.	Protein loss due to exuvia	Lipid loss due to resp.	Protein loss due to excr.	Protein loss due to exuvia
		(micrograms per larva)			(calories x 10^{-3})		
MALE LARVAE							
I	6	0·004	0·004	0·040	0·034	0·017	0·226
II	18	0·007	0·011	0·040	0·070	0·062	0·226
III	24	0·010	0·013	0·040	0·098	0·072	0·226
IV	48	0·023	0·023	0·040	0·219	0·130	0·226
Cyp.	48	0·010	0·013	0·147	0·095	0·071	0·264
					Total calories = 2·04 x 10^{-3}		
FEMALE LARVAE							
I	6	n. d.	0·002	0·027	n. d.	0·014	0·151
II	18	0·010*	0·007	0·027	0·093	0·040	0·151
III	24	0·010	0·008	0. 027	0·089	0·046	0·151
IV	48	0·018	0·017	0·027	0·171	0·098	0·151
Cyp.	48	0·011	0·009	0. 033	0·104	0·053	0·188
					Total calories = 1·50 x 10^{-3}		

Calorific equivalents were from Crisp (1971); lipid = 9·45 cal. $mg.^{-1}$, protein = 5·65 cal. $mg.^{-1}$ and 1·0 ml. O_2 at N. T. P. = 4·8 cals.

Key: I, II, III, IV = nauplius stages; Cyp. = cypris stage; * = this lipid loss due to respiration was calculated over 24 hours; excr. = excretion; resp. = respiration.

Figure 3. **Protein and lipid present as % of organic fraction in *S.carcini* larvae.**

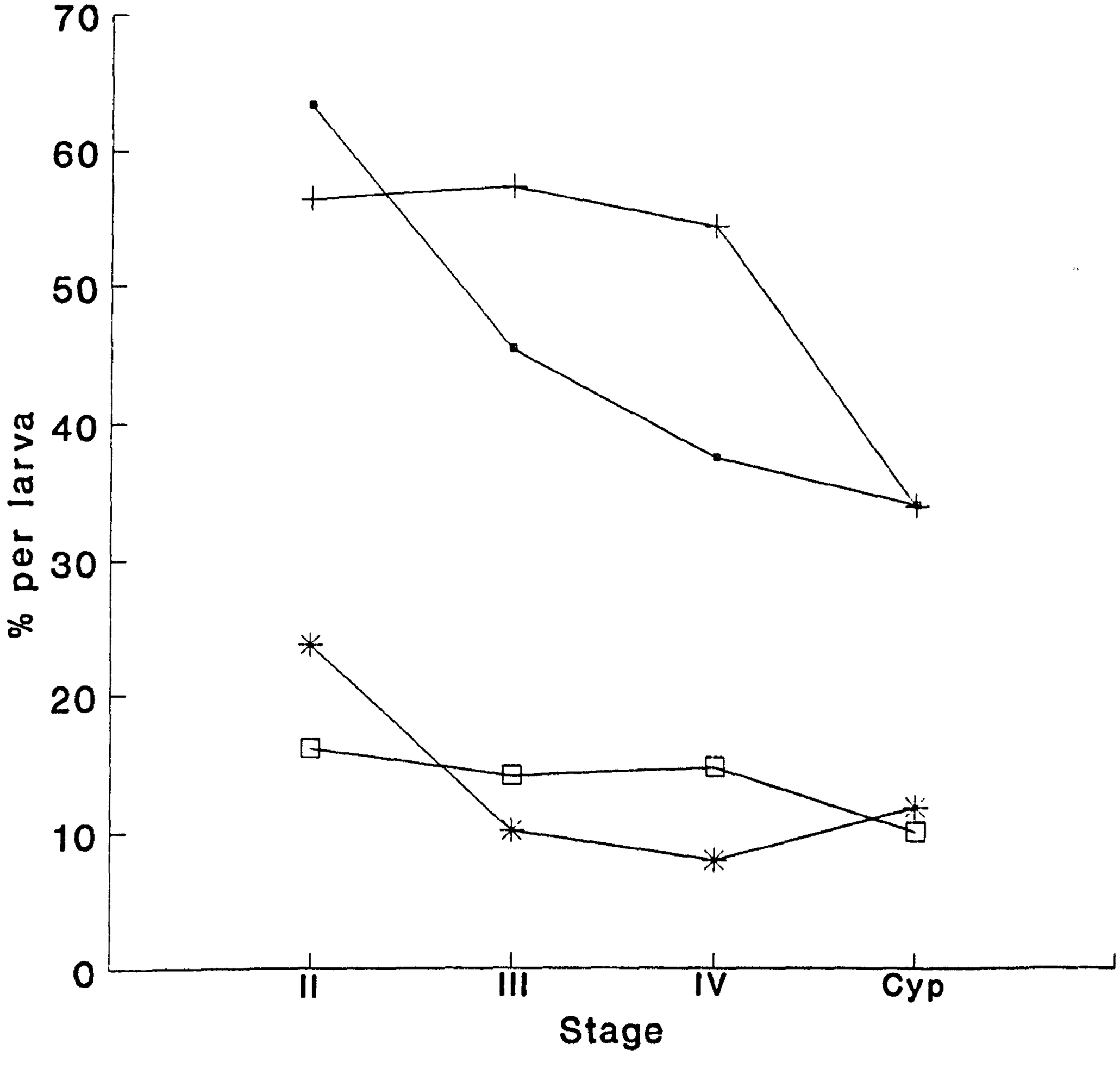


Figure 4. **Protein and lipid present in the organic fraction of *S.carcini* larvae**

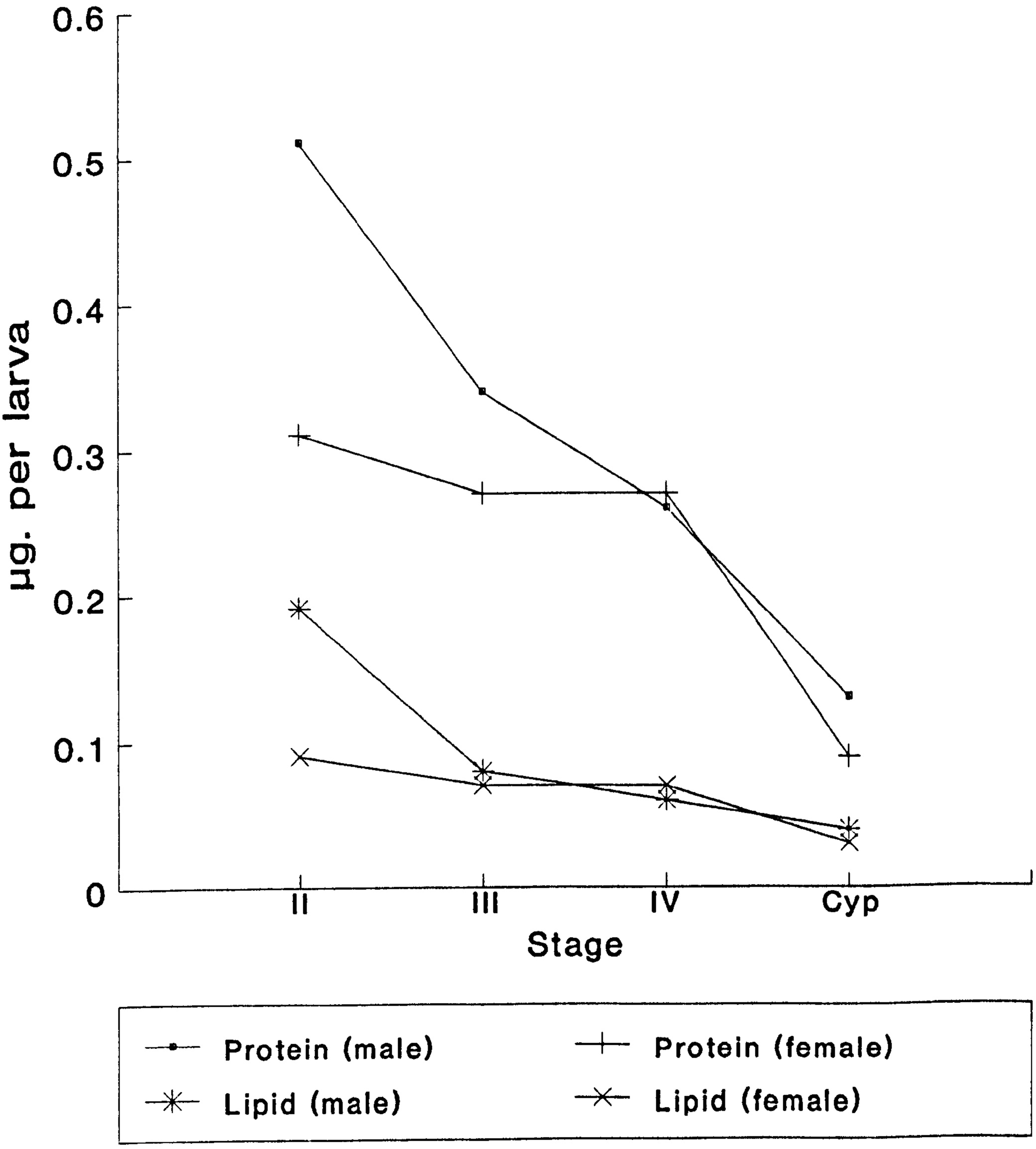


Figure. 5 Equations relating the energy budget of *Sacculina carcini* from the beginning of embryogenesis through to the cyprid and inoculation stage.

Energy equation to the cypris stage

$$TC = A + (B - [C^{o} + C^{1} + C^{2} + C^{3} + M^{o}])$$

Energy equation to the inoculation stage

$$TC = A - (A-A^{1}) + ([B+B^{1}] - [C^{o} + C^{1} + C^{2} + C^{3} + M^{o} + M^{1}])$$

Key: TC = the total calorific content in the egg at the beginning of embryogenesis;

A = the calorific content of the basic cell constituents of the egg;

A^{1} = ditto for the inoculation stage i.e. kentrogon or trichogon, being much less than the larval basic cell constituents.

B = the available energy store of the egg, expressed as calories;

B^{1} = the difference between A and A^{1} which will be available as an extra energy store in the cyprid;

C^{o} = the energy expressed as calories, utilised during embryogenesis in terms of respiration, excretion and the loss of the egg case;

C^{1} = the energy expressed as calories, utilised by the first stage nauplius in terms of respiration, excretion, exuvia loss and secretions;

C^{2} = ditto for the stage II nauplius;

C^{3} = ditto for the stage III nauplius;

M^{o} = the energy expressed as calories utilised by the stage IV nauplius for respiration, excretion, exuvia loss and secretions together with the extra energetic cost for the metamorphosis to the cyprid;

M^{1} = ditto for the cyprid together with the extra energetic cost for the metamorphosis to the trichogon or kentrogon.

GENERAL DISCUSSION

The presence of the parasitic barnacle *Sacculina carcini* on the common shore crab, *Carcinus maenas*, displays a disparate distribution. From field work carried out in this study (Chapter II) it was clear that centres of very high parasite prevalence, such as 50% - 60% and occasionally 90%, were a characteristic of the population distribution. The larvae of *S. carcini* are lecithotrophic, they develop to the cyprid within 5 days at 18°C and are capable of settlement on the host, 2 days later. It was noted that retention of the parasite larvae, within a semi-enclosed body of water would increase the probability of the parasite finding a suitable settlement target. The parasite was judged to be particularly successful within such systems, especially in the harbour at Roscoff, L'Etang de Thau and the sheltered area of the Baie de Quberon. Further study to calculate flushing times for these bodies of water, together with parasite prevalence, using a consistent capture method, would provide interesting results. However, conditions within these coastal systems are more variable than the open ocean and it was important to discover the temperature and salinity tolerances of the larvae. From experiments carried out in the laboratory it was clear that *S. carcini* larvae are capable of survival at very low temperatures, although they were unable to complete development below 7°C - 8°C for the males and 8°C - 9°C for the females. In order to test larval tolerance to low salinity it was necessary

to acclimate the larvae before beginning the experiment. The male nauplii developed through to the cypris stage within 9 days at 21‰, and at 22‰ the females developed to cyprids in 6 days. Larval activity at these low salinities was reduced and at 18‰ and 19‰ the nauplii developed to stage III, however by Day 4 they were incapable of moulting and remained within the stage III cuticle. This encapsulated state encouraged protozoan contamination and consequent larval death. Although the population of *S. carcini* on *C. maenas* in the lagoon at Aveiro is subjected to salinities less than 10‰ in some areas, the highest parasite prevalence was at salinities between 18‰ and 30‰. The larvae of *S. carcini* used in the laboratory were from mature externae on crabs maintained in 33‰ seawater. It may be possible that when crabs, infected with *S. carcini* are acclimated to lower salinities the parasite could also develop a greater tolerance. This would be advantageous in an estuary system where there is a landward flow of higher salinity water at the bottom, which balances the seaward flow of lower salinity water in the upper layer. The larvae of *Rhithropanopeus harrisii*, the mud crab, which is parasitised by the rhizocephalan barnacle, *Loxothylacus panopaei*, utilise this system by vertical migration and consequently are retained within the estuary (Cronin & Forward 1986 and Walker, Clare, Rittschof & Mensching - unpublished data).

From the morphological study of the nauplii of *S. carcini* (Chapter III) it was shown that the larvae bore the standard cirripede limbs, but they were simple in structure and lacked

gnathobases. These features are characteristic of lecithotrophic larvae (see Crisp 1986). The frontal horns were open at the tip and a median pore was observed together with a labral papilla, which is the exit for the functional labral glands. The presence of the vestigial ventral thoracic process was evident on the stage III nauplius and more prominent on the stage IV nauplius. These features support the argument that the rhizocephalan nauplius fits well within the overall pattern of thoracican nauplius form, ranging from the specialised planktotrophic forms to the simplified lecithotrophic forms. The presence of the ventral thoracic process is further certification that rhizocephalans should be included in the Cirripedia *sensu stricto* (see Newman 1987 for counter argument).

The final investigation to produce an energy budget for the larval stages of *S. carcini* proved to be a challenge. An essential part of an energy budget is to measure an accurate dry weight per larva. It is virtually impossible to obtain an absolute dry weight as there will always be moisture in the atmosphere. From the methods tested to weigh the larvae it was seen that it was more important to minimise handling time than to use many replicates. When preparing larvae for weighing and biochemical analysis, it was essential to quickly wash them free of seawater using an isotonic solution of ammonium formate, as they were seen to quickly burst and release their body fluids. This may have been the reason for some of the low results for biochemical constituents recorded in the colorimetric analysis. From the final table and equations in Chapter VI, it appeared

that the larvae remained in budget. The loss of calories at each stage due to larval secretions is an important consideration. Observations of cyprid behaviour showed that they are very 'sticky' and once they have made contact with one another, they have to make a considerable effort to push themselves apart using their antennules. Also the cyprids are hydrophobic which suggests that lipid may be present on the outside of the cuticle.

Unlike most other lecithotrophs, the larvae of *S. carcini* are produced in large numbers and are comparatively small. The strategy is obviously geared to maximise the probability of the settling larval stage reaching its settlement site, by producing large numbers of propagules able to disperse in limited areas. The larvae are very active initially, increasing their chances of dispersal into the surface waters, but they only have a finite energy reserve on board. The settlement target for the female cyprid is the base of a seta on a crab carapace. The male cyprid needs to search for a virgin externa which has erupted on the abdomen of a crab previously infected by a female. The virgin externa has to be the more ephemeral settlement target. Consequently male larvae are larger and have been shown to have a greater energy store. Successful male settlement is the crucial point in the life-cycle of *S. carcini*. If a male cyprid is unable to settle on a virgin externa it will perish, and if a virgin externa is not inoculated within 8 months by male cyprids, it will also die (Walker 1987). Consideration has to be given for the migrations which *C. maenas* seasonally undergoes. These migrations may be an added reason for parasite prevalence to vary

both temporally and spatially. Within an enclosed body of water the parasite is very successful, as in L'Etang de Thau, but the more open the environment, the less probable it is that the larvae, with their finite energy reserves, will reach their designated settlement site and metamorphose to allow the life-cycle to continue.

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APPENDICES

Key for Tables 1 ⇒ 4 :-

All weights are in milligrams.

A = weight of net + larvae (average of two weighings);
B = weight of net (ditto);
C = weight of larvae;
D = weight per larva;
E = number of larvae on net (average of three counts);
• = clean net used to calculate average weight per stage;
I, II, III, IV = nauplius stages; Cyp = cypris stage.

Table 1. Data from weighing a single brood of female *S. carcini* larvae throughout their development, using ten replicates at each stage (No. 5FY).

	II	III	IV	Cyp.
A	6·74680	6·88660	6·89490	8·25580
B	6·38380	6·75220	6·75670	8·18580
C	0·36300	0·13440	0·13820	0·07000
D	0·00072	0·00089	0·00105	0·00099
E	(505)•	(151)•	(132)	(71)
A	7·91440	6·39730	7·10040	7·53930
B	7·62860	6·29000	6·99470	7·49200
C	0·28580	0·10730	0·10570	0·04740
D	0·00082	0·00110	0·00095	0·00051
E	(348)•	(102)	(112)	(93)•
A	6·70660	6·53730	6·46000	8·28880
B	6·36460	6·40470	6·37150	8·25290
C	0·34200	0·13260	0·08850	0·03600
D	0·00091	0·00075	0·00103	0·00025
E	(377)•	(176)•	(86)	(147)•
A	6·95360	7·19530	6·64100	7·28410
B	6·65520	7·06710	6·48700	7·26300
C	0·29840	0·12830	0·15410	0·02120
D	0·00079	0·00082	0·00085	0·00014
E	(379)•	(157)•	(181)•	(151)•
A	6·27190	6·75730	7·92940	7·27820
B	6·02080	6·65690	7·79670	7·08780
C	0·25110	0·10050	0·13270	0·10050
D	0·00091	0·00090	0·00083	0·00225
E	(276)•	(111)•	(160)•	(81)

Table 1 continued:-

A	7·27460	6·24210	8·93460	7·27920
B	7·02490	6·12210	8·75870	7·22070
C	0·24980	0. 12000	0·17590	0·05850
D	0·00091	0·00081	0·00191	0·00163
E	(274)•	(149)•	(92)	(36)
A	6·96900	7·47350	7·89280	8·94630
B	6·79220	7·36020	7·65170	8·94620
C	0·16990	0·11330	0·24110	0·00010
D	0·00110	0·00115	0·00221	0·00002
E	(154)	(99)	(109)	(41)
A	7·34370	6·75670	7·51380	7·12820
B	7·12630	6·65250	7·36420	7·06710
C	0·21740	0·10430	0·14970	0·06110
D	0. 00087	0·00096	0·00075	0·00071
E	(249)•	(109)•	(200)•	(86)•
A	7·96760	8·98750	7·42870	7·53250
B	7·63110	8·84990	7·23910	7·52090
C	0·33650	0·13760	0·18960	0·01160
D	0·00086	0·00110	0·00190	0·00022
E	(391)•	(126)	(100)	(53)•
A	6·48170	6·55860	7·84570	6·65800
B	6·17200	6·43260	7·54840	6·62950
C	0·30970	0·12610	0·29730	0·02850
D	0·00090	0·00075	0·00259	0·00026
E	(346)•	(168)•	(115)	(108)•

Table 2. Data from weighing a single brood of female *S. carcini* larvae throughout their development, using ten replicates at each stage (No. 3M).

	II	III	IV	Cyp.
A	8·55360	-	7·32070	6·76250
B	8·25250	-	7·14780	6·67310
C	0·30110	-	0·17270	0·08940
D	0·00070	-	0·00076	0·00043
E	(430)•	-	(227)•	(208)•
A	6·87730	-	6·74320	6·65850
B	6·67600	-	6·66310	6·54810
C	0·20130	-	0·08020	0·11040
D	0·00050	-	0·00057	0·00039
E	(343)•	-	(141)•	(287)•
A	9·68440	-	6·30440	6·42380
B	9·28440	-	6·07960	6·32690
C	0·40000	-	0·22480	0·96900
D	0·00064	-	0·00054	0·00044
E	(623)•	-	(415)•	(218)•
A	8·62550	-	6·32920	6·98130
B	8·30060	-	6·14650	6·83640
C	0·32490	-	0·18280	0·14490
D	0·00058	-	0·00046	0·00048
E	(557)•	-	(395)•	(301)•
A	8·57880	-	6·24760	6·96110
B	8·36340	-	6·06590	6·82100
C	0·21550	-	0·18180	0·14010
D	0·00056	-	0·00046	0·00062
E	(388)•	-	(393)•	(225)•

Table 2 continued:-

	A	8·90440	-	6·80220	7·20590
	B	8·56030	-	6·59770	7·01950
	C	0·34410	-	0·20450	0·18640
	D	0·00059	-	0·00067	0·00072
	E	(587)•	-	(306)•	(259)
	A	9·02000	-	6·99380	6·15340
	B	-	-	6·69140	6·06370
	C	-	-	0·30250	0·08970
	D	-	-	0·00046	0·00042
	E	(579)	-	(652)•	(215)•
	A	8·90480	-	7·16280	6·45290
	B	8·61350	-	7·04910	6·33340
	C	0·29160	-	0·11370	0·11950
	D	0·00067	-	0·00051	0·00043
	E	(436)•	-	(223)•	(280)•
	A	6·95090	-	6·39080	6·62610
	B	6·56220	-	6·27640	6·50480
	C	0·38870	-	0·11440	0·12140
	D	0·00054	-	0·00048	0·00048
	E	(719)•	-	(238)•	(253)•
	A	7·70250	-	5·78530	5·21050
	B	7·45510	-	5·62760	5·12000
	C	0·24740	-	0·15770	0·09050
	D	0·00058	-	0·00051	0·00039
	E	(425)•	-	(306)•	(211)•

Table 3. Data from weighing a single brood of male *S. carcini* larvae throughout their development, using ten replicates at each stage (No. 2F).

	II	III	IV	Cyp.
A	7·33960	8·76490	7·89470	9·50420
B	7·12500	8·35280	7·78780	9·3953
C	0·21460	0·41210	0·10690	0·10890
D	0·00079	0·00160	0·00064	0·00074
E	(272)•	(257)	(168)•	(147)•
A	7·85530	9·52040	8·68450	8·78630
B	7·62150	9·29960	8·48090	8·42140
C	0·23380	0·22080	0·2036	0·36490
D	0·00086	0·00089	0·00063	0·00166
E	(272)•	(249)•	(324)•	(220)
A	8·96130	7·92140	7·69070	9·11420
B	8·74720	7·75160	7·55260	9·02740
C	0·21410	0·26980	0·13810	0·0868
D	0·00080	0·00102	0·00065	0·00064
E	(265)•	(269)	(212)•	(135)•
A	6·70550	7·96550	7·83080	7·35720
B	6·53440	7·69460	7·58540	7·14020
C	0·17110	0·27090	0·24540	0·21700
D	0·00070	0·00082	0·00067	0·01219
E	(245)•	(331)•	(365)•	(178)
A	8·18990	7·80180	7·74620	8·92240
B	7·95230	7·59470	7·63010	8·76120
C	0·23760	0·20710	0·11610	0·17140
D	0·00090	0·00081	0·00062	0·00093
E	(261)•	(256)•	(186)•	(184)

Table 3 continued:-

A	7·51210	10·36920	8·40620	9·13760
B	7·34790	10·10670	8·31790	8·99660
C	0·1642	0·26250	0·08830	0·14100
D	0·00084	0·00082	0·00061	0·00087
E	(196)•	(319)•	(145)•	(162)
A	8·32110	8·09260	9·39390	8·96390
B	8·14190	7·80310	9·21660	8·87290
C	0·17920	0·28950	0·17730	0·09100
D	0·00079	0·00084	0·00167	0·00064
E	(227)•	(346)•	(106)	(143)•
A	8·82430	8·58130	9·43830	8·53350
B	8·64740	8·38700	9·21680	8·43940
C	0·17690	0·19430	0·22250	0·09410
D	0·00078	0·00088	0·00063	0·00064
E	(227)•	(222)•	(357)•	(147)•
A	8·20320	7·41820	8·74970	8·64270
B	7·96280	7·24110	8·42270	8·55110
C	0·24040	0·17710	0·32700	0·09160
D	0·00082	0·00080	0·00069	0·00059
E	(292)•	(220)•	(474)•	(156)•
A	9·11000	9·02830	9·53720	8·38220
B	8·91970	8·81610	9·27730	8·10150
C	0·19030	0·21220	0·25990	0·28070
D	0·00084	0·00083	0·00063	0·00180
E	(227)•	(255)•	(411)•	(156)

Table 4. Data from weighing a single brood of male *S. carcini* larvae throughout their development, using ten replicates at each stage (No. 6M).

	I	II	III	IV	Cyp.
A	7·71100	9·73500	8·30600	7·11700	7·86900
B	7. 60000	9·21000	8·09900	6·84300	7·38000
C	0. 11100	0·52500	0·20700	0·27400	0·48900
D	0. 00067	0·00135	0·00071	0·00131	0·00281
E	(167)•	(388)	(293)•	(209)	(174)
A	7·22700	8·10200	7·71900	7·42600	7·65000
B	7·16200	7·98000	7·57000	7·04000	7·58100
C	0·06500	0·12200	0·14900	0·03860	0·06900
D	0·00048	0·00072	0·00062	0·00141	0·00059
E	(136)•	(170)•	(242)•	(274)	(117)•
A	7·79500	9·64800	10·19200	7·66000	7·06200
B	7·56200	9·28900	9·98700	7·43200	6·95500
C	0·23300	0·35900	0·20500	0·33400	0·10700
D	0·00061	0·00241	0·00082	0·00147	0·00084
E	(383)•	(148)	(249)•	(228)	(128)•
A	9·64000	8·04000	8·50900	9·59000	8·00500
B	9·61100	7·70500	7·99200	9·30400	7·68800
C	0·02900	0·33500	0·51700	0·28600	0·31700
D	0·00035	0·00147	0·00298	0·00140	0·00293
E	(83)•	(228)	(174)	(204)	(108)
A	8·01800	7·75800	8·04400	7·26900	7·36900
B	7·78700	7·42000	7·81600	7·09800	7·33500
C	0·23100	0·33800	0·22800	0·17100	0·03500
D	0·00153	0·00147	0·00064	0·00083	0·00027
E	(151)	(230)	(355)•	(205)•	(126)•

Table 4 continued:-

A	7·64600	7·65800	7·90200	8·13900	7·05000
B	7·58000	7·30500	7·69000	7·56500	6·99500
C	0·06600	0·35300	0·21200	0·57400	0·05500
D	0·00032	0·00083	0·00061	0·00392	0·00047
E	(209)•	(427)•	(350)•	(146)	(117)•
A	8·94000	9·24300	7·79000	7·48100	8·59500
B	8·86100	8·84000	7·58700	7·33000	8·51600
C	0·07900	0·40300	0·20300	0·15100	0·07900
D	0·00043	0·00148	0·00055	0·00089	0·00056
E	(182)•	(273)	(369)•	(170)•	(141)•
A	7·72100	7·89700	7·20600	7·47000	7·19300
B	7·59600	7·66000	6·86000	7·04000	6·89000
C	0·12500	0·23700	0·34600	0·43000	0·30300
D	0·00063	0·00099	0·00245	0·00352	0·00219
E	(197)•	(239)•	(141)	(122)	(138)
A	10·47300	8·56400	9·48400	8·40200	7·85100
B	10·36400	8·31400	9·39100	8·29300	7·79100
C	0·10900	0·25000	0·09300	0·10900	0·06000
D	0·00049	0·00117	0·00088	0·00089	0·00041
E	(223)•	(214)	(105)•	(123)•	(146)•
A	7·77500	7·46400	8·81700	7·38800	6·98600
B	7·73900	7·24600	8·65500	7·06600	6·67800
C	0·03600	0·21800	0·16200	0·32200	0·30800
D	0·00025	0·00130	0·00090	0·00187	0·00367
E	(145)•	(168)	(180)•	(172)	(84)

Table 5a. The weights of *S. carcini* larvae during their development using the aluminium foil boat method.
I, II, III, IV = nauplius stage; Cyp. = cypris stage.

	II	III	IV	Cyp.
MALE LARVAE				
Boat + Larvae (mg.)	26·280	25·110	26. 213	26·046
Boat (mg.)	26·225	25·132	26·165	26·035
Larvae (mg.)	0·055	0·022	0·048	0·011
Number of larvae	(65)	(28)	(65)	(27)
Wt. per larva (µg.)	0·85	0·79	0·74	0·41
FEMALE LARVAE				
Boat + Larvae (mg.)	26·279	26·142	25·987	25·984
Boat (mg.)	26·241	26·126	25·973	25·970
Larvae (mg.)	0·038	0·016	0·014	0·014
Number of larvae	(67)	(32)	(26)	(49)
Wt. per larva (µg.)	0·57	0·50	0·54	0·29

Table 5b. The ash weights of male and female *S. carcini* larvae at each stage of their development.
I, II, III, IV = nauplius stage; Cyp. = cypris stage.

Stage	Boat and larvae (mg.)	Boat (mg.)	Larvae (mg.)	Boat and ashed larvae (mg.)	Ashed larvae (mg.)	% Ash
MALE LARVAE						
I	15·035	12·041	2·944	12·213	0·172	5·75
	13·207	10·930	2·277	11·061	0·131	5·75
	13·772	10·982	2·790	11·138	0·156	5·59
II	16·324	12·217	4·107	12·426	0·209	5·09
	15·587	12·215	3·372	12·392	0·177	5·25
	15·736	12·087	3·649	12·277	0·190	5·21
III	13·869	12·458	1·411	12·552	0·094	6·66
	12·421	10·912	1·402	11·091	0·107	7·63
	13·870	12·176	1·694	12·288	0·112	6·61
IV	20·014	17·548	2·466	17·690	0·142	5·75
	20·165	17·741	2·424	17·873	0·148	6·11
	20·261	17·833	2·428	17·981	0·148	6·10
Cyp.	13·785	12·247	1·538	12·381	0·134	8·71
	12·742	10·908	1·835	11·073	0·165	9·00
FEMALE LARVAE						
Egg	14·464	12·230	2·234	12·371	0·141	6·31
	14·154	12·174	1·980	12·293	0·119	6·01
I	15·036	10·967	4·069	11·245	0·279	6·84
	14·512	10·978	3·534	11·231	0·254	7·17
	15·130	11·107	4·023	11·384	0·227	6·89
II	16·431	10·953	5·477	11·296	0·343	6·25
	17·430	10·913	6·517	11·291	0·379	5·81
	16·661	10·917	5·744	11·254	0·338	5·88
III	12·762	10·941	1·821	11·060	0·119	6·56
	13·134	10·906	2·228	11·072	0·166	7·45
IV	11·126	10·793	0·333	10·818	0·025	7·53
	11·312	10·863	0·449	10·895	0·032	7·02
	11·196	10·923	0·274	10·946	0·023	8·56
Cyp.	11·836	10·937	0·899	10·985	0·047	5·26
	11·501	10·976	0·533	11·003	0·027	5·12
	11·404	10·915	0·489	10·957	0·042	8·51

Table 6. The respiration rates of several cirripede species converted to S.T.P. The temperatures at which these rates were measured are included.

Species	Temp. (°C)	Stage	Dry wt. larva^{-1} (µg.)	O_2 consumption (µl. O_2h.$^{-1}$ind.$^{-1}$) x10^{-3}	O_2 consumption (ml. O_2h.$^{-1}$g.$^{-1}$ dry weight)	Reference
B. bal.	10·0	I	0·63	3·19	5·07	Lucas (1980)
		II	1·24	3·82	3·08	
		III	2·16	3·43	1·59	
		IV	5·12	9·48	1·85	
		V	8·41	19·11	2·27	
		VI	14·81	31·57	2·13	
		Cyp	33·22	35·28(s)	1·06	
				20·27(e)	0·61	
B. bal.	10·0	I	n. d.	3·76	-	Davenport (1976)
		VI	n. d.	31·06	-	
B. ebu.	25·0	I	0·27	4·47	16·56	Jorgensen & Vernberg (1981)
		IV	0·68	15·13	22·25	
		VI	1·50	45·98	30·64	
		Cyp.	2·18	8·59	3·94	
E. mod.	16·0	II	0·3	1·39 to 2·05	4·64 to 6·84	Bhatnagar & Crisp (1965)
E. mod.	12·0	II	0·39	0·69 & 0·91	1·77 & 2·33	Harms (1987)
		III	0·71	1·60	2·25	
		IV	1·20	2·82	2·35	
		V	2·33	5·11	2·19	
		VI	4·27	6·97	1·63	
		Cyp.	4·56	6·51	1·43	
	18·0	II	0·41	1·24 & 2·18	3·02 & 5·32	
		III	0·75	3·40	4·53	
		IV	1·47	7·21	4·91	
		V	2·62	9·33	3·56	
		VI	5·19	11·35	2·19	
		Cyp.	5·81	9·39	1·62	
	24·0	II	0·39	2·09 & 2·63	5·36 & 6·74	
		III	0·70	4·95	7·07	
		IV	1·06	9·73	9·20	
		V	2·45	15·03	6·14	
		VI	4·39	16·85	3·84	
		Cyp.	4·38	12·77	2·92	

Key: *B. bal.* - *Balanus balanoides*; *B. ebu.* - *Balanus eburneus*; *E. mod.* - *Elminius modestus*; s- swimming cyprid; e- exploring cyprid; The first rates shown (Harms 1987) for Stage II nauplii, are for newly hatched larvae.

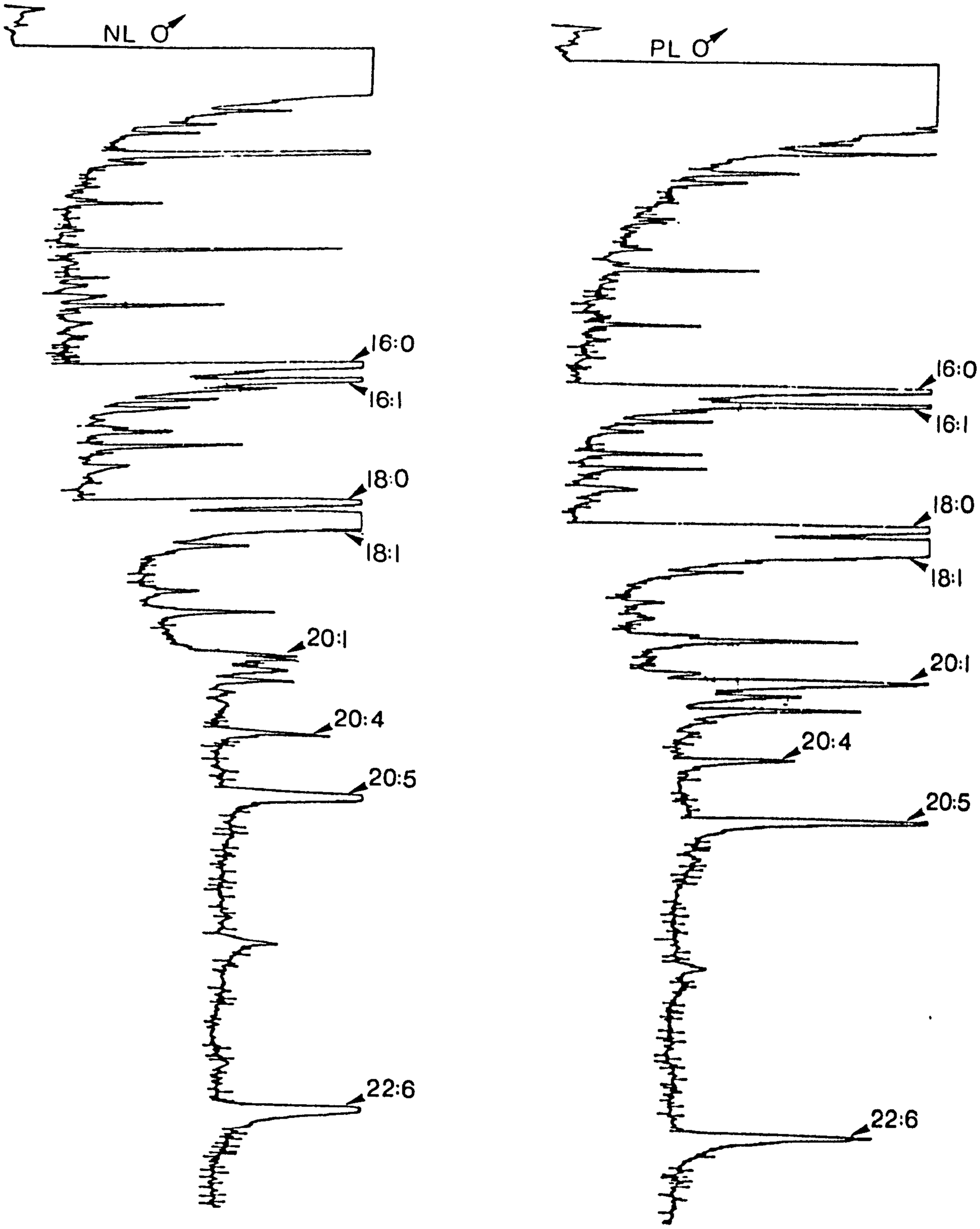

Figure 1a. The traces for the fatty acid composition of male stage II *S. carcini* nauplii, analysed by gas liquid chromatography (from Chapter VI, Table 3.). NL = neutral lipid ; PL = phospholipid.

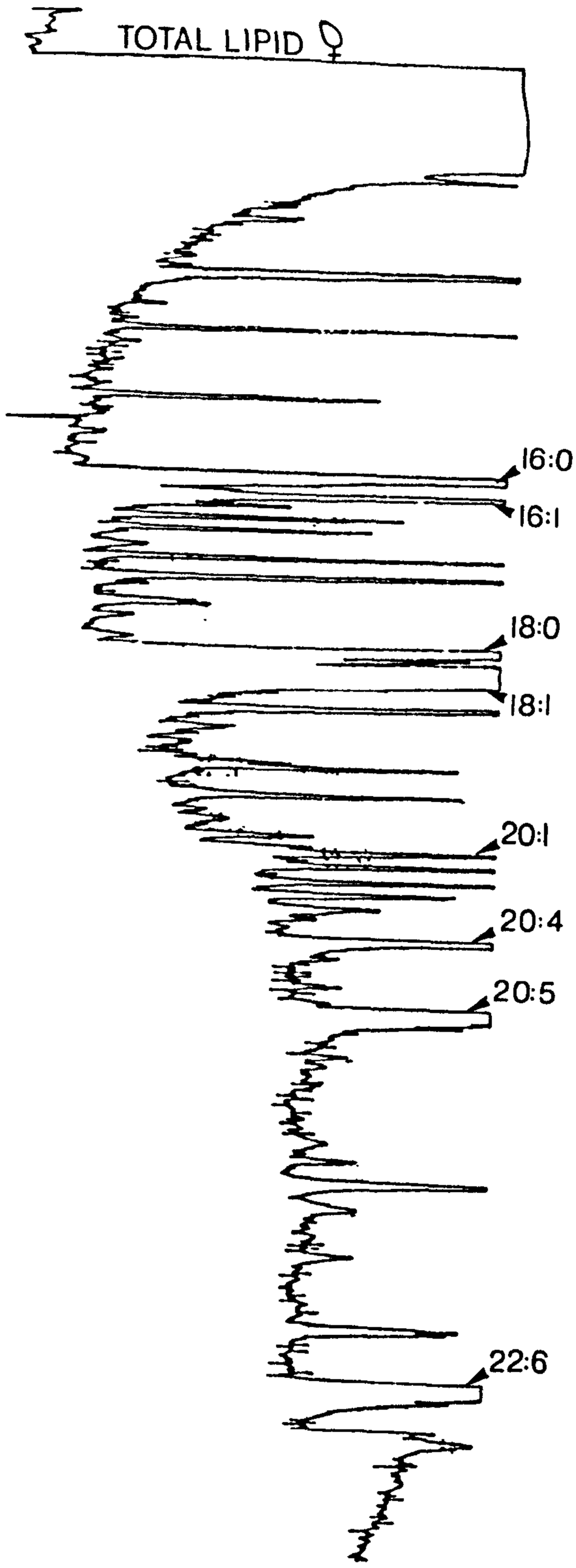

Figure 1b. The trace for the fatty acid composition of the total lipid fraction of female stage II *S. carcini* nauplii, analysed by gas liquid chromatography (from Chapter VI, Table 3.).