DOCTOR OF PHILOSOPHY

Feeding and energetic relationships of Pollicipes pollicipes (Gemlin, 1790) (Cirripedia; Lepadomorpha).

Norton, Rachel J.

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Feeding and energetic relationships of *Pollicipes pollicipes* (Gmelin, 1790) (Cirripedia; Lepadomorpha).

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Rachel J. Norton.

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School of Ocean Sciences
University College of North Wales,
Askew Street, Menai Bridge,
Gwynedd. LL59 5EH
U.K.

Summary

Field and laboratory studies on the morphology, gut contents, ingestion rates and digestion efficiency of *Pollicipes pollicipes* were combined to obtain estimates of the likely range and quality of materials required to sustain this species .

Orientation of *P. pollicipes* on the shores of south-west Portugal appeared to be determined by microtopography. Animals generally faced into the wave backwash. Orientation could be temporarily altered by torsion of the peduncle in response to changes in flow direction, permitting more efficient filter feeding.

Cirral and mouthpart morphology suggested that *P. pollicipes*, *Capitulum mitella* and *Lepas anatifera* were omnivores. Size-related changes in cirral morphology made small juveniles better equipped than adults to feed on small particles. Cirral activity of *P. pollicipes* was investigated. Very slow rhythmic cirral extension (or 'beating') was observed in all *P. pollicipes*, but only in relatively still water; once flow rates exceeded 14 cm s^{-1} all barnacles exhibited prolonged cirral extension. The 'beat' rate was temperature dependent in most animals and larger animals exhibited a lower extension frequency than juveniles. It was concluded that 'beating' was primarily respiratory in function and not a feeding mechanism.

The gut contents of wild *P. pollicipes* included animal and algal material but little inorganic matter. Small organic material predominated in small juveniles while large organic material predominated in adults. The rates of faecal production and growth were much higher in barnacles feeding on zooplankton than on algae and although algal cells were ingested in high numbers, the energy intake was so low that animals barely maintained their body weight.

Digestive efficiencies varied with diet but little with barnacle size. A wide range of digestive enzymes were identified in *P. pollicipes* and *L. anatifera* suggesting that a variety of foods may be digested. Specific enzyme activity was low, characteristic of more carnivorous animals.

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Chapter 1

General Introduction

Barnacles are members of the Cirripedia - the only group of sessile crustaceans. In general, they are found permanently attached to surfaces, such as rock, shell, coral or floating wood, although there are also commensal and parasitic forms. They are, because of their sedentary lifestyle, one of the most aberrant groups of Crustacea. In fact, it was not until 1830, when the larval stages were discovered, that barnacles were recognised as crustaceans at all (Barnes, 1987). It was Louis Agassiz who described barnacles as "nothing more than a little shrimp-like animal, standing on its head in a limestone house and kicking food into its mouth". Barnacles show many adaptations to a sessile existence the most obvious being cirri for suspension feeding, a very long tubular penis and protective calcareous shell plates.

The Class Cirripedia can be divided into three orders: Acrothoracica, Rhizocephala and Thoracica. The first three are all naked forms that burrow into rocks or parasitise invertebrates while the last includes all the common forms. The Thoracican barnacles are non-parasitic or live commensally and the order may be further divided into three Suborders: the Lepadomorpha (stalked or pedunculated forms), the Verrucomorpha and the Balanomorpha. The latter two are known as the sessile or acorn barnacles.

Stalked barnacles consist of a capitulum, sitting on top of the peduncle. The capitulum is generally an oval-shaped carapace which contains all but the pre-oral part of the body; it contains a sac (mantle lining) which, together with the upper part of the peduncle, encloses the animal's body. The lepadomorph carapace consists of many pairs of calcareous shell plates, the largest being the terga and scuta, connected with strips of cuticular membrane characteristic of all crustacean exoskeletons (see Darwin, 1851). The carapace valves may be held together for protection, or opened to permit the protrusion of the thoracic appendages (cirri). The mantle sac lining consists of a chitinous membrane under which a double layer of chorion is held by short, strong transverse bundles of fibres branched at both ends (Darwin, 1851). There is a cuticular membrane, which covers the shell plates of most barnacles but is rapidly abraded from the surface. The peduncle is a muscular, near cylindrical, stalk, bounded by a thick, transparent membrane which may be naked or, in some species, such as *Pollicipes*, covered with whorls of calcareous spines. The peduncular spines are not moulted except in *Lithotrya*. The peduncle is lined with three layers of muscle,

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* Unless designated, all taxonomic authorities are as given in Howson (1987)

longitudinal, transverse and oblique, none of which have transverse striae characteristic of voluntary muscle (Darwin, 1851), these run from the bottom of the peduncle to the base of the capitulum in most species. The peduncle increases in length from the top, just under the capitulum e.g. *Pollicipes polymerus* Sowerby (Chaffee & Lewis, 1988).

The acorn barnacles have no peduncle. The body is attached directly to the substratum at the basis, which may be membranous or calcareous. There is a wall of plates that encircles the animal and is generally topped by two pairs of opercular plates (the terga and scuta). According to Darwin (1851), another difference between acorn and pedunculated cirripedes is that the lepadomorphs have three layers of striaeless muscles continuously surrounding the peduncle but not attached to the terga and scuta. Acorn barnacles have five longitudinal bundles of voluntary muscle (with striae) fixed to the terga and scuta which give the valves power of independent movement. In the pedunculates, the lower shell valves or (if absent) the membranous wall of the capitulum moves when the terga and scuta open or close. The cirri and mouth parts of the lepadomorphs closely resemble those of the balanomorphs, particularly the chthamalids (Darwin, 1851).

Most barnacles cannot move around in search of food and must rely on waves and currents bringing planktonic food to them. Barnacles generally feed by means of cirri: typically six pairs of long, biramous, thoracic appendages that may be extended upwards, through the opening in the mantle cavity into the overlying water, where they are used for suspension feeding. It is movement of the cirri, together with that of the operculum and mouthparts that are the most obvious manifestations of barnacle activity (Anderson & Southward, 1987). The appendages may be divided into two classes according to their function; captorial cirri and maxillipedes. The former are usually the last three or four pairs of cirri and are longer than the first two or three pairs, the maxillipedes. The activity of these two sets of cirri is dissimilar, but together they are responsible for feeding (Crisp & Southward, 1961; Anderson, 1981: Anderson & Anderson, 1985; Anderson & Southward, 1987). Successful feeding also relies on the action of the mouthparts and oral cone, and on co-ordination between these structures and the maxillipedes (Crisp & Southward, 1961; Anderson, 1980a; 1981; Anderson & Southward, 1987). The lepadomorph barnacles feed primarily by cirral extension, relying on external water currents, whilst balanomorphs exhibit cirral beating, actively sweeping the overlying water for food particles.

The stalked barnacles

Darwin (1851) found no grounds for dividing the Lepadidae (the family that existed before being renamed Suborder Lepadomorpha) into subfamilies because of the close affinity between several genera and although he divided the Lepadidae into eleven genera, no one part or set of organs afforded sufficient diagnostic characteristics. Since then, the Lepadomorpha have been divided into 12 extant families (70 genera) and 3 extinct families (12 genera) (see Foster & Buckeridge, 1987). The family Scalpellidae contains 2 extinct and 8 extant subfamilies, including the Pollicipinae, Scalpellinae and Calanticinae.

Nearly half the pedunculate cirripedes are found attached to floating objects, the rest attach to fixed substrata. The lepadomorphs extend all over the world, with many species having large geographic ranges, particularly those that attach to floating objects. A greater number of species inhabit the warmer temperate and tropical seas. *Scalpellum* species are generally found in deep water, while most *Pollicipes*, *Ibla* and *Lithotrya* are littoral. *Lithotrya* burrow into rock, coral and shell. *Analasma* lives with its peduncle embedded into shark flesh and *Conchoderma* attaches to Cetaceans (Darwin, 1851).

The stalked barnacles are considered more primitive than the non-stalked thoracicans. The ancestral barnacle was probably a cypris-like, bivalved crustacean attached to the substratum by its first antennae (Barnes, 1987). Initially, each valve was covered by a single plate which became divided, perhaps to permit the opening of the valves and cirral extension without exposing the whole body. The ventral aperture became guarded by the terga and scuta. From the ancestral barnacle, two lines evolved, one becoming the existing stalked, scalpellid barnacles and the other to the sessile barnacles (Newman, 1987).

According to Darwin (1851), the Lepadidae are much more ancient than the Balanidae and were at their most diverse during the Cretaceous when many species of *Scalpellum*, *Pollicipes* and *Loricula* existed. *Pollicipes* is the oldest genus, found in the lower Oolite, perhaps in the Lias.

Pollicipes

Prior to 1851, the genus *Pollicipes* had been variously known as *Lepas*, *Anatifa*, *Mitella*, *Ramphidiona*, *Polylepas* and *Capitulum* (see Darwin, 1851). Although *Mitella* and *Ramphidiona* were both prior to *Pollicipes* Leach, the third name had been universally adopted throughout Europe and North America and extensively used in

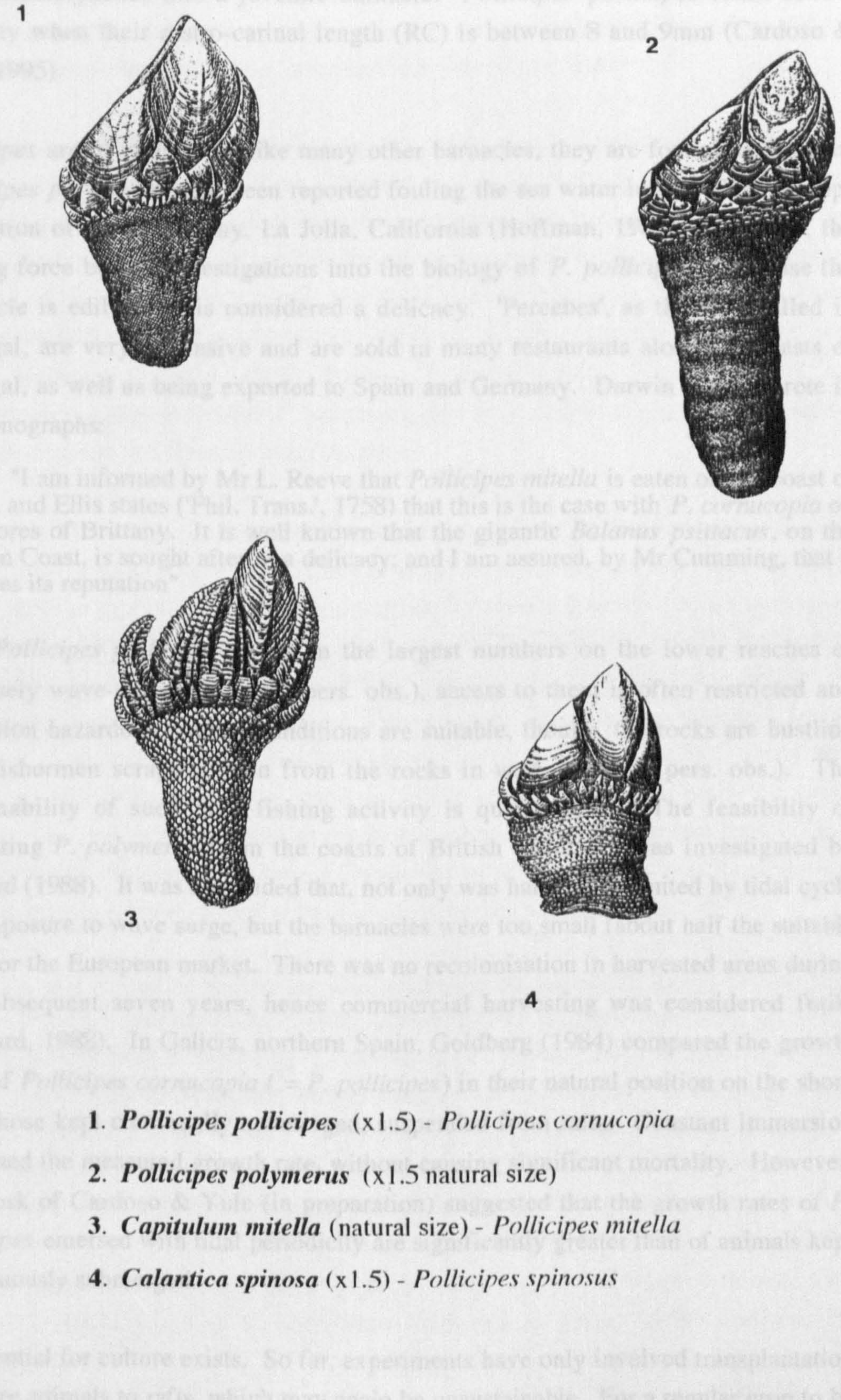
geology so Darwin decided it was futile to attempt to change the name in observance of British Association Rules of Nomenclature. The genus *Pollicipes* was originally proposed by Sir John Hill in 1752 (see Darwin, 1851).

When Darwin wrote his monograph on the pedunculated cirripedes in 1851, he presented the genus *Pollicipes* as containing six species: *P. cornucopia*, *P. polymerus*, *P. elegans*, *P. mitella*, *P. sertus* and *P. spinosus*. However, he suggested that these species could naturally be divided into three different genera: one containing *P. cornucopia*, *P. polymerus* and *P. elegans*; the second containing *P. mitella* and the third *P. spinosus* and *P. sertus*. The genus *Pollicipes* now contains only three species *P. cornucopia*, renamed *P. pollicipes*, Gmelin *P. polymerus* and *P. elegans*. *P. mitella* was renamed *Capitulum mitella* (Foster & Buckeridge, 1987). The examination of large numbers of the three described species of *Pollicipes* from New Zealand (*P. spinosus*, *P.sertus* Darwin, 1851 and *P. darwinii* Hutt) led Batham (1945) to conclude that all the *Pollicipes* in New Zealand were the same species and that priority should be given to the name *Pollicipes spinosus*. *P. spinosus* was renamed *Calantica spinosa* (Quoy & Gaimard). Thus the division of *Pollicipes* into three different genera occurred as suggested by Darwin. Figure 1.1 shows the drawings of *Pollicipes pollicipes* and related barnacles that appeared in Darwin's monograph (1851). The three present *Pollicipes* species are closely related not only to their old con-generics but also to *Scalpellum* and *Lithotrya* (Darwin, 1951).

Pollicipes pollicipes has appeared in the literature under several names: *P. cornucopia* Leach, 1824, *Pollicipes smythii* var Leach, *Lepas pollicipes* Gmelin, 1789 and *Lepas gallorum* Spengler, 1790. *Pollicipes pollicipes* is found on rocky outcrops on exposed, wave-swept coasts in France, Portugal, Spain and North Africa. Although Darwin (1851) also reported that specimens had been found in England (on the bottom of a wrecked vessel in Dartmouth), in Ireland (attached to woodwork near Dublin) and in Scotland (on driftwood in the Firth of Forth), they are not indigenous in the British Isles. *P. pollicipes* are found forming dense clumps, attached directly to rock, mussel shells or other sessile animals. Juveniles are often attached to the peduncle of conspecific adults.

The ecology of this species is very similar to that of *Pollicipes polymerus* (Sowerby) which is found in large numbers in exposed situations particularly on the *Mytilus californianus* beds of the Pacific coast of North America, from Washington State to Baja California (Barnes & Reese, 1959; Lewis & Chia, 1981). *P. polymerus* is usually restricted to between mid- and low-tide marks in exposed situations and is often absent in quiet and sheltered areas (Barnes & Reese, 1959).

Figure 1.1. Drawings of *Pollicipes pollicipes* and closely related barnacles that were at one time all classified *Pollicipes* (from Darwin, 1851).



1. ***Pollicipes pollicipes*** (x1.5) - *Pollicipes cornucopia*
2. ***Pollicipes polymerus*** (x1.5 natural size)
3. ***Capitulum mitella*** (natural size) - *Pollicipes mitella*
4. ***Calantica spinosa*** (x1.5) - *Pollicipes spinosus*

Pollicipes are hermaphrodite but are obligate cross-fertilisers (A.C. Cardoso pers.comm.). They breed in the wild from April to November and produce nauplii with six planktotrophic stages which moult into the cypris larva. The cyprid settles and metamorphoses into a juvenile barnacle. *Pollicipes pollicipes* reach sexual maturity when their rostro-carinal length (RC) is between 8 and 9mm (Cardoso & Yule, 1995).

Pollicipes are of interest as, like many other barnacles, they are fouling organisms. *Pollicipes polymerus* have been reported fouling the sea water intake of the Scripps Institution of Oceanography, La Jolla, California (Hoffman, 1985). However, the driving force behind investigations into the biology of *P. pollicipes* is because the peduncle is edible and is considered a delicacy. 'Percebes', as they are called in Portugal, are very expensive and are sold in many restaurants along the coasts of Portugal, as well as being exported to Spain and Germany. Darwin (1851) wrote in his monographs:

> "I am informed by Mr L. Reeve that *Pollicipes mitella* is eaten on the coast of China, and Ellis states ('Phil. Trans.', 1758) that this is the case with *P. cornucopia* on the shores of Brittany. It is well known that the gigantic *Balanus psittacus*, on the Chilean Coast, is sought after as a delicacy; and I am assured, by Mr Cumming, that it deserves its reputation"

With *Pollicipes pollicipes* living in the largest numbers on the lower reaches of extremely wave-exposed shores (pers. obs.), access to them is often restricted and collection hazardous. When conditions are suitable, though, the rocks are bustling with fishermen scraping them from the rocks in vast numbers (pers. obs.). The sustainability of such high fishing activity is questionable. The feasibility of harvesting *P. polymerus* from the coasts of British Columbia was investigated by Bernard (1988). It was concluded that, not only was harvesting limited by tidal cycle and exposure to wave surge, but the barnacles were too small (about half the suitable size) for the European market. There was no recolonisation in harvested areas during the subsequent seven years, hence commercial harvesting was considered futile (Bernard, 1988). In Galicia, northern Spain, Goldberg (1984) compared the growth rates of *Pollicipes cornucopia* (= *P. pollicipes*) in their natural position on the shore with those kept continually submerged, suspended from rafts. Constant immersion increased the measured growth rate, without causing significant mortality. However, the work of Cardoso & Yule (in preparation) suggested that the growth rates of *P. pollicipes* emersed with tidal periodicity are significantly greater than of animals kept continuously submerged.

A potential for culture exists. So far, experiments have only involved transplantation of shore animals to rafts, which may again be unsustainable. For a regular crop to be

farmed, some means of exploiting the large numbers of larvae released into the sea that never successfully settle, metamorphose or grow to adulthood would be desirable. Alternatively, the spawning of adults, rearing of larvae and obtaining good rates of settlement, spat survival and growth in an artificial environment are required. Until more is known about the biology of *P. pollicipes* and particularly of the young, more sensitive animals the feasibility of farming these barnacles is unknown.

The current study focuses on aspects of the feeding and digestion of the species, looking at both adults and juveniles. The distribution of *Pollicipes pollicipes* on the shore and more particularly the orientation of animals with regard to wave direction have not been documented. *P. polymerus* on the shores of North America have been found to orientate with their cirral nets facing away from oncoming waves thus filtering particles from the water as it runs back off the rocks after the wave has broken (Howard & Scott, 1959; Barnes & Reese, 1960). The similarity of morphology and habitat between *P. polymerus* and *P. pollicipes* might suggest similar micro-distribution and orientation on the shore.

Although all barnacles have six feeding limbs which may be divided into captorial cirri and maxillipedes, the detailed structure of the cirri, mouthparts and oral cone vary from species to species and have been described for several species of acorn barnacles (see Darwin, 1854; Stubbings, 1975; Anderson, 1978; Anderson, 1980b; Anderson, 1981; Anderson & Anderson, 1985). A brief description of the limb morphology of many pedunculate barnacles including *Pollicipes cornucopia* (= *P. pollicipes*), *Lepas anatifera*, *P. mitella* (= *Capitulum mitella*) was given by Darwin (1851). Cirral and mouthpart morphology tends to correlate with diet and ecology in many barnacle species. The current study aimed to produce a more detailed comparison of the limb morphology of *Pollicipes pollicipes*, *Capitulum mitella* and *Lepas anatifera* with a view to assessing potential food sources and mechanisms of capture.

The cirral activity of *P. polymerus* was described by Barnes & Reese (1959) and Lewis (1981). A juvenile to adult shift in feeding strategy characterised by a change in cirral activity from limb beating to constant limb extension was described by Lewis (1981) and a similar phenomenon was noted in a small number of *P. pollicipes* by Hui (1983). In addition to observation of cirral activity in juvenile and adult *P. pollicipes*, the present study examined the cirral morphology in *P. pollicipes* of various sizes to investigate the morphological evidence for any shift in diet or feeding strategy.

Little was known about the diet of *P. pollicipes* although it was believed that they are primarily carnivorous like *P. polymerus* (Howard & Scott, 1959) and can be

maintained in the laboratory, virtually indefinitely, on a diet of *Artemia* alone (A.B. Yule, pers comm.). The diet of *P. pollicipes* was investigated during the present study by examination of the gut contents of wild animals and through feeding experiments in captivity. It was the aim to obtain further evidence to support or refute the proposed shift in feeding strategy (Lewis, 1981). Much work has been done on the digestive efficiency and the gut enzymes of Crustacea, particularly those species that are widely cultured for human consumption. Digestive efficiencies of over 90% have been reported for several species of barnacle (see Kuznetsova, 1973; Wu & Levings, 1978 and Page, 1983). However, it seems that some dietary components (e.g. protein of animal origin) are digested and absorbed more readily than others (e.g. pure fats and starch) (Crisp & Southward, 1961). The ability of *P. pollicipes* to digest and absorb the nutritional components of its diet may give some indication of whether efficiencies could be increased by the use of enriched diets. Reference is made in the literature to the digestive enzymes of two species of acorn barnacle (DeVillez & Buschlen, 1967; Kristensen, 1972), no published material appears to be available on the digestive enzymes of pedunculate barnacles. The range of enzyme activity associated with size or accompanying different diets in *P. pollicipes* was therefore investigated.

The general aims of the study are thus to combine field and laboratory studies on the morphology, gut contents and ingestion rates of *P. pollicipes* to obtain reasonable estimates of the likely range and quality of materials needed to sustain healthy adults. A focus is clearly made on the juveniles which would represent a critical stage for any aquaculture project for the species. An understanding of the post-ingestion processes, i.e. digestion, is also essential for optimising feeding practice and dietary composition if farming *P. pollicipes* is to be successful.

Chapter 2

General materials & methods

Juvenile collection and maintenance

Juvenile *Pollicipes pollicipes* were collected from the south-west coast of Portugal and were brought back to Menai Bridge during the summer of 1992. At various times during the subsequent 3 years more animals were collected and brought back. Individuals were attached to small squares of Velcro by means of Superglue. Each animal was numbered and then mounted on a strip of Velcro stuck to the inside walls of a plastic container. A strong recirculating water current was passed through the container which was maintained in a seawater bath at 20°C. A trickle of fresh seawater maintained a constant turnover of water in the bath. *Artemia* sp. were added daily (~ 8 x 10^5 animals) and a 2 litre mixed algal culture of *Rhinomonas reticulata* (Lucas) Novarino (1000 cells μl^{-1}) and *Skeletonema costatum* Cleve (2000 cells μl^{-1}) was added continuously from a drip tank.

The population was continually fouled by small anemones which had to be removed gently by brushing the barnacles with an old toothbrush every two months. At similar intervals the animals were removed from their tank which was then cleaned with sodium hypochlorite solution, rinsed and refilled. The cleaning reduced the anemone fouling and controlled potential pathogens. Despite cleaning, the entire barnacle population was wiped out in the Spring of 1994 by a white fungal infection which initially appeared to invade any damaged peduncular tissue then rapidly spread to apparently healthy animals.

New stock animals were thence kept in a larger tank, once again with recirculating water and trickled throughflow. The barnacles were again glued onto Velcro but this time the other piece of Velcro was glued onto a length of monofilament fishing line which in turn was attached to a cane that was laid across the top of the tank (see Fig 2.1a & b). Animals were identified by numbers on the cane and on the Velcro to which the animal was attached. It was hoped that the larger body of water surrounding the animals would prevent any catastrophic spread of infection before curative action could be taken. The animals were however, subjected to less water flow. Subsequent mortality rates were low (roughly two animals per month). *Lepas anatifera* washed up in South Wales attached to lengths of rope (see Fig 2.2) were transported to Menai Bridge and maintained in the laboratory in the tank with *P. pollicipes*. The animals were left on the rope until they were required and they were

then gently detached without damaging the peduncle and attached to Velcro and treated the same as the *P. pollicipes* (Figure 2.1 a & b).

Changes in the animals appearance occurred once they were brought into the laboratory. *P. pollicipes* in the wild are rarely fouled and opercular flaps are often bright red in colour. After two months in the laboratory the animals began to get fouled by algae, hydrozoans and anemones and the bright coloration of the opercular flaps faded. After many months, even though moulting was regular, the capitular plates developed white translucent deposits around their margins, extending from the edges. The deposits appeared to be growth but of malformed shell. A similar phenomenon was observed in the *Lepas anatifera* kept in the laboratory. For these reasons recently collected animals were used for experiments when possible as it was felt that animals kept under what are obviously suboptimal conditions for any length of time may exhibit abnormal behaviour.

Measurement of *Pollicipes pollicipes.*

The distance from the base of the rostrum to the point were the carinal plate is adjoined by a secondary scute (rostro-carinal length RC see Fig. 2.3) was measured by vernier calipers on all animals and was used as a representative measure of animal size. A similar measure of size was used by Lewis (1981) and RC is roughly proportional to the cubed root of weight. In addition, the distance from the top of the tergal plate to the base of the secondary lateral scutes was also measured (capitulum height - Fig. 2.3). These measures were chosen as they were found to be least variable (A.C. Cardoso).

Seawater

In the laboratory at Menai Bridge all seawater used is allowed to stand in settling tanks for three days to remove silt before being passed through a sand and gravel biological filter which removes large organic and inorganic particles. Three in-line Gelman pleated filters of 10, 1 and 0.2 μm mesh size remove organisms. The water then passes through a 245 nm UV radiation 'sterilising' unit which kills many of the remaining micro-organisms, and is stored in a 300 l reservoir. Stored water passes through the UV unit once more before use. The system is steam cleaned on a weekly basis to kill any contaminating bacterial populations. This UV-irradiated, fine-filtered sea water (UVFSW) was used for algal culture and all experimental procedures and has an almost invariant salinity of 32 ppt.

Figure 2.1. The system used for the storage of *Pollicipes pollicipes* and *Lepas anatifera* in the laboratory in Menai Bridge.

a) *P. pollicipes* and *L. anatifera* suspended on lengths of monofilament.

b) Tank in which *P. pollicipes* and *L. anatifera* are maintained at a constant temperature of 20°C. The batons from which the barnacles are suspended can be seen running across the top of the tank.

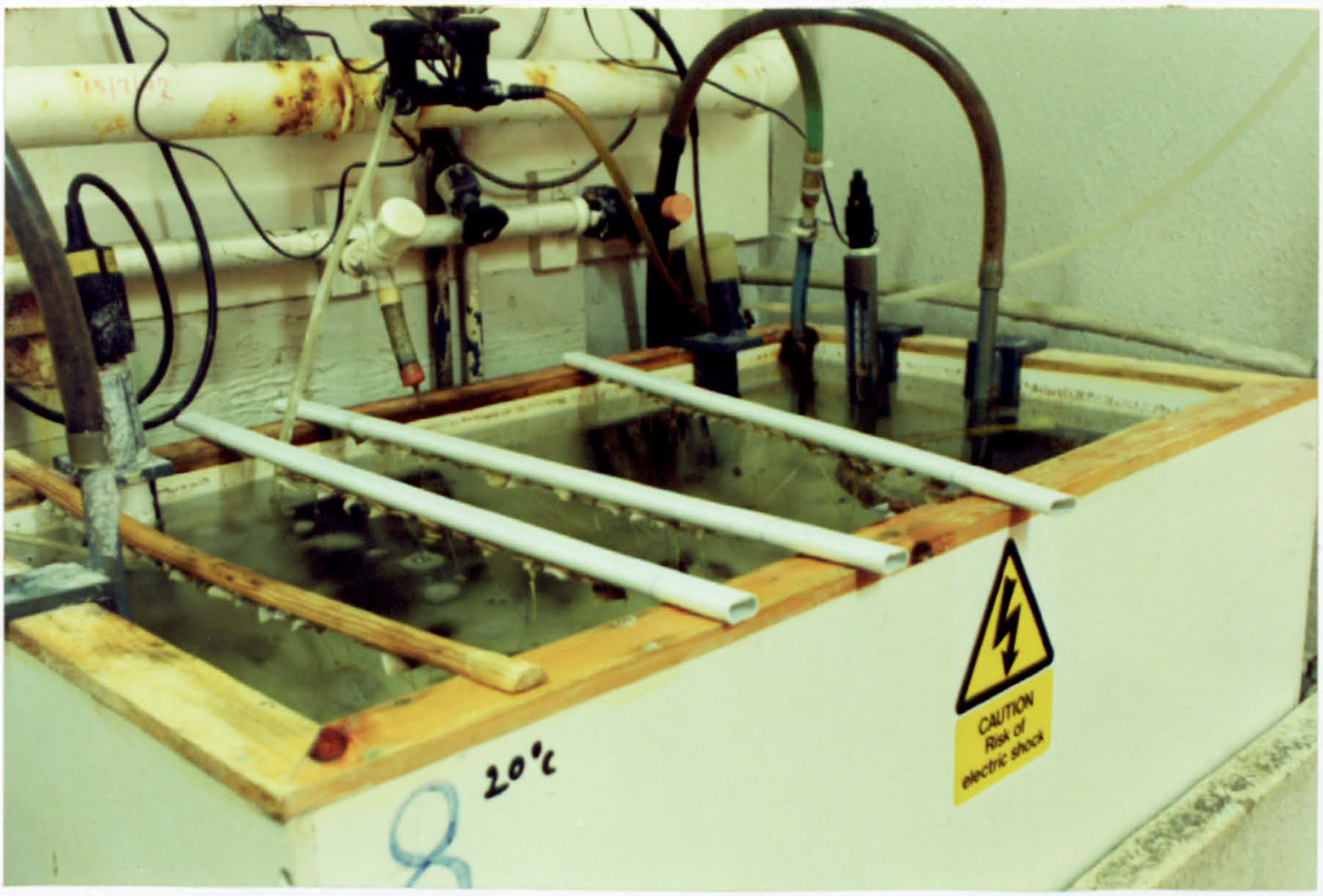
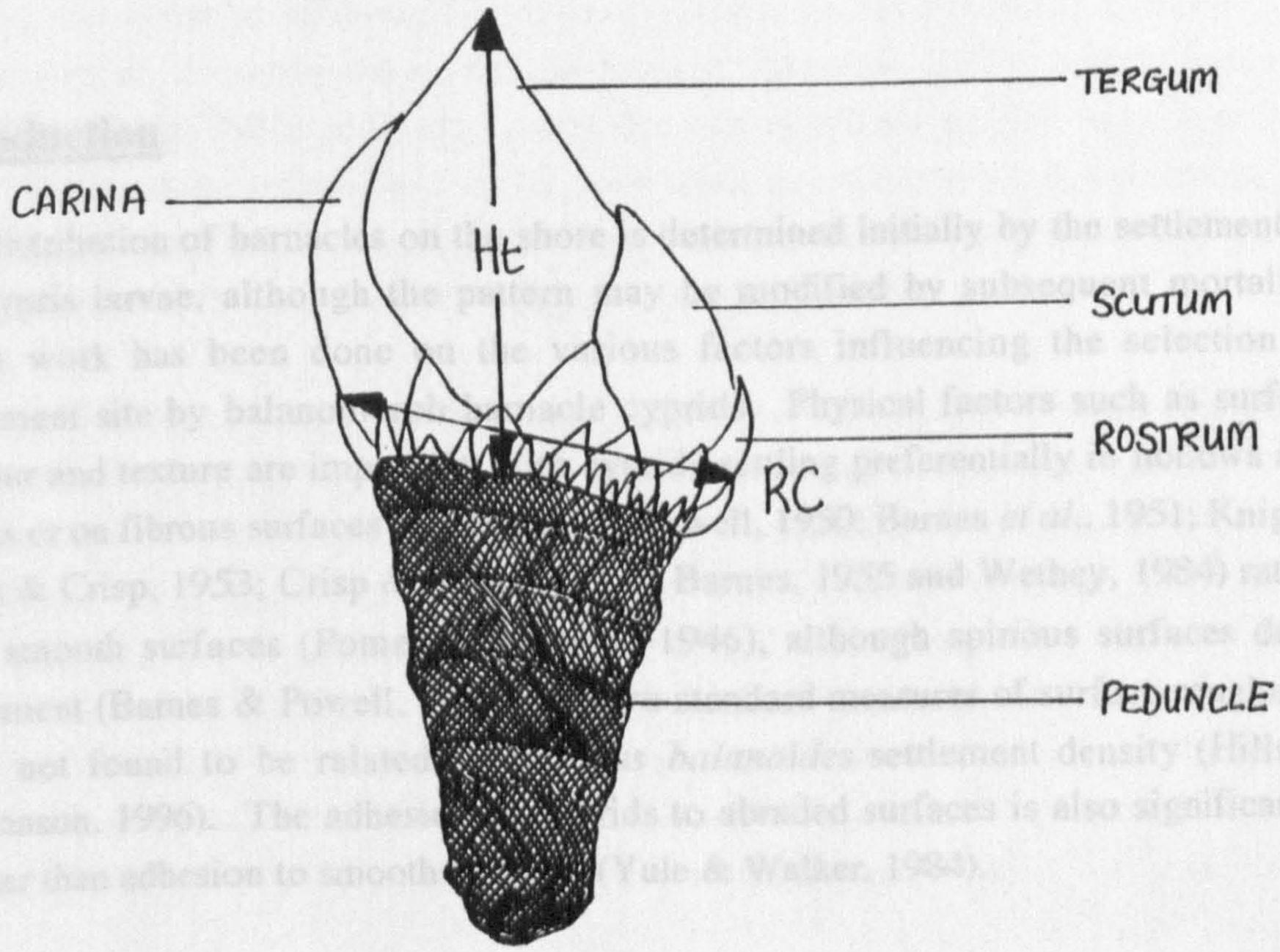


Figure 2.2. Length of rope bearing *Lepas anatifera*.

Figure 2.3. Diagram of a *P. pollicipes* indicating the measured capitular dimensions - capitulum height (Ht) and rostro-carinal length (RC).



Chapter 3

The distribution and orientation of *Pollicipes pollicipes* on the shore.

Introduction

The distribution of barnacles on the shore is determined initially by the settlement of the cypris larvae, although the pattern may be modified by subsequent mortality. Much work has been done on the various factors influencing the selection of settlement site by balanomorph barnacle cyprids. Physical factors such as surface contour and texture are important, with cyprids settling preferentially in hollows and cracks or on fibrous surfaces (see Barnes & Powell, 1950; Barnes *et al.*, 1951; Knight-Jones & Crisp, 1953; Crisp & Barnes, 1954; Barnes, 1955 and Wethey, 1984) rather than smooth surfaces (Pomerat & Weiss, 1946), although spinous surfaces deter settlement (Barnes & Powell, 1950) and two standard measures of surface roughness were not found to be related to *Balanus balanoides* settlement density (Hills & Thomason, 1996). The adhesion of cyprids to abraded surfaces is also significantly greater than adhesion to smooth surfaces (Yule & Walker, 1984).

Light has also been shown to influence settlement in *Balanus eburneus* Gould with cyprids settling preferentially on dark surfaces (Pomerat & Reiner, 1942). Similar settlement is exhibited by *B. improvisus* (Weiss, 1947). The cyprids of *Balanus balanoides* (Barnes *et al.*, 1951) and *Elminius modestus* (Crisp & Ritz, 1973) are photopositive at settlement whereas the cyprids of many barnacle species prefer to settle on darker surfaces (Visscher, 1928; McDougall, 1943; Smith, 1948; Skerman, 1958) or on surfaces shaded from light (Gregg, 1945). Yule & Walker, (1984) found the number of *B. balanoides* cyprids exploring and the force required to remove temporarily attached cyprids, was significantly less for white, yellow and blue panels than black and red panels. Visscher & Luce (1928) showed that the peak spectral sensitivity of barnacle cyprids to be in the blue/green part of the spectrum.

Water currents are also involved in barnacle settlement as cyprids are responsive to both the magnitude and direction of flow (Moore, 1933; Crisp, 1955; Rittschof *et al.*, 1984; Eckman *et al.*, 1990) and it was suggested by Forbes *et al.* (1971) that the relative importance of the physical factors in influencing settlement are contour>light>current. However, the presence of a chemical factor may override the physical stimulus given by a surface (see Yule & Walker, 1984).

Moore (1939) observed that barnacles colonised bare areas of substratum more slowly than areas partially covered with other organisms. Burton (1949) suggested that *Balanus balanoides*, as well as many other marine organisms, settle gregariously, that is, settle preferentially close to conspecifics. Gregarious settlement of *Elminius modestus* and other cyprids in nature and in the laboratory has been clearly demonstrated by Knight-Jones & Stevenson (1950), Knight-Jones (1953) and Crisp (1961) and it was concluded that cyprid recognition of quinone-tanned protein in the epicuticle of the barnacle shell was responsible (Knight-Jones, 1953; Knight-Jones & Crisp, 1953). Knight-Jones (1953) suggested that the settlement factor was also present throughout the body tissues.

Aqueous extracts of barnacle and arthropod tissues, adsorbed onto slate panels, were found to be highly active settlement promoters (Crisp & Meadows, 1962) but not when in solution (Crisp & Meadows, 1963). The settling factor was cautiously identified by Crisp & Meadows (1962) as the water soluble protein fraction of the cuticle, classed as arthropodin by Fraenkel & Rudall (1940; 1947). Purification of extracts revealed groups of protein-carbohydrate and protein-nucleic acid complexes (Gabbott & Larman, 1971; Larman & Gabbott, 1975; Larman *et al.*, 1982). Indeed, such complexes were found in all the animal extracts which induced cyprid settlement (Larman & Gabbott, 1975; Larman, 1984).

Crisp & Meadows (1962) found that the cyprids of *Balanus balanoides* settle on extracts of cirripedes in preference to extracts of other arthropods. *B. balanoides* and *B. amphitrite* cyprids were found to discriminate between extracts of different barnacle species (Crisp & Meadows, 1962; Larman & Gabbott, 1975; Crisp, 1990) while *Elminius modestus* did not (Larman & Gabbott, 1975). The precise mechanism by which cyprids are able to recognise adsorbed protein is unknown although two theories have been proposed (see Crisp & Meadows, 1963; Nott & Foster, 1969; Gibson & Nott, 1971 and Crisp, 1974; 1975).

Yule & Crisp (1983a) measured the strength of temporary adhesion of *Balanus balanoides* cyprids and found that the force required to detach cyprids from arthropodin-treated slates was significantly greater than that required to detach them from clean slates. Such evidence tends to support Crisp's (1974) suggestion that cyprids are able to assess the physico-chemical properties of a substratum from the strength of adhesion between their antennules and the substratum. Although molecular adhesion is undoubtedly measured, voluntary action on the part of the cyprid can precipitate detachment (Yule & Crisp, 1983a; Yule & Walker, 1984),

magnitude of the forces measured ~~are~~ is a combination of adhesion and cypris behaviour (Neal & Yule, 1994).

Once cyprids have settled and metamorphosed into spat, their distribution may be modified by mortality mediated by predation, desiccation and inter- and intraspecific competition. The cyprids of *Pollicipes polymerus* tend not to settle on rocky substrata (Hoffman, 1988) but generally settle on the peduncles of conspecifics (Lewis, 1975; Hoffman, 1984, 1988; Chaffee & Lewis, 1988). The larvae tend to aggregate at the proximal end of the peduncle under the carinal and rostral margins of the capitulum (Hoffman, 1988). This is a region of growth where new capitulum is produced and it may be that the new cuticle attracts the cyprids (Darwin, 1851; Crisp, 1965, 1974; Hoffman, 1984). It is also a region of the barnacle that is not in contact with adjacent animals and is therefore available as a settlement site (Hoffman, 1988). The interlocking peduncular scales may also provide a favourably textured surface for the settling cyprids. From this position on the adult peduncle the juveniles are protected from predation and desiccation while still being able to feed. As the cyprids grow they produce peduncular extensions (Hoffman, 1984) and slowly migrate down onto the rocky substrata where they ultimately become attached when about 9 mm in rostro carinal length (Hoffman, 1988). *Pollicipes pollicipes* larvae also settle on the peduncles of adult conspecifics and their ability to move following settlement has been demonstrated by Kugele & Yule (1993).

The factors once thought to limit the population size and distribution of *Pollicipes polymerus* on the Pacific coast of North America were the shortage of areas with suitable hydrodynamic conditions (Barnes & Reese, 1960) and settling space (Paine, 1966, Dayton, 1971; Paine 1974; Hoffman, 1984). *P. polymerus* are generally found in dense communities with *Mytilus californianus* Conrad (Ricketts *et al.*, 1985) and competition with *M. californianus* influences the distribution of *P. polymerus* on the shore. Recent work suggests that the interaction of interspecific competition and bird predation determines *P. polymerus* distribution (Meese, 1993; Wootton, 1993), with bird predation the primary factor on some shores, and competition with mussels more important on others (Meese, 1993).

The orientation of barnacles is often related to the direction of prevailing conditions of water movement. Crisp (1953) noted that adult populations of *Balanus balanoides*, *Balanus crenatus* and *Elminius modestus* are orientated so that the majority have the carina pointing away from the prevailing current and the cirral net facing the current. Naturally occurring populations of epizoic barnacles generally orientate to external

water currents or the respiratory water currents of the host (Crisp & Stubbings, 1957; Dinamani, 1964; Bowers, 1968; Ross & Jackson, 1972). Current, to some extent, also influences the orientation of cyprids at settlement. Settling balanomorph cyprids have been observed to orientate with their posterior end into the prevailing flow so that after metamorphosis the current is directed into the cirral net (Moore, 1933; Crisp, 1953; 1955). However, water movement also promotes rotation of metamorphosed balanomorph barnacles, modifying orientation through subsequent growth (Crisp & Stubbings, 1957).

Water movement was found to be the major orientation stimulus for the sub-littoral barnacle *Balanus trigonus* Darwin, which orientates at right angles to the axis of wave surge. The cirral net is able to rotate through 90° in each direction to allow the exploitation of the incoming and outgoing wave water flow (Ayling, 1976). The intertidal barnacle, *Tesseropora rosea* Krauss exhibits strongly directional orientation on vertical and sloping surfaces (Otway & Underwood, 1987), such that their cirral nets always face into the wave backwash.

Orientation in response to water currents has also been seen in lepadomorph barnacles and may result from any of three factors: orientation at settlement, modification of orientation by growth subsequent to metamorphosis and changes in body attitude, under muscular control. In the field, *Pollicipes polymerus* were found, in all but sheltered locations, to orientate with their cirral net at 180° to the direction of incoming waves. The anterior face of the net filtered the wave water as it poured off rocks (Howard & Scott, 1959; Barnes & Reese, 1960). The orientation observed was inconsistent with respect to the shore or general wave direction, but was determined by the microtopography at each site. In the laboratory, Barnes & Reese (1960) showed that when a jet of water was directed laterally at the cirral net, the whole capitulum slowly twisted until the net faced the jet. No twisting occurred if the jet was aimed at the peduncle or when the capitulum was tied shut with twine. Barnes & Reese (1960) speculated that the receptors of the stimulus were located either on the cirri themselves or on the body of the animal which is exposed to the current during feeding.

Preliminary laboratory studies on the feeding behaviour of *Pollicipes pollicipes* (M. Cottam, pers. comm.) suggested orientation to current. When an unfed specimen was placed in sub-optimal orientation to a current, more favourable orientation was effected by torsion of the peduncle. Torsion through 180° was observed in just 10 minutes in the starved animal, resulting in the presentation of the full cirral net to the water flow. No such torsion or orientation behaviour was apparent in an animal that

had been fed to satiation prior to the experiment. The torsion was under muscular control and therefore only temporary. It has been shown that *Pollicipes pollicipes* placed at 180° to the direction of water flow would, over a 4-6 week period, turn around by swivelling the base of the peduncle relative to the substratum (M. Kugele, pers comm.). The reorientation is only reversible after a 4-6 week period with the flow in the opposite direction.

The aim of the study was to assess the distribution of *Pollicipes pollicipes* on the shore at several sites in south-west Portugal and to investigate orientation to prevailing water currents. The field observations were supplemented by manipulation of orientation in the laboratory. The factors influencing the ability of *P. pollicipes* to temporarily modify body attitude over short periods of time were also investigated.

Materials & Methods

Distribution on the shore

Distribution of animals on the shore was observed primarily at 3 sites on the south-west coast of Portugal; Praia do Castelejo, Ponta da Fisga and at a shore, between Praia do Tonel and Ponta da Alheta near to Sagres (see Fig 3.1). Several other shores around Cabo de São Vicente were also visited.

General, subjective, observations were made regarding the distribution of animals on the shore, the position on the shore, zonation, degree of wave exposure, distribution in relation to topography and the position of juveniles within the population. Photographs were taken where possible to illustrate the features.

Orientation on the shore

Groups of animals on the shore were examined and observations made regarding the orientation of animals within groups and between nearby groups. The sites were observed when the tide was high and the directions of water movement noted as waves broke and then ran off the rocks. Photographs were taken at Ponta da Fisga (see Fig 3.2) and near Sagres (Fig 3.4 b). Scale drawings were made of small areas (~ 50 x 30 cm) at Ponta da Fisga to illustrate the orientation of barnacles in relation to the microtopography and flow of water from breaking waves.

The direction and degree of orientation exhibited was quantified using the technique previously employed by Barnes *et al.* (1951) and Crisp & Barnes, (1954) for assessing the orientation of balanomorph barnacles and by Barnes & Reese (1960) for *Pollicipes*

Figure 3.1. Map of Southwest Portugal with the three main study sites indicated. Inset shows area covered by main map.

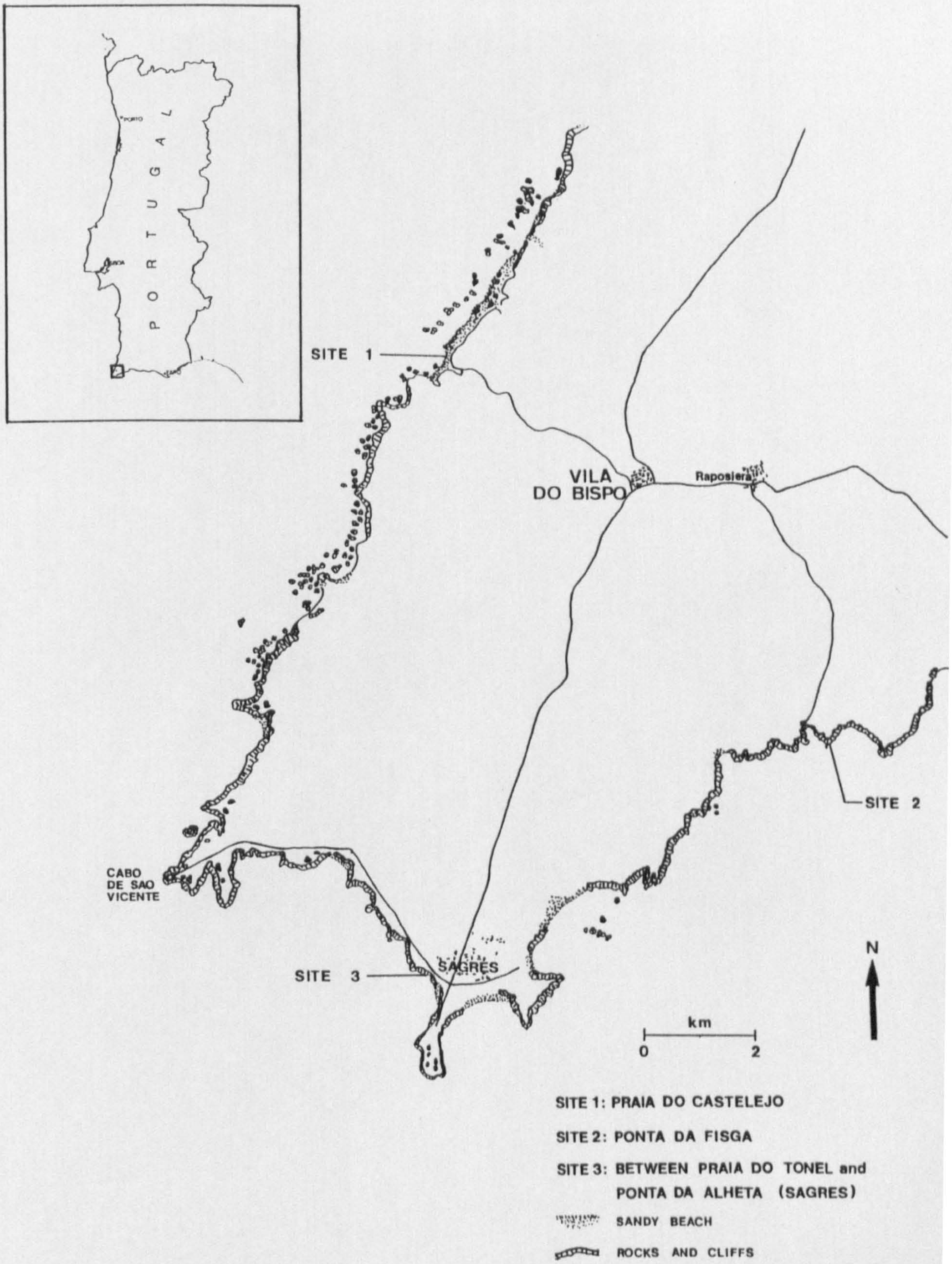


Figure 3.2. Sample site 2 at Ponta da Fisga.

polymerus. Photographs were taken of selected areas with the lens held parallel to the rock surface. A Nikon EM with wide angle (35 mm 1:2.5) lens and macro lens (55mm 1:2.8) was used and the camera was held ca. 10 cm away from the rock surface. No tripod was used due to the uneven rock surfaces and the ever present danger from large Atlantic waves. Areas photographed were as flat as possible to avoid the inevitable distortion on photographs of curved surfaces. The areas were observed at high tide in an attempt to determine the dominant direction of water flow (incoming wave direction and backwash). On each photograph, the angle of opening of the capitulum to the line of global flow (i.e. that of wave direction or run-off rather than that caused by the microtopography) was measured, using a protractor, for 20 - 33 randomly selected individuals. An animal with its cirral net at right angles to the current, anterior face intercepting the stream was designated 180°. The orientation angles (0 - 360°) were treated as vector quantities (with respect to 'cross' and 'along' current components) resolved and summed and the mean values calculated as follows:

If ϕ_i = orientation angle of the i_{th} individual and N = number of individuals.

mean component along current $$A = \frac{\sum_i (\cos \phi_i)}{N}$$

mean component across current $$B = \frac{\sum_i (\sin \phi_i)}{N}$$

The mean direction of sample Ø is given by $$\tan^{-1} \emptyset = B / A$$

The mean vector length $r = \sqrt{(A^2 + B^2)}$ together with the sample size (N) were analysed, using Rayleigh's tables which tests whether the population from which the sample was drawn differs significantly from randomness. Assuming the sample to be reasonably unimodal and symmetric with respect to the mode (i.e. approximating a sample drawn from a von Mises distribution) confidence limits for the mean angle of orientation were calculated (see Appendix 1). The technique could only be used to look at the orientation of the adults as the juveniles were distributed on the adult peduncles, hidden by the capitula and, therefore, not visible in photographs.

Orientation relative to water flow in the laboratory

A unidirectional flow of UVFSW (15 l min^{-1}, equivalent to ca. 10 cm s^{-1}) was passed down a length of plastic guttering (8 cm diameter, 40 cm long) and the water recirculated (see Fig. 3.3a). Even without animals in the gutter, the Velcro anchorages (Fig. 3.3b) disrupted the flow causing turbulence. Animals were secured by their Velcro anchorages onto the inside surface of the gutter and their angle of orientation relative to the direction of water flow was measured. The gutter faced away from a NE facing window and was covered with a translucent perspex sheet to diffuse the light. The initial placement orientation was designated thus:- 0° = cirral net facing into current, 90° = net opening at right angles to the current (left or right) and 180° = cirral net opening away from the current. Prior to use in the observation, animals were maintained in a 1 litre crystallising dish containing aerated UVFSW with a low density of *Artemia* nauplii, at room temperature for 24 hours. Room and water temperature, prior to and during the observations, varied between 10 and 20°C (warmest during the day and coolest at night).

The alteration of orientation by adult and juvenile *Pollicipes pollicipes* over 24 hours.

Ten similarly sized adult *Pollicipes pollicipes* were placed down the length of gutter, 1-2 cm on either side of the midline. All were at 90° to the direction of flow, half facing left and half right. The angle of orientation was measured after 1, 2, 3, 5, 7 and 24 hours. The method of re-orientating to water flow shown by *Pollicipes pollicipes* was described. The observations were then repeated using ten juveniles.

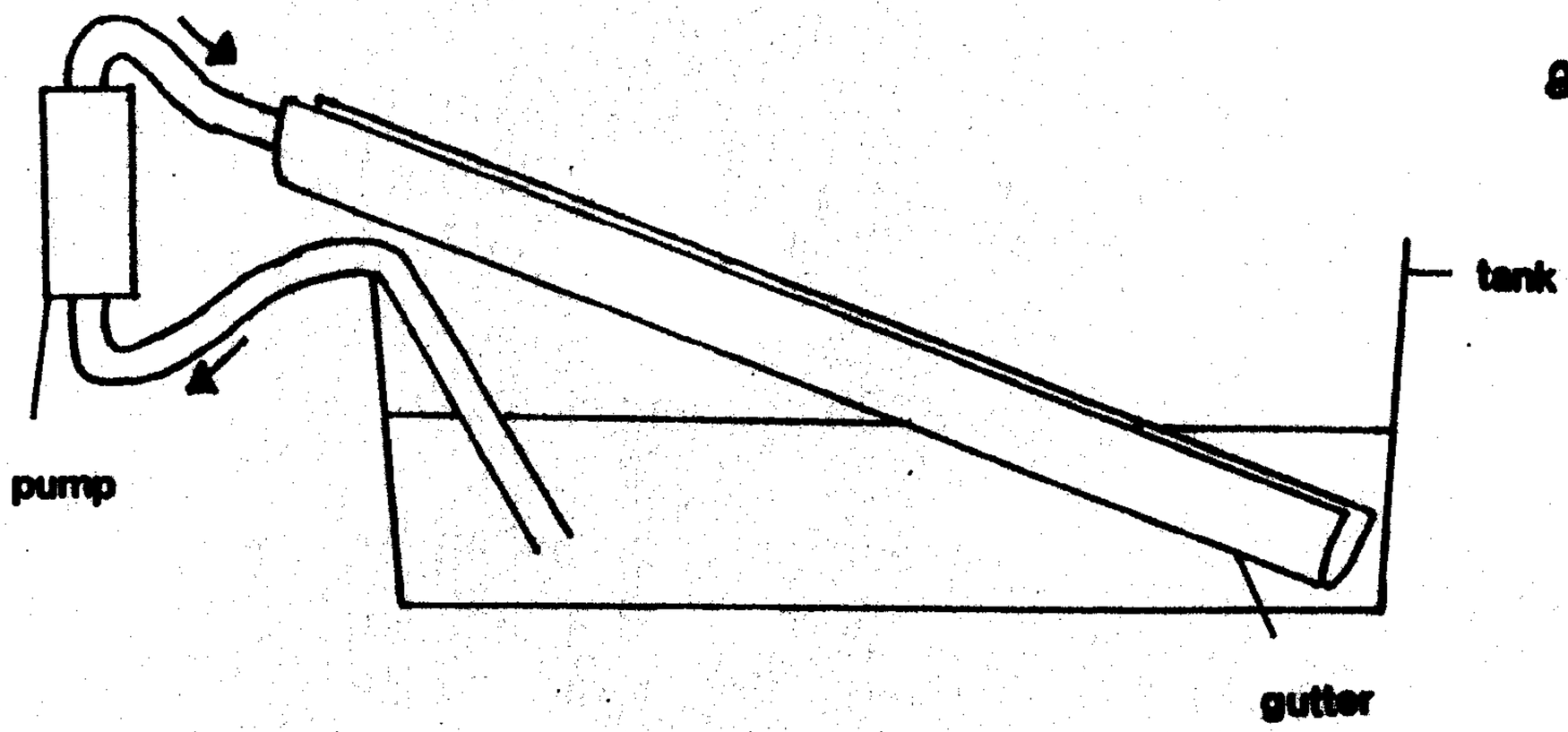
The effect of initial orientation on the degree of turning.

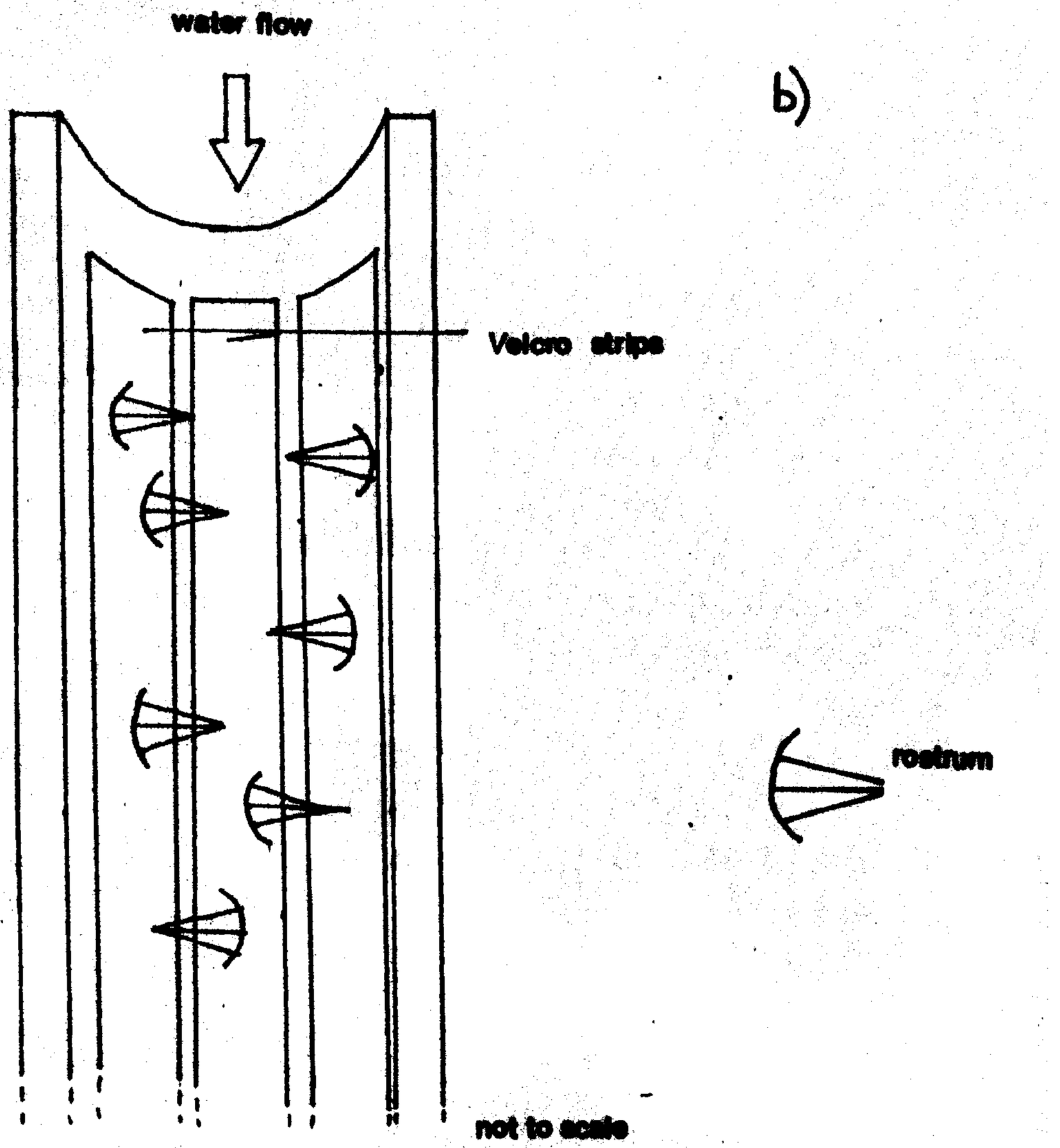
Adult *P. pollicipes* were secured in the gutter at initial angles ranging from 40 - 180° to the flow and their orientations after 24 hours measured. Five adults with similar rostro-carinal lengths were used for each initial orientation and the same five individuals used throughout. Animals were fed with *Artemia* in a crystallising dish for 24 hours between each change in initial orientation.

Size and orientation.

Ten *P. pollicipes* from each of the following size categories <4.00, 4.05 - 6.00, 6.05 - 9.00, >9.00 mm rostro-carinal length (RC) were fed as before and then were placed in the gutter at 90° to the direction of water flow. Single size categories were tested in the gutter at any one time. After 24 hours the angle of orientation was measured.

Figure 3.3 a) Diagram showing the tank, inclined gutter and recirculation pump used for the orientation experiments in the laboratory. b) Diagram of the experimental gutter, viewed from above, indicating how the barnacles were positioned in the experiments.





Orientation with closed opercula

Thirty adult *P. pollicipes* of similar rostro-carinal length were placed in the gutter, ten at a time, at 90° to the direction of water flow. Half the animals had their capitular valves tied shut with twine. After 24 hours the angle of orientation was measured.

Aggregation and orientation

P. pollicipes adults of approximately the same size were maintained in the gutter for 24 hours at three different levels of aggregation. The same 15 adults were placed at 90° to the current flow at distances of 2.5, 1.5 and 0.5 cm apart. The angle of orientation after 24 hours was again measured.

Starvation and orientation

Ten *Pollicipes* were fed overnight on *Artemia* nauplii. They were then placed at 90° to the direction of water flow and after 8 hours their angle of orientation to the flow was measured. Eight hours was chosen as there was no further change in orientation angle after seven hours in the previous tests. The experiment was repeated after the same ten *P. pollicipes* had been starved for 48 hours.

Results

Distribution on the shore

Pollicipes pollicipes in the Algarve were found on very exposed shores. Even on calm days these shores experience considerable residual swell (see Figs 3.4 and 3.5). On the less exposed shores visited, in the lee of Cabo de Såo Vicente, *P. pollicipes* were only found in very localised areas where water movement was expected to be highest (e.g. in crevices and surge channels). The barnacles were found on cliffs and boulders, on the low shore, in a zone about 1.5 m in vertical height and were not conspicuous subtidally. In places where incoming wave water was channelled into a crevice causing an uprushing of water, *P. pollicipes* were found inhabiting a broader zone. Such was the case in a gully at Castelejo (see Fig. 3.6) where the samples were taken for gut contents analysis.

A small number of animals were found inhabiting the edges of low shore rock pools. Few *P. pollicipes* were seen on horizontal surfaces, the majority being located on vertical surfaces. An obvious exception was a large horizontal fissure at Sagres (see Fig. 3.7) where *P. pollicipes* were found encrusting both the upper and lower surfaces. On open horizontal surfaces *Mytilus* was found to dominate. At all three sites, lower numbers of *Pollicipes* were found on open rock surfaces, normally attached to

depressions or cracks in the rock (see Fig. 3.8 a and b) rather than attached to smooth, open surfaces. The greatest densities of *Pollicipes* were found in locations where water movement was highest; in narrowing crevices, vertical channels, under overhangs, tunnels and gullies (Fig. 3.9 a and b).

Pollicipes were also frequently found living within large clumps of small mussels (Fig. 3.10). Animals on the open surfaces were generally found in ones or twos whereas those found in crevices, overhangs and under boulders were found in much larger clumps, 'rosettes' or swathes. Animals living in such shaded localities were generally free from epibionts and often had very bright red mantle flaps. Animals found on open rock surfaces (illuminated) were frequently heavily fouled with filamentous and encrusting red algae, green algae and acorn barnacles and did not have such red flaps.

Pollicipes pollicipes juveniles were not found exclusively attached to adults. Many were attached to adult peduncles and capitula but many more were attached to the encrusting red algae that cover the rock and directly onto the rock itself. A few were even found attached to mussel shells (there were cement tracks, showing evidence of movement). Mussels surrounding small groups of barnacles were removed from the substratum and frequently revealed several tiny juveniles attached to the rock close to the adults, but with no evidence that they had travelled there from the adult peduncles.

Figure 3.4 (a) and (b) The exposed nature of the shore at Ponta da Fisga (Site 2) even on a calm day in late June.

(a) Wave breaking over the sample site shown in Fig. 3.2.

(b) After a wave has broken water drains back down the channels and gullies.

Figure 3.5 (a) and (b). The exposed shore at Site 3, near Sagres. Ponta de Alheta can be seen in the distance in b).

Figure 3.6. The crack at Praia do Castelejo from which animals were taken for gut contents analysis and where *Pollicipes* distribution is extended vertically by the uprushing of water. Scale: 20cm

Figure 3.7. The large horizontal crevice near Sagres from which animals were taken for gut content analysis and where *Pollicipes* were found in unusually large numbers on horizontal surfaces. Scale: 10 cm

Figure 3.8 (a) ***Pollicipes pollicipes*** **living under an overhang and in a crevice at Ponta da Fisga. Scale:** 8 cm

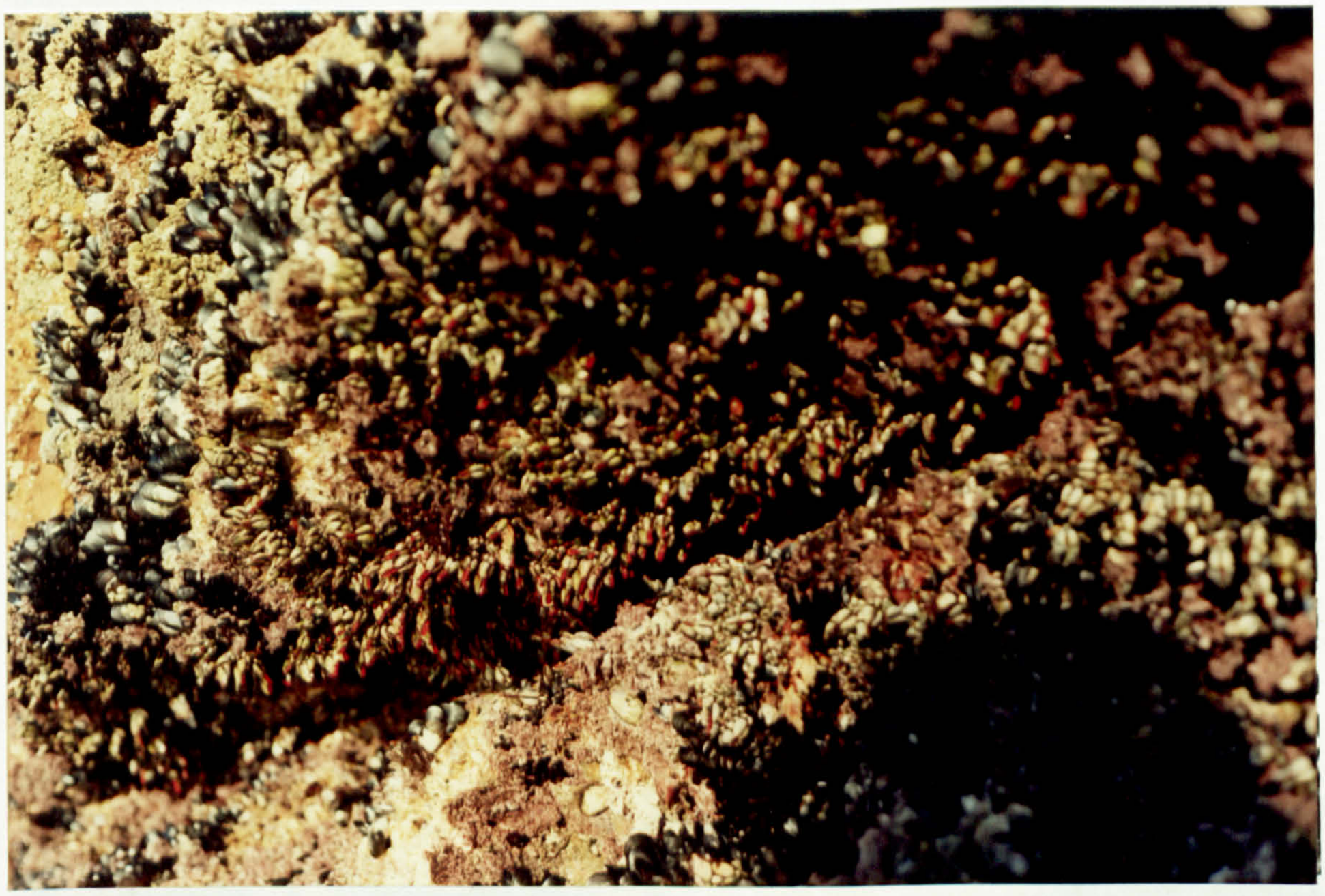
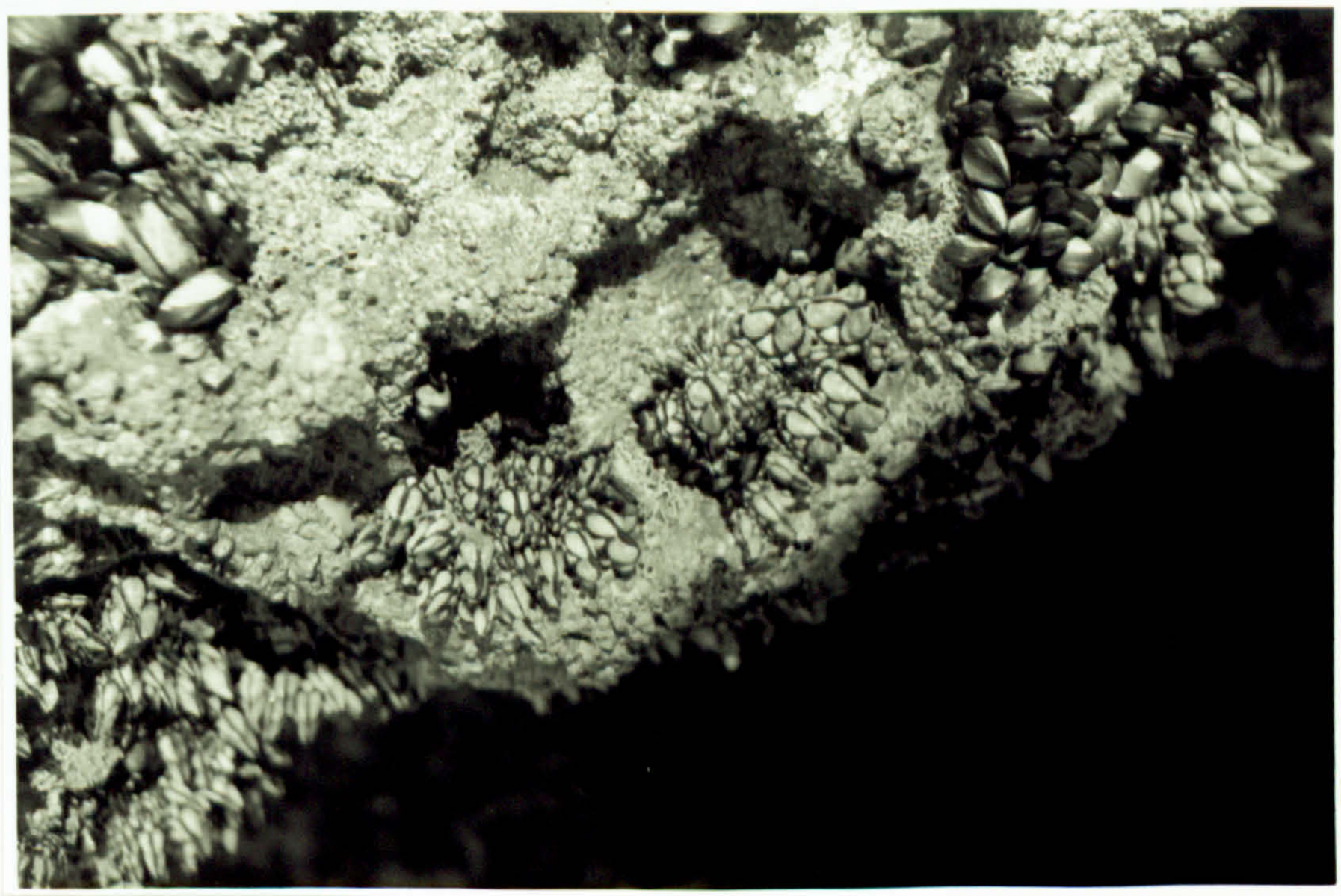
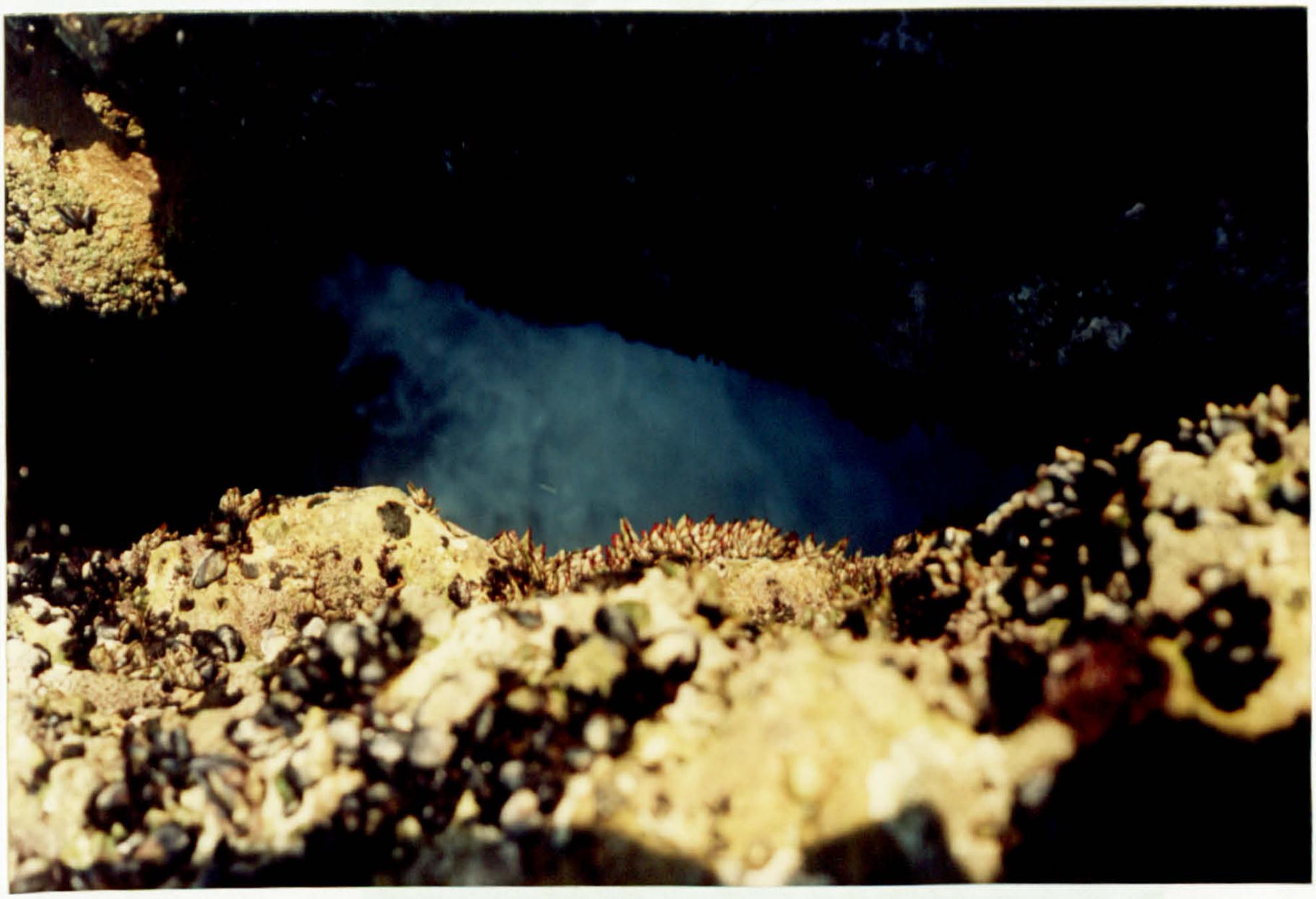


Figure 3.8 (b) ***P. pollicipes*** **living in clumps within depressions in the rock at Ponta da Fisga. Scale:** 2 cm



Figures 3.9 (a) and (b). The concentration of *P. pollicipes* in regions of high water movement.

(a) Vertical crack behind a boulder at Site 3, near Sagres. Barnacles can be seen in greater numbers within the crack. Scale: 6cm



(b) Tunnel-like hole in leading edge of rock at Ponta da Fisga. *Pollicipes* did not occur on the open rock surface but were densely packed inside the tunnel. Scale: 6cm

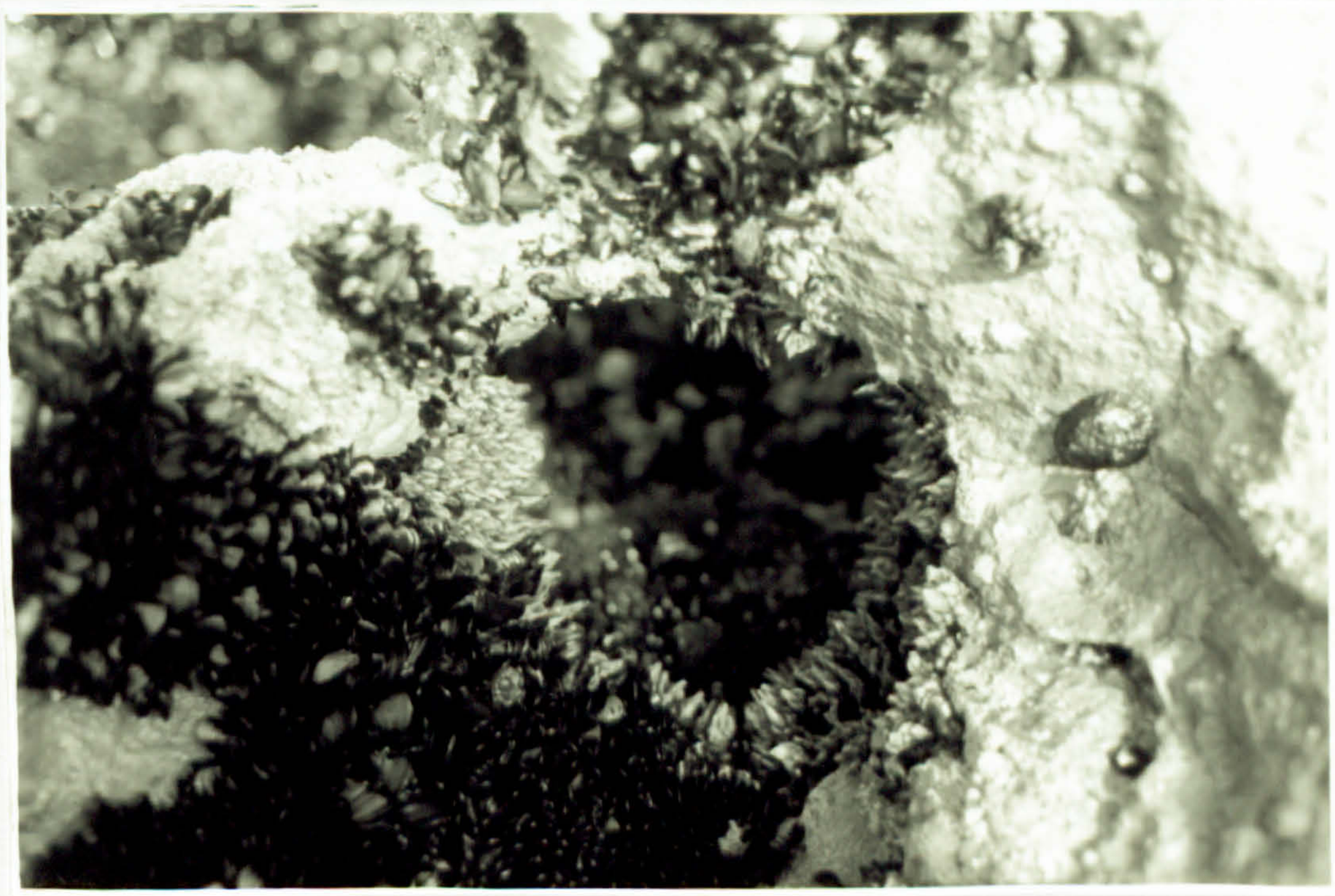
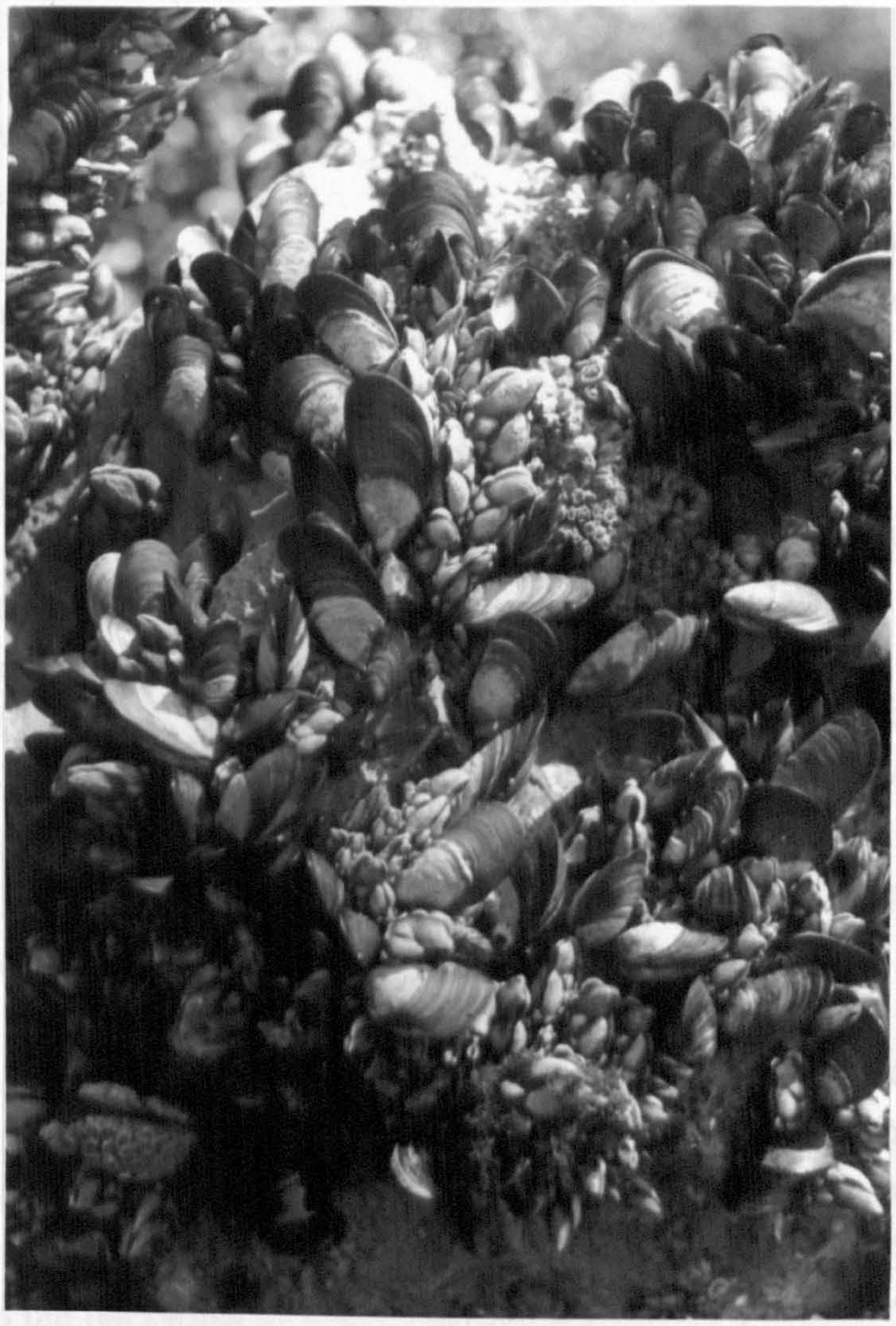


Figure 3.10. ***Pollicipes pollicipes*** **individuals and clumps amongst the mussels on the front of a boulder at Site 3, near Sagres. Scale: 1cm =** ⟵⟶



Orientation on the shore

At both sites, most or all of the animals within clumps were similarly orientated. However, the direction of orientation was not consistent between adjacent clumps of barnacles. Nor was orientation consistent with the general topography of the shore or with the direction of the waves.

Fig. 3.11 (a) shows a drainage channel on the shore at Ponta da Fisga. Fig. 3.11 (b) shows scale drawing of a section of the channel showing the orientation of the barnacles within the channel. Barnacles generally orientate such that their open cirral nets face into the backwash that streams down the channel after a wave has broken.

Fig. 3.12 is a scale drawing of the top end of a gully at Ponta da Fisga indicating the orientation of barnacles in the gully. Barnacle orientation depends largely on the microtopography with evident differences in orientation within a single clump of barnacles. At the top of a clump (A) animals orientate into the backwash. At the bottom of the same clump animals are facing into the incoming waves and uprush across the sloping surface. However at (B), where animals are located under an overhang, every individual in the clump is orientated into the uprushing water of incoming waves whereas nearby animals on a sloping surface orientate in a similar fashion to those at (A). In the immediately adjacent channel, (C) all the uppermost animals are orientated to face into the backwash.

The variability in orientation due to water movement channelled by microtopography is illustrated in Fig. 3.13. The photograph is of a 14 x 9 cm area on the leading edge of a large boulder at Site 3, near Sagres. The clump of animals (A) are situated in a drainage channel which leads from a small pool and are all facing upwards with their cirral nets pointing into the backwash of water that drains from the pool after a wave has broken. The exception being the two small animals (s) to the bottom left of this clump which are sheltered from the backwash by the larger animals above them, and hence are orientated into the uprush of the incoming wave. In position (B) the four animals are sheltered from the backwash by the overhanging piece of rock and these are orientated into the incoming wave water.

Fig. 3.14 is a scale diagram of the leading edge of a boulder at Site 3, showing the directions of water flow and the microtopography. At position (A) the animals are sheltered from wave backwash and orientate into the incoming wave water. At position (B) the animals are on a lip and are therefore able to exploit the backwash. In position (C) all the animals are facing into the incoming wave as the water rushes into the channel between adjacent boulders.

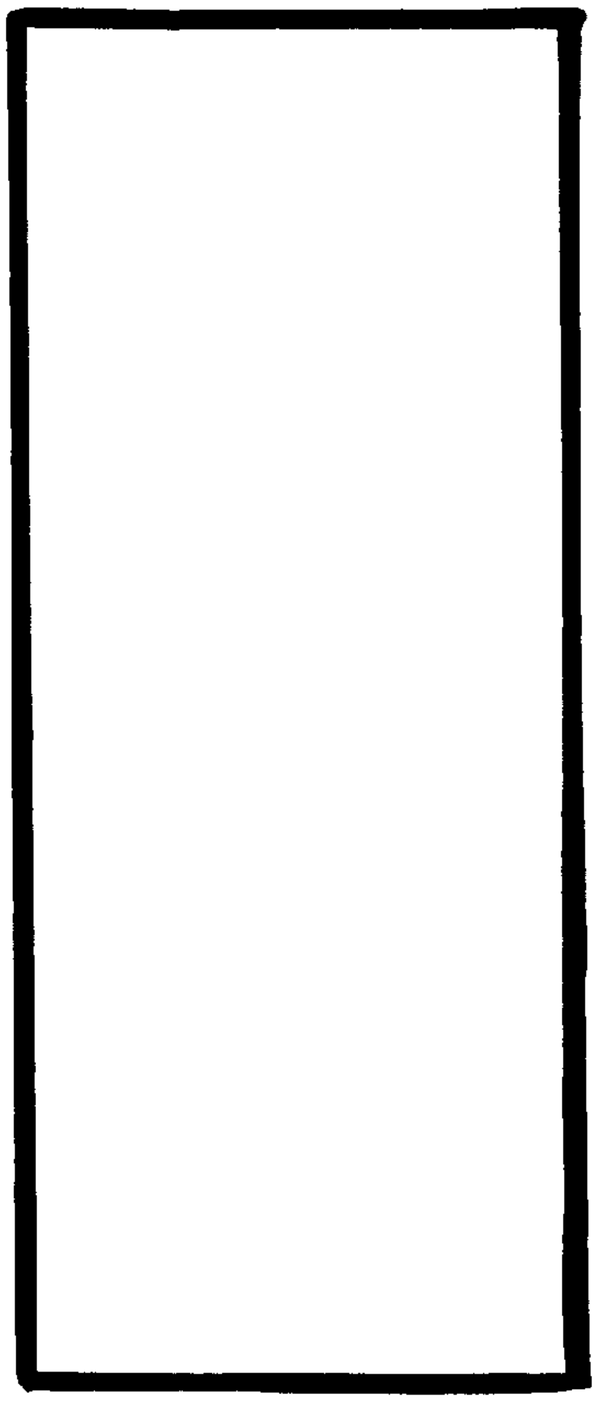
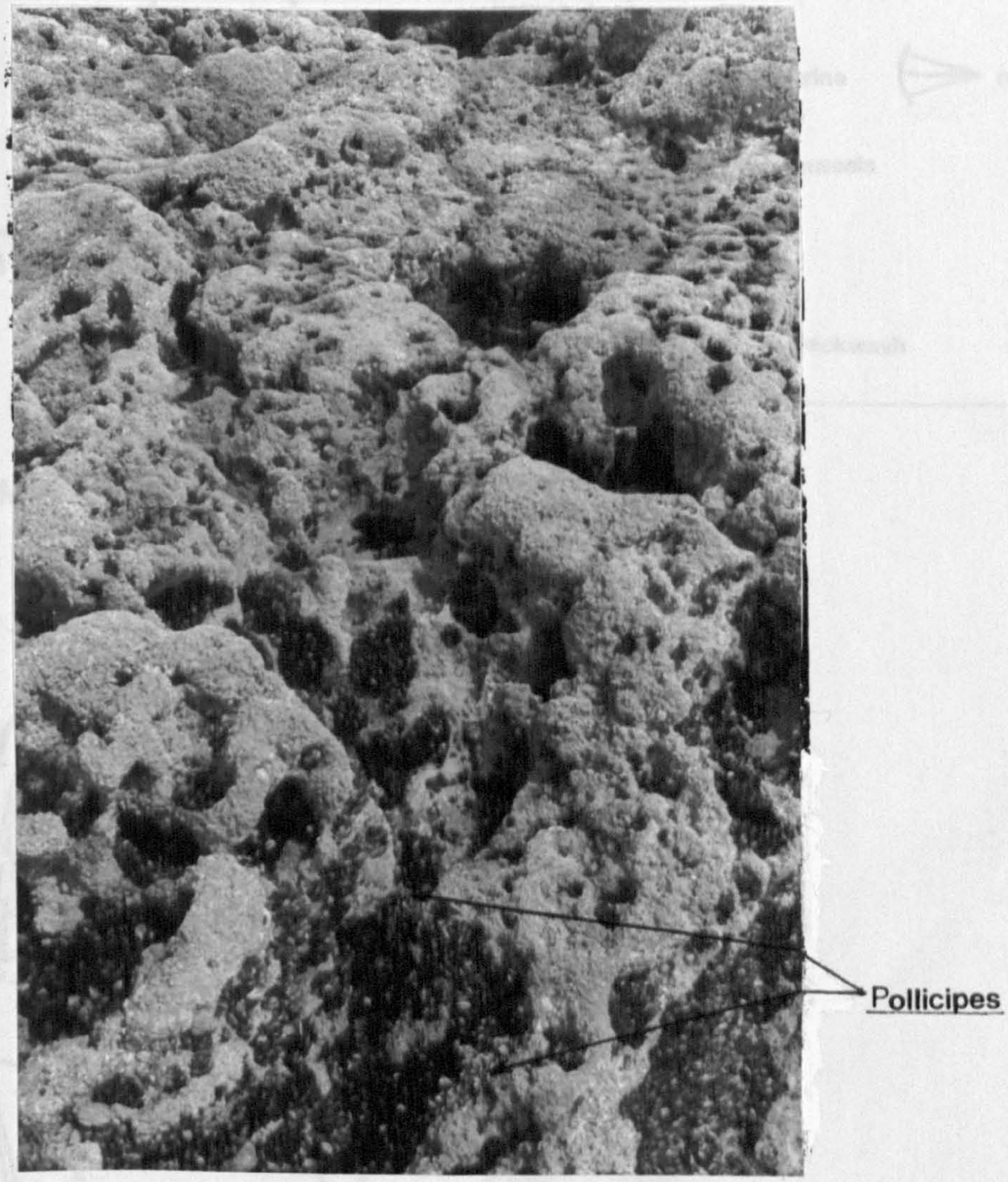


Figure 3.11 (a). The drainage channel on an open rock surface at Ponta da Fisga. It can be seen that there are no *Pollicipes* on the open rock surface but are several within the gully. The box designates the position of the gulley drawn in Fig. 3.11b.



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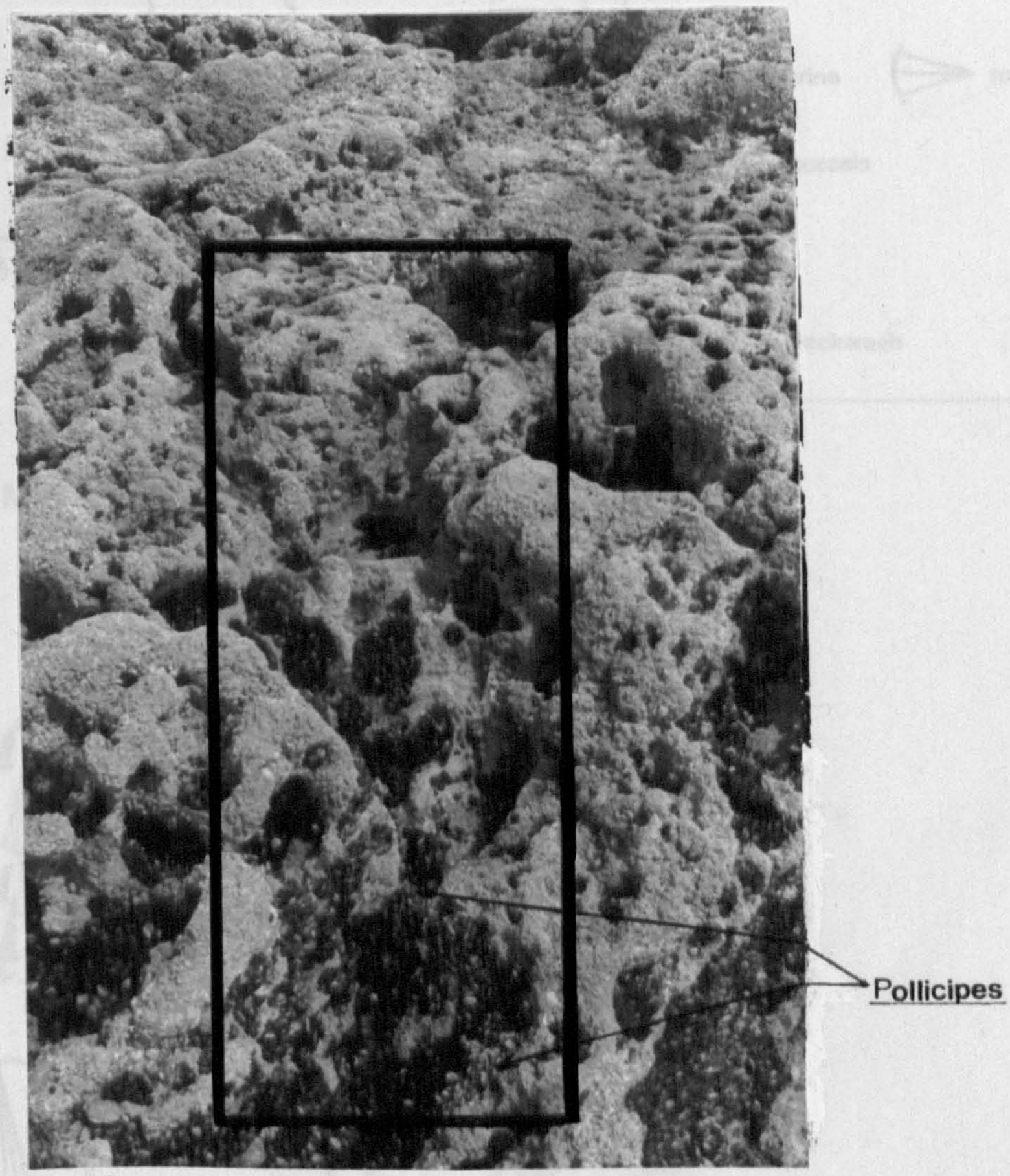


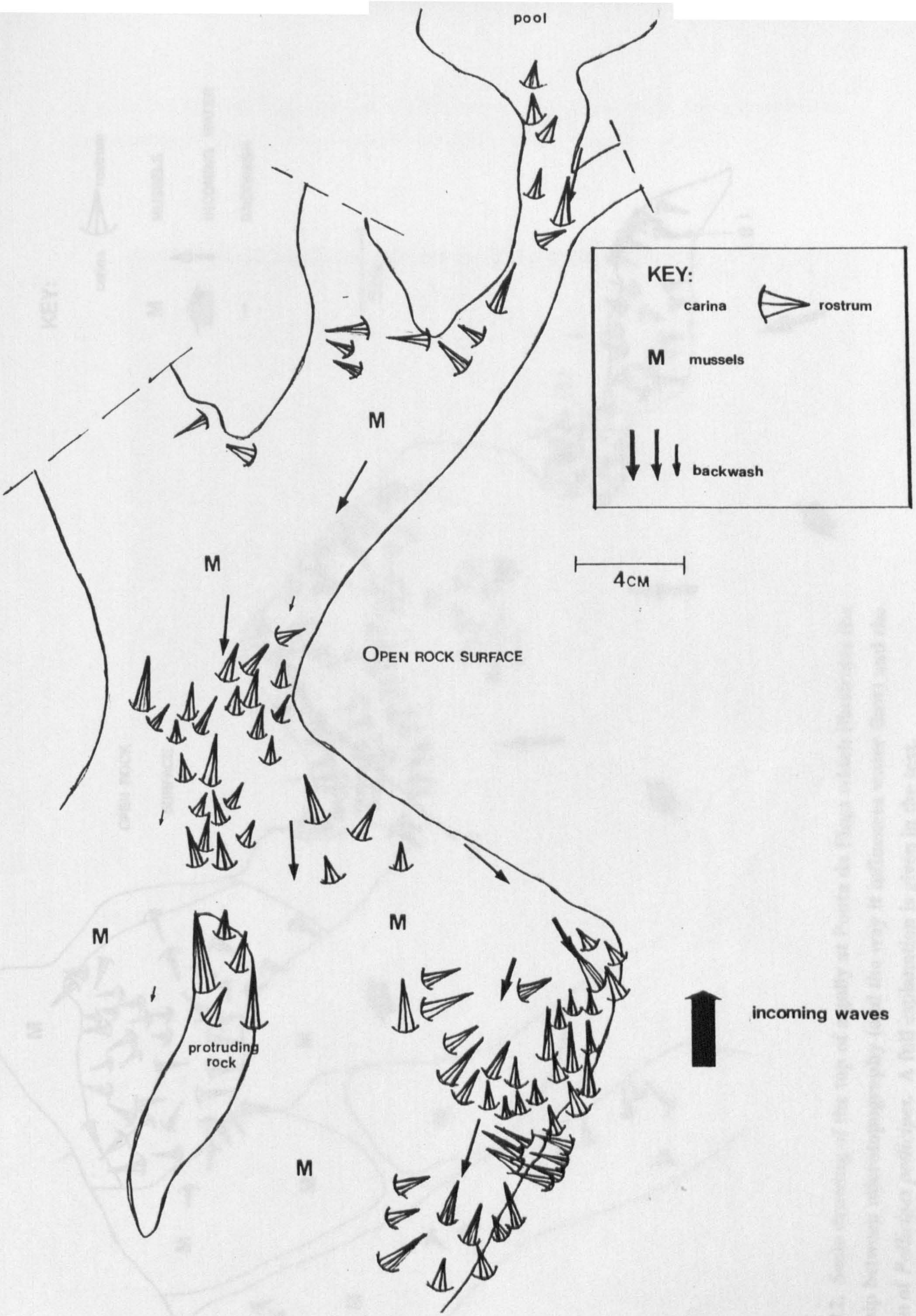


Figure 3.11(b). Scale drawing of the drainage channel pictured in 3.11(a) showing the orientation of *Pollicipes pollicipes* in relation to the directions of water flow.

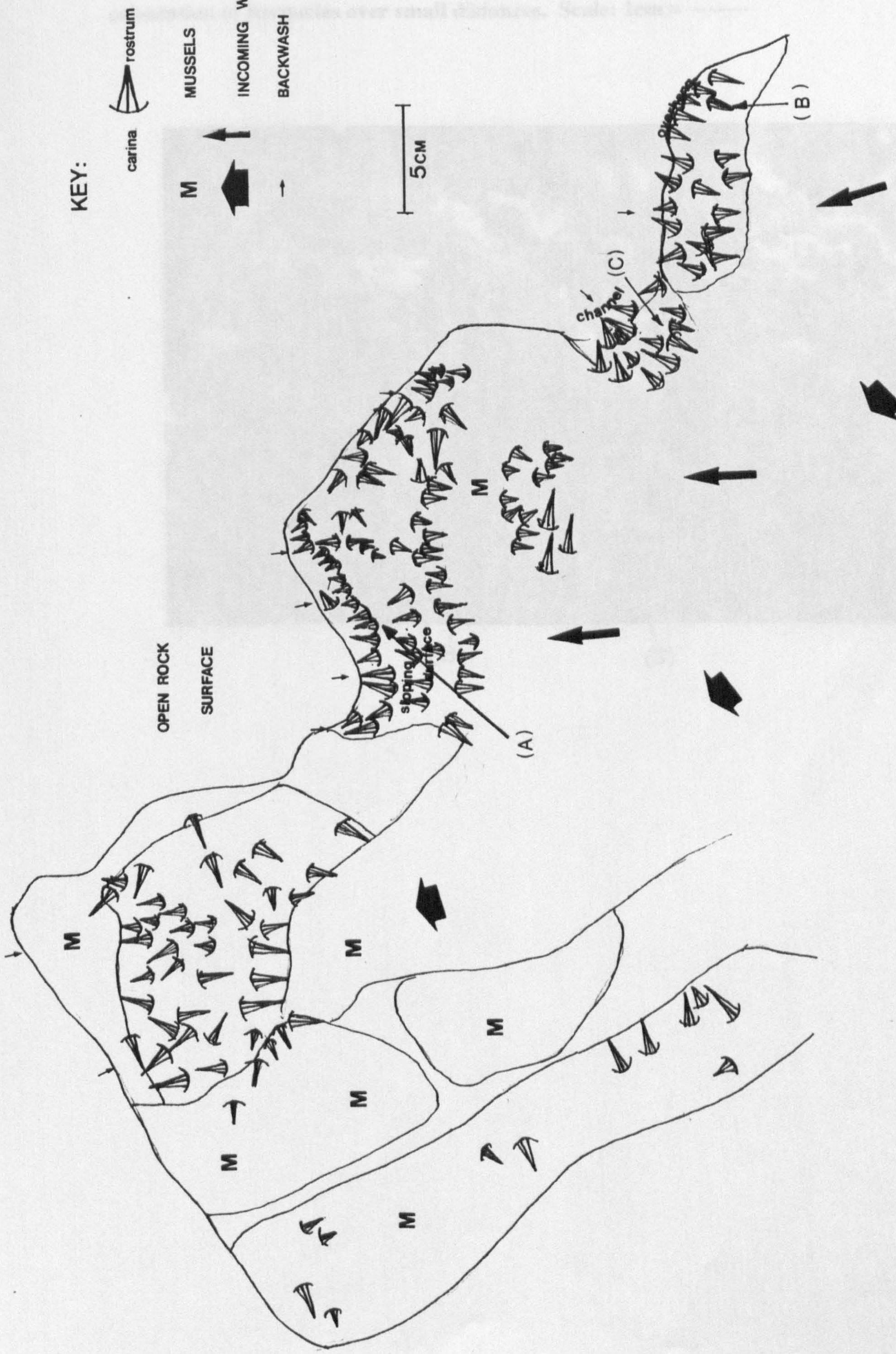
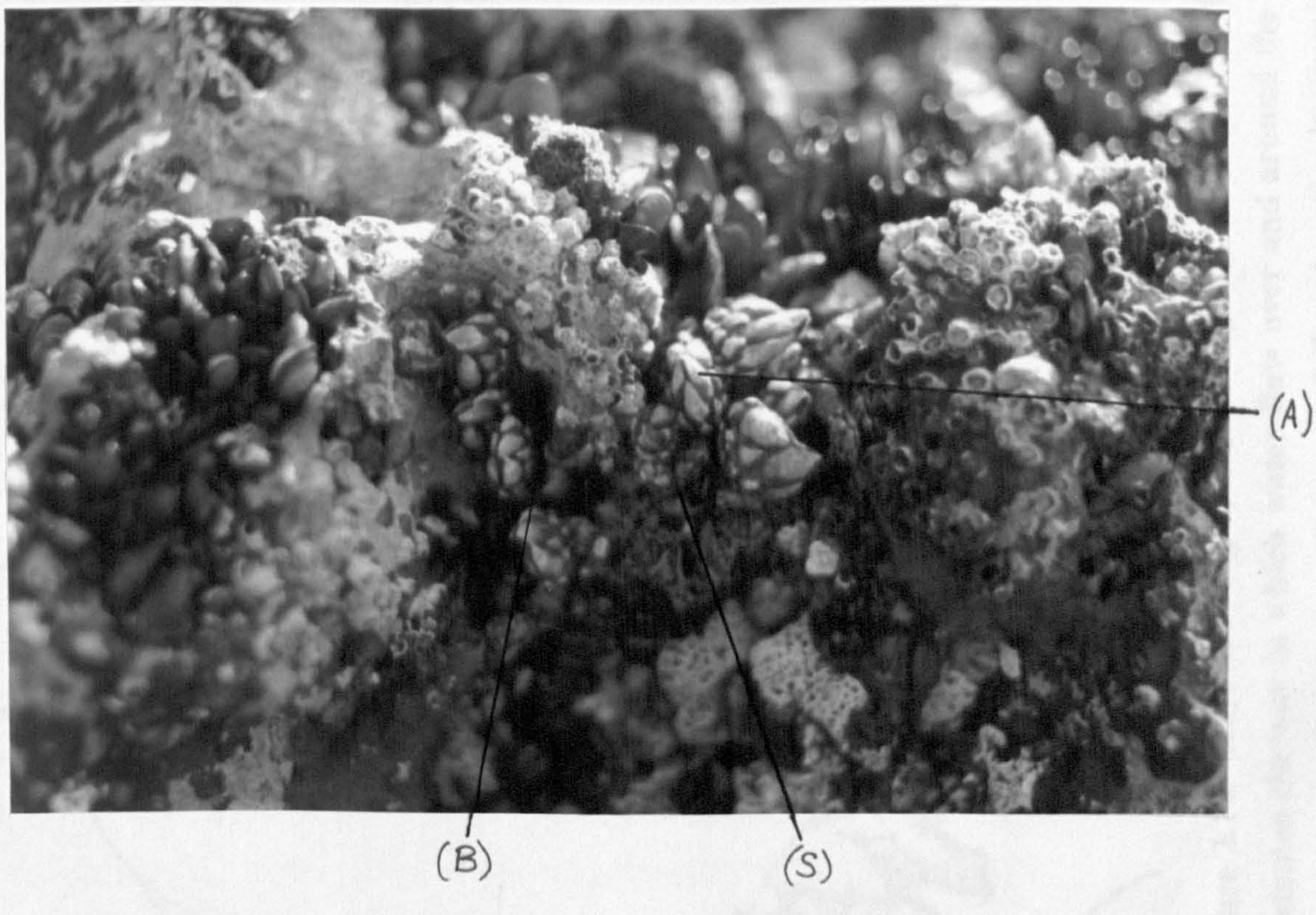


Figure 3.12. Scale drawing of the top of a gully at Ponta da Fisga which illustrates the relationship between microtopography (and the way it influences water flow) and the orientation of *Pollicipes pollicipes*. A full explanation is given in the text.

Figure 3.13. Leading edge of a boulder at Sagres showing the variation in orientation of barnacles over small distances. Scale: 1cm = ——



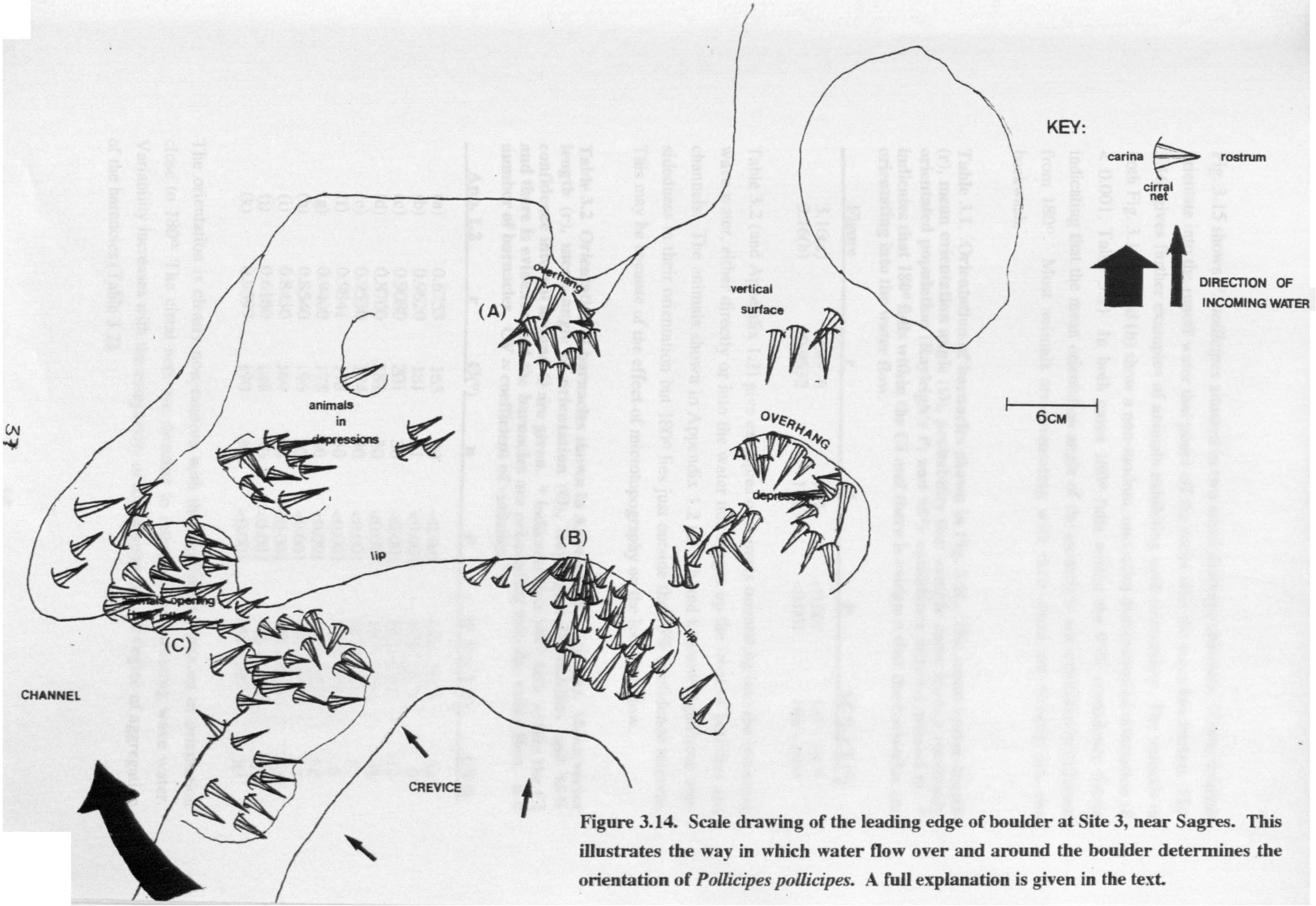


Figure 3.14. Scale drawing of the leading edge of boulder at Site 3, near Sagres. This illustrates the way in which water flow over and around the boulder determines the orientation of *Pollicipes pollicipes*. A full explanation is given in the text.

Fig. 3.15 shows *P. pollicipes* situated in two small drainage channels. These animals orientate into the runoff water that pours off the rocks after the wave has broken. Fig. 3.16 gives further examples of animals exhibiting such orientation. The animals in both Fig. 3.16 (a) and (b) show a non-random, one-sided distribution of orientation ($P < 0.001$, Table 3.1) In both cases 180° falls within the 95% confidence limits indicating that the mean orientation angle of the animals is not significantly different from 180°. Most animals are orientating with their cirral net directly into the backwash.

Table 3.1. Orientation of barnacles shown in Fig. 3.16. The mean vector length (r), mean orientation angle (Ø), probability that sample came from a randomly orientated population (Rayleigh's P) and 95% confidence interval around Ø. * indicates that 180° falls within the CI and there is evidence that the barnacles are orientating into the water flow.

Figure	r	Ø	P	95 % CI (°)
3.16(a)	0.8976	177	<0.001	167 - 187*
3.16(b)	0.9632	172	<0.001	164 - 180*

Table 3.2 (and Appendix 1.2) give examples of animals orientating into the incoming wave water, either directly or into the water that surges up the front of boulders and channels. The animals shown in Appendix 1.2 (c, f, h and k) show significant one-sidedness in their orientation but 180° lies just outside the 95% confidence interval. This may be because of the effect of microtopography on the localised flow.

Table 3.2 Orientation of barnacles shown in Appendices 1.2 (a)-(k). Mean vector length (r), mean angle of orientation (Ø), Rayleigh's Probability and 95% confidence interval around Ø are given. * indicates that 180° falls within the CI and there is evidence that the barnacles are orientating into the water flow. n = number of barnacles. CV = coefficient of variation.

App. 1.2	r	Ø(°)	n	P	95 % CI (°)	CV%
(a)	0.6753	165	30	<0.001	144 - 186*	34
(b)	0.9620	181	31	<0.001	175 - 187*	9
(c)	0.9080	201	30	<0.001	192 - 210	12
(d)	0.8700	181	30	<0.001	169 - 193*	18
(e)	0.9530	174	30	<0.001	167 - 181*	11
(f)	0.9841	173	30	<0.001	169 - 177	6
(g)	0.9400	175	30	<0.001	167 - 183*	12
(h)	0.8540	195	30	<0.001	183 - 207	17
(i)	0.8430	169	30	<0.001	157 - 181*	19
(j)	0.6160	189	30	<0.001	166 - 208*	33
(k)	0.9597	190	30	<0.001	183 - 197	10

The orientation is clearly non-random with the significant direction of orientation close to 180°. The cirral nets are directed to intercept the incoming wave water. Variability increases with the complexity of topography and the degree of aggregation of the barnacles (Table 3.2).

Figure 3.15. ***Pollicipes pollicipes*** **inhabiting two small drainage channels at Ponta da Fisga. All animals are orientated to intercept the runoff water as it pours off the rocks after the wave has broken. Scale: 1cm = ⟵⟶**

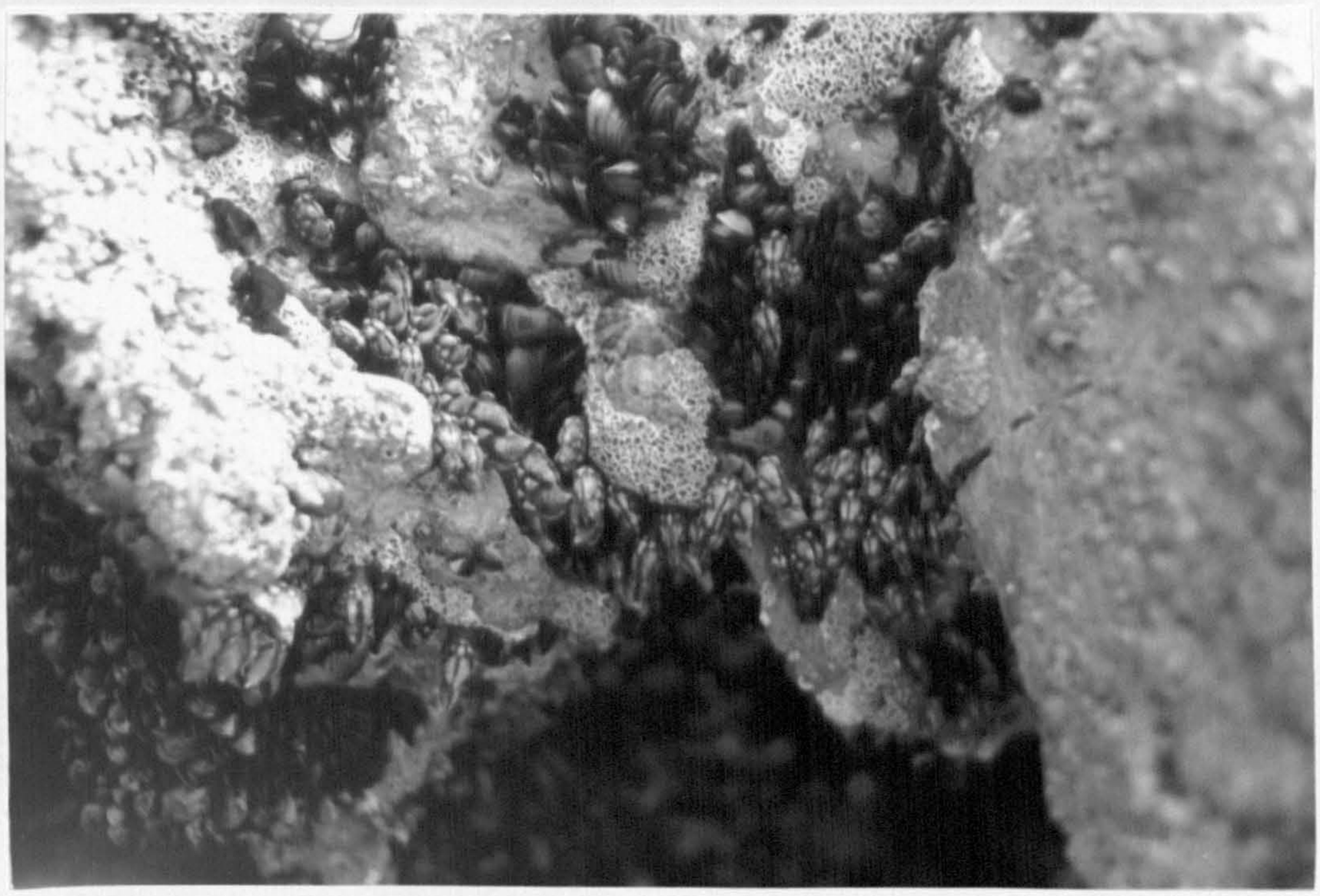


Figure 3.16 (a). *Pollicipes* above an overhang on the cliff at Ponta da Fisga. Although there is variability all animals orientate into the backwash. Scale: 1cm = ⌊——⌋

Figure 3.16 (b). *Pollicipes* in a drainage gutter running diagonally across the leading edge of a boulder at Site 3. All barnacles are orientating so as to intercept the water as it runs down the gutter after a wave has broken. Scale: 1cm = ⌊——⌋

Orientation in the laboratory.

P. pollicipes were found to twist the peduncle and thereby temporarily alter the orientation of the capitulum, hence the cirral net, when exposed to unidirectional water flow. The response to flow was temporary since the original orientation was resumed once the barnacles were placed in still water (hence light was not thought to have a marked effect on the orientation behaviour). The temporary torsion of the peduncle was occasionally accompanied by bending of the peduncle (see Fig. 3.17).

Change in orientation of juvenile and adults in relation to time in a unidirectional flow.

Adult *P. pollicipes* appeared to turn more than juveniles (Fig. 3.18) although because of the variability in angle measurements (coefficients of variation ranged from 0.57 to 2.7 for juveniles and from 0.32 - 1.08 for adults), only at 7 hours are there significant differences between juveniles and adults. The high variability is attributable to the small number of juveniles turning during the first three hours (only two or three out of ten) but all turning after 24 hours. Most adults show a turning response during the first three hours (6, 7 and 8 out of 10, respectively) and all do subsequently.

Figure 3.18 shows no significant changes in adult or juvenile orientation angle for the first five hours however, the change in angle occurring between 5 and 7 hours was significant. It took longer for all juveniles to respond to water flow and change their orientation than adults but neither group showed further significant alteration in their body positioning after seven hours.

Orientation to flow with respect to initial orientation.

Table 3.3 gives the mean changes in orientation for barnacles with different initial orientations. There is a general trend for animals with an initial orientation furthest from facing into the flow to show the greatest change in orientation. Fig. 3.19 shows the results as a polar diagram indicating very small changes in orientation for animals that are initially facing less than 90° away from the oncoming flow. The greater the initial orientation angle to the water flow the greater the change in orientation angle over 24 hours. Animals initially placed at 40, 60 and 100° to the direction of water flow showed no significant change in their orientation angle (although one animal placed at 100° did turn by 50°), all animals initially placed at angles of 110° and over to the prevailing water flow did show significant changes in their orientation (Table 3.3).

Figure 3.17. Modification of *Pollicipes pollicipes* orientation by change in body attitude in response to water flow.

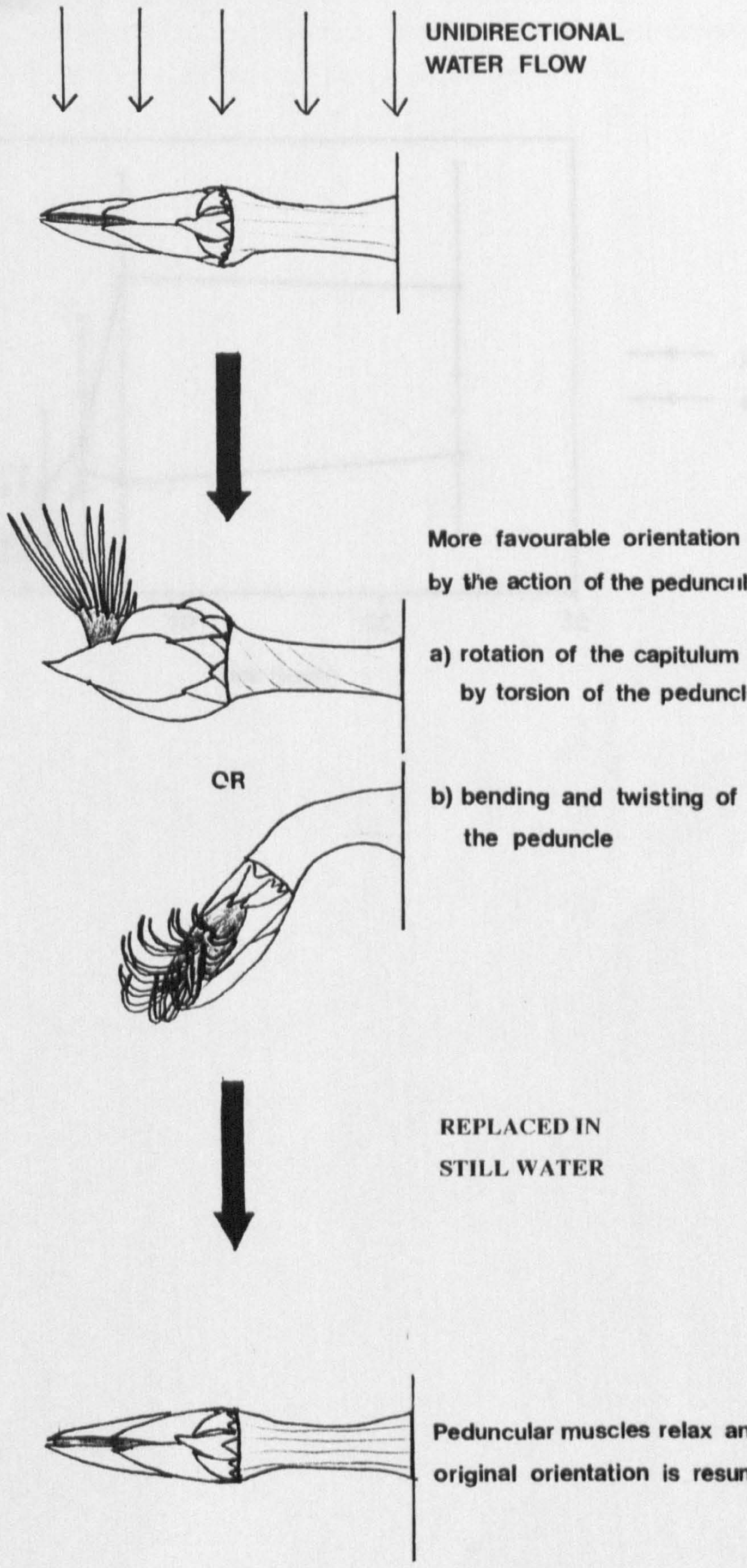


Figure 3.18 Mean (± SD) change in orientation angle of juvenile and adult *P. pollicipes* during a 24 hour period. Initial orientation was at 90° to the direction of water flow.

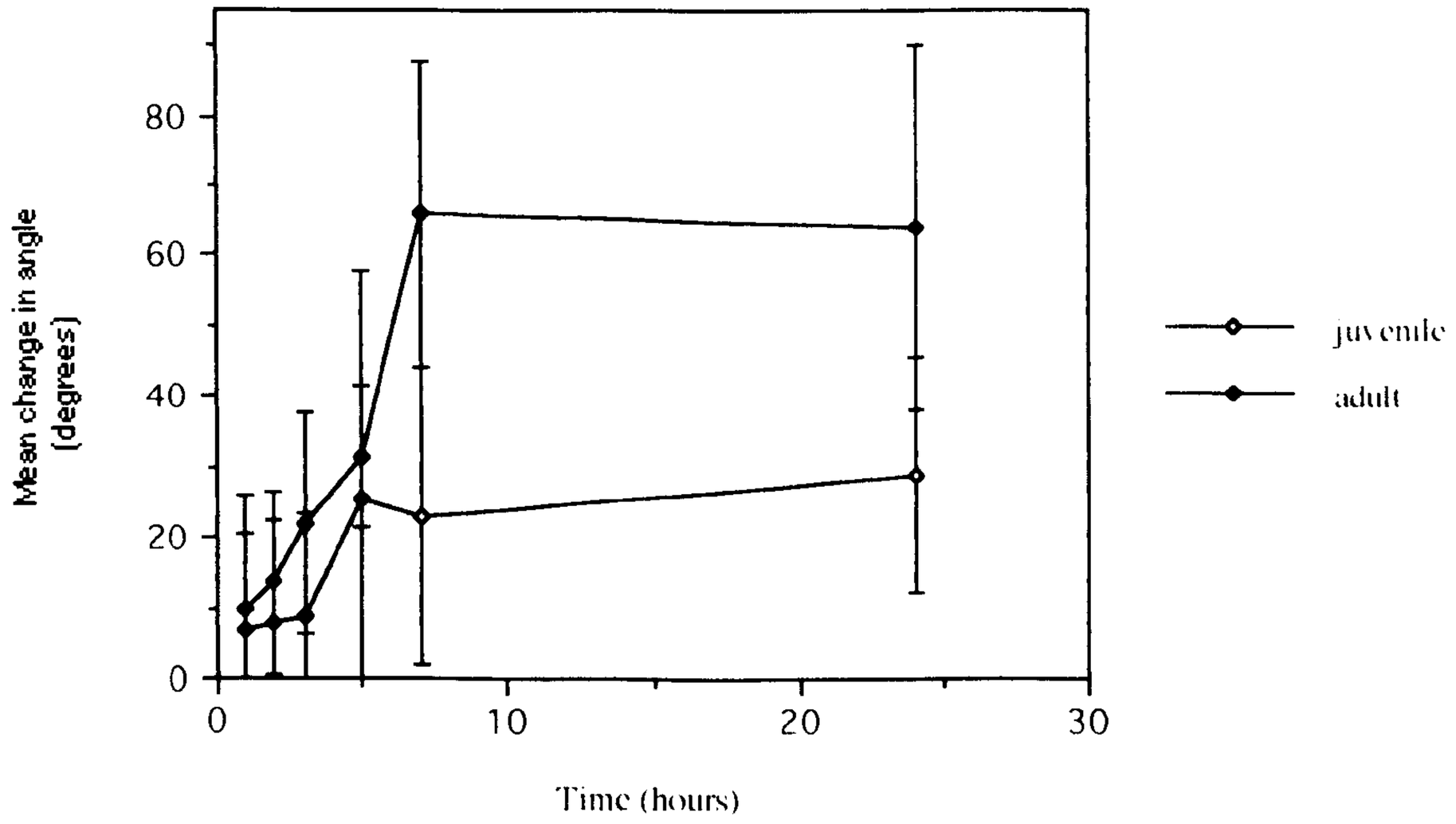


Figure 3.19. Polar diagram showing the change in mean orientation angle of *P. pollicipes* against initial orientation to the prevailing direction of water flow. Concentric circles denote the change in orientation angle after a 24 hour period. Line direction indicates initial orientation in relation to prevailing flow.

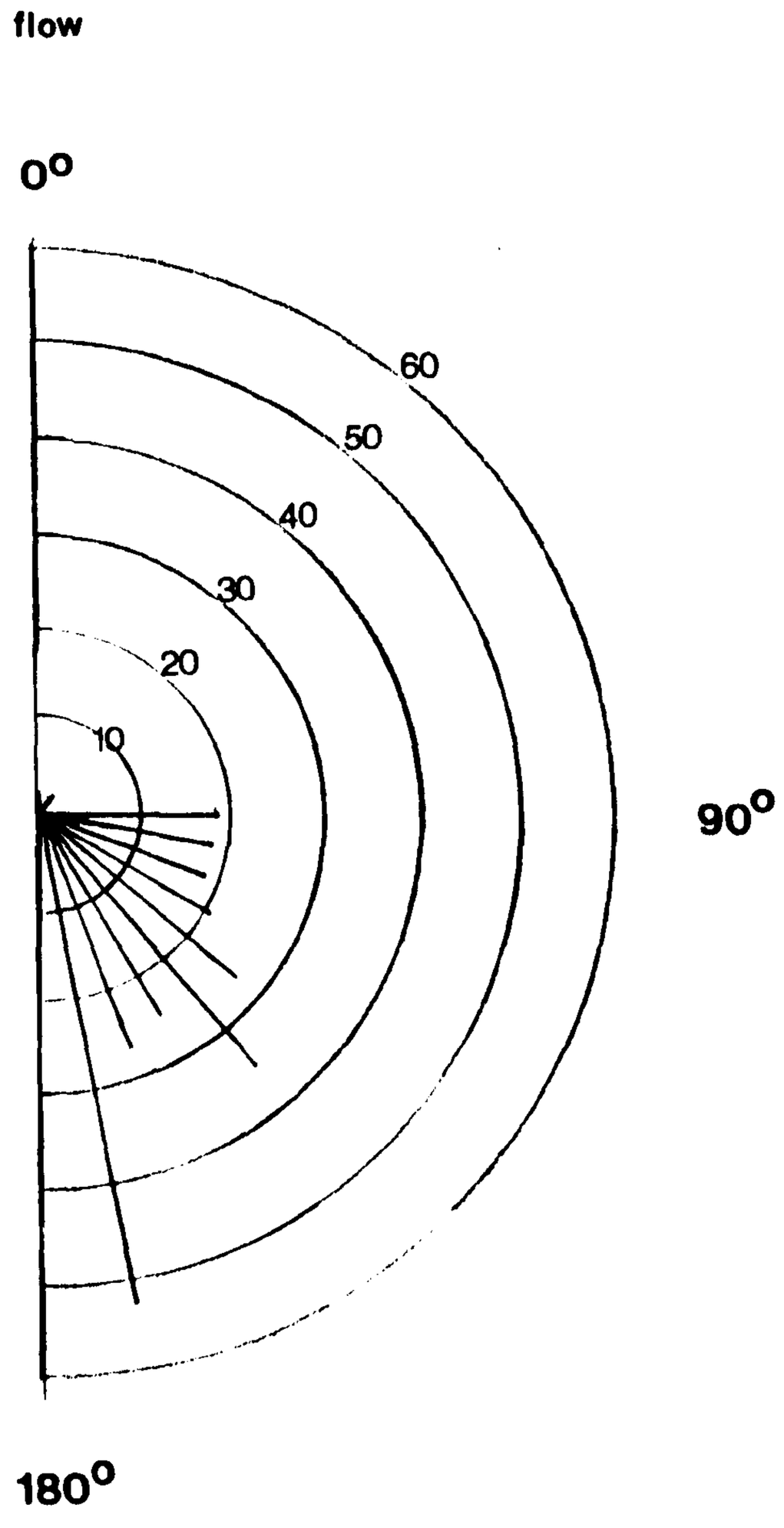


Table 3.3. The effect of initial orientation of *P. pollicipes* on the change in orientation to flow over a 24 hour period. Mean change ΔØ ± SD, mean angle Ø, are given, as is the paired sample t value for change in orientation angle. N = number of observations. * denotes a significant change in orientation (t >2.776).

Initial angle	N	ΔØ	SD	Ø	t
40	5	2	4.47	38	1.001
60	5	0	0.00	60	0
90	5	18	8.37	72	4.809*
100	5	18	19.24	82	2.092
110	5	18	8.37	92	4.809*
120	5	20	10.00	100	4.472*
130	5	26	18.17	104	3.200*
140	5	34	11.40	106	6.669*
150	5	24	15.17	126	3.538*
160	5	26	15.00	138	3.876*
170	5	52	34.20	118	3.400*
180	5	34	25.10	146	3.029*

Orientation to flow with tied valves.

Table 3.4. shows the mean angles of orientation at the end of the experimental period of *P. pollicipes* with tied and untied capitular valves.

Table 3.4. The mean angle of orientation Ø (°) for *Pollicipes pollicipes* after 24 hours with tied and untied capitular valves. Initial orientation = 90° to water flow. Standard deviations and coefficients of variation are also given. N = number of observations.

	N	Ø	SD	CV
Untied valves	15	67	17.09	0.26
Tied valves	15	78	15.68	0.20

There was no significant difference between the final angle of orientation for animals with their valves tied and those with untied capitular valves ($t = 1.836$, 28 df, $P = 0.08$). In both cases the barnacles altered their orientation significant to the flow (untied $t = 5.212$, 14 df, $P < 0.001$, tied $t = 2.964$, 14 df, $P = 0.01$). Although the response appeared slightly less for animals with tied valves it is clear that some appreciation of current direction can be perceived by the barnacles even when the soma and cirri are enclosed in the capitulum.

Aggregation and orientation

Table 3.5 gives the mean change in orientation angle for animals that are distributed with varying proximity to one another. The animals that are 1.5 cm apart appear to turn the most while animals 2.5 cm apart turn the least. The variability is high (CV = 0.53, 0.45 and 0.63) but there is no significant heterogeneity of variance (Cochran's C = 0.4166 for 3 and 14 df, $P > 0.05$).

Table 3.5. The mean change in orientation angle, ΔØ (from 90°) of *Pollicipes pollicipes* with differing proximity to other animals. The standard deviations are given. N = number of observations.

Distance (cm)	N	ΔØ	SD	CV
0.5	15	36.0	19.19	0.53
1.5	15	52.7	23.74	0.45
2.5	15	32.7	20.50	0.63

Because of the high variability, an analysis of variance suggested that the distance between *Pollicipes pollicipes*, over the range investigated here, had no significant effect on the ability of the barnacles to temporarily modify their orientation to water flow (Table 3.6, $F = 0.79$, $df = 2$, $P = 0.461$).

Table 3.6. Analysis of variance for the effect of separation distance on the ability of *Pollicipes pollicipes* to turn in response to water flow.

Source	df	Sum Sq.	Mean Sq.	F-Value	Prob.
Separation	2	1151.1	575.6	0.79	0.461
Error	42	31626.7	729.2		
Total	44	31777.8			

Starvation and orientation

Animals which had been starved before the experiment turned round more than twice as far as those which were fed as normal (Table 3.7). The variability in angle measurements was, however, very high (CV = 1.32 and 0.68) although no significant heterogeneity of variance was evident (Cochran's statistic = 0.5148 for 2 and 10 df, $P > 0.05$). Because of the high variability a two sample t-test between starved and fed animals showed no significant difference between mean angles ($t = 1.897$, $df=20$, $p = 0.07$).

Table 3.7. Mean change in orientation angle (ΔØ from 90°) for *Pollicipes pollicipes* that had been previously starved or fed, the standard deviations and coefficient of variation are given. N = number of observations.

Status	N	Mean ΔØ	SD	CV
Fed	11	22.73	30.03	1.32
Starved	11	48.18	32.81	0.68

Discussion

Pollicipes pollicipes inhabit very exposed rocky shores similar to those occupied by *Pollicipes polymerus* in North America. Exposed shores were found by Bustamante & Branch (1996) to have a water turnover seven times greater than on sheltered shores and consequently a potentially higher food supply. *Pollicipes* are found on the low- to mid-shore, competing for space with mussels. However, unlike *P. polymerus*, the *P. pollicipes* on the coast of Portugal are heavily exploited by man with such heavy fishing pressure that it is impossible to determine whether the *P. pollicipes* distribution patterns result largely from exploitation, differential settlement or subsequent 'natural' mortality. At Site 3 there were two adjacent horizontal fissures in the cliff. One fissure was too narrow to allow human access and the surfaces were covered with *P. pollicipes*. The other was wider, allowing easy access to the surfaces and there were only a few *P. pollicipes*. It was striking that the dense stands of *P. pollicipes* all occurred in very inaccessible places; under boulders, in narrow cracks or in places only accessible through a restricted opening. *Pollicipes* on open surfaces occurred individually or in very small clumps usually of small barnacles, rarely over 15 mm RC. Larger animals were to be found either in inaccessible places or cryptically camouflaged by macro-algae and mussels.

The orientation of many species of barnacle has been shown to be related to water flow (Crisp, 1953; Howard & Scott, 1959; Barnes & Reese, 1960; Otway & Underwood, 1987). Some species such as *Balanus balanoides*, *B. crenatus* and *Elminius modestus* (Crisp & Stubbings, 1957) orientate primarily into the incoming wave water while others such as *Tesseropora rosea* (Otway & Underwood, 1987) and *Pollicipes polymerus* (Howard & Scott, 1959; Barnes & Reese, 1960) face the wave backwash. It was noted by Otway & Underwood (1987) that in regions of lower wave energy, *Tesseropora rosea* showed a bimodal orientation with respect to incoming breaking waves and backwash. When the anterior face of the cirral net is at right angles to the water flow the feeding efficiency is maximal.

P. pollicipes, like *P. polymerus*, shows strong orientation into water flow, but the orientation is inconsistent with respect to shore and wave direction and more

dependent on microtopography at each site. Howard & Scott (1959) and Barnes & Reese (1960) noted that *P. polymerus* in all but the most sheltered locations orientate into the wave backwash. *P. pollicipes*, on the other hand, was found to orientate into the predominant direction of water flow, whether from the incoming waves or the runoff. When the animals were sheltered from the backwash by rocks, mussels or other barnacles they faced the incoming wave water. Presumably the breaking wave has more kinetic energy than the backwash and it is unclear how successfully *P. pollicipes* can hold the cirral net open against such force. Filtering the backwash should be a good strategy at Ponta da Fisga since waves there produced an incoming flow that lasted for one eighth the time that the backwash took to drain down the channels. The back-flowing water was channelled into gutters and crevices where *P. pollicipes* were most abundant and indeed the barnacles in drainage channels were still in the backwash as the next wave arrived. Although the volume/unit time passing through the drainage channels is probably no greater, there is less energy in the backwash which should make filtration easier.

Barnes & Reese (1960) suggested that a certain degree of water movement was necessary to trigger feeding activity in *Pollicipes polymerus*. Cirral extension and maintenance was stimulated by the incoming wave in readiness for filtering the backwash of the retreating wave. The results of the current study show that *P. pollicipes* orientate towards prevailing currents (be it incoming or retreating wave) but are equally capable of twisting in response to persistent alteration in the prevailing direction. This ensures maximal capturing capacity. The twisting response was generally seen to be slow hence indicative of a change in posture which could accommodate a wind or storm driven change in general flow direction over hours or days rather than a short timescale adjustment of the feeding posture in relation to micro-flow fluctuations as might be expected from variable turbulence with tidal flow.

Barnes & Reese (1960) observed that isolated *P. polymerus* rotate through an angle of 90° over 24 hours when subjected to a sustained jet of water directed laterally onto the cirral net. *P. pollicipes* were able to rotate by up to 90° in 24 hours, although most animals actually turned quicker (in 1-7 hours) and to a much lesser degree (10 - 50°). The original orientation is resumed on removal of the lateral flow. Temporary twisting, through rotation of the capitulum by the peduncular muscles, is quite unlike the permanent basal movement described by Kugele & Yule (1993) where the barnacle can detach and grow through new basal extensions into a new position or orientation under prolonged stimulation.

Barnes & Reese (1960) suggested that minor differences in wave direction caused by shifts in the wind would induce slight orientation changes in *P. polymerus* that would improve their feeding efficiency. However, in the sites occupied by *P. pollicipes*, the direction of water flow is determined by the local topography rather than the direction of the incoming waves. Moreover, my results indicate that the degree of rotation depends on the initial orientation to the direction of flow with more rotation for larger angles and that *P. pollicipes* require at least a 90° switch in flow direction for reorientation to occur. An animal whose angle of orientation, although not optimal, allows the interception of the flow will still be able to feed. There will be an energetic cost for twisting into the flow so when there is little advantage to be gained, little modification of body attitude occurs. However, an animal whose cirral net is facing away from the flow will presumably be unable to feed and torsion will be essential for survival.

The importance to a passively filter feeding animal of being able to modify permanently its orientation is obvious. Experiments with *Tesseropora rosea* indicated that animals maintained in sub-optimal orientations lost weight (Otway & Underwood, 1987). *T. rosea* are unable to modify the orientation of the shell after metamorphosis and although they can rotate the cirral fan through 180° to either side of the rostro-carinal axis when newly settled, they are only able to swivel it through 90° to either side of the axis once they reach 2 months old (Anderson & Buckle, 1983). For *T. rosea*, ideal orientation at settlement is essential for long-term survival. *P. pollicipes*, left in sub-optimal orientation to water flow for several months will 'permanently' alter its orientation by rotation of its peduncle relative to the substratum (M. Kugele, pers.comm.). Whether the temporary modification of orientation I observed is an end in itself or is just a necessary precursor to permanent turning is unclear. The long term torsion of the peduncle by the peduncular muscles may exert a force on the adhesive which may be sufficient, over time, to allow detachment and then rotation through growth of the base and the cement system.

It is difficult to conceive of a short-lived alteration of the course of predominant water flow that lasts for many hours and alters the flow by more than 90° relative to the barnacle. More permanent changes to the predominant direction of water flow are more easily imagined. These might include: the movement of boulders; those on which the barnacle is situated or ones nearby that alter the course of water flow, the rapid growth of mussels in a channel above *Pollicipes* which could shelter the barnacles from the backwash. In such cases the ability to turn into flowing water is necessary for survival. The evidence suggests that the peduncular twisting is

primarily the initiation of permanent turning although it does enable feeding in the meantime.

Barnes & Reese (1960) suggested that the flow receptors of *P. polymerus* were situated on the cirri or body of the barnacle and not on the stalk. Their evidence was the lack of a turning response in barnacles whose capitular valves had been tied (closed) with twine, but I found that tying the valves of *P. pollicipes* had no significant effect on the turning response. Animals with tied valves changed orientation in response to water flow and, within the variability of the results, the degree of response was not significantly different from that of unrestrained animals. These results suggest that flow receptors on the cirri are not necessary hence there must be sensors on the peduncle or opercular flaps which are exposed even when the capitulum is tied shut. Barnes & Reese (1960) suggested, having observed *P. polymerus* behaviour, that there were receptors on the peduncle but that these detect only water pressure, not direction. However, drag results from the substantial pressure difference between the leading and trailing edges of an object in flow, so detecting pressure differences on either side of the peduncle could clearly indicate flow direction.

Barnes & Reese (1960) found that the turning response was more obvious in isolated *Pollicipes polymerus* individuals and those closest to the water jet. They found that the current affecting animals further from the source, although not measured, was insufficient to stimulate complete reorientation. My experiment was performed in an inclined gutter (Barnes & Reese used a horizontal chamber) so any removal of momentum from the fluid by a barnacle would have been counteracted to some degree through the acceleration due to gravity. There were no significant differences between the change in orientation of closely packed animals and those that were more widely spaced. It might have been anticipated that more closely spaced animals would reduce water flow velocity reaching subsequent animals . Although the flow rates were not measured in front and behind individuals the response of the animals suggested there was little influence.

On rocky shores where flow rates of 14 m s^{-1} (Jones & Demetropoulos, 1968 - recalculated by Vogel (1981) as 16 m s^{-1}) have been measured for waves 6.5 m in height and flow rates estimated by Denny (1988) to fluctuate between 0.5 m s^{-1} and 20 m s^{-1} with the oscillation of the waves, the proximity of other animals may not greatly alter flow rates. Reynolds numbers for juveniles vary from 5×10^{3} - 1×10^{5} at flow rates 1 and 20 ms^{-1} and for adults between 5×10^{4} and 1×10^{6}. Therefore, most of the time adult, and some of the time juvenile, *P. pollicipes* are likely to be subjected to turbulence. Barnes & Reese (1960) found that *P. polymerus* living in turbulent

conditions showed no obvious orientation. There was no significant difference between the ability of adults and juveniles to orientate to flow. The degree of turning response was very variable from one animal to another, particularly in juveniles because some individuals did not show any rotation in response to flow.

In summary, *P. pollicipes* are well suited to take maximum advantage of the extreme flow conditions they inhabit and are able to reorientate their feeding apparatus to cope with changes in the direction of flow.

Chapter 4

Limb morphology of Lepadomorph barnacles.

Introduction.

Most barnacles feed by means of a cirral net, typically consisting of six pairs of long, biramous appendages that may be extended through the opening in the mantle cavity into the overlying water to filter particles. Movement of the cirri, the operculum and the mouthparts are the most obvious manifestations of barnacle activity (Anderson & Southward, 1987). The thoracic appendages may be classified according to their function either as captorial cirri or maxillipedes (see Southward, 1955a). The former are usually the last three or four pairs of cirri which are longer than the rest. The activity of these two sets of cirri is dissimilar but together they are responsible for feeding (Crisp, 1961; Anderson, 1981; Anderson & Anderson, 1985; Anderson & Southward, 1987). Successful feeding also relies on the action of the mouthparts and oral cone and on co-ordination between these structures and the maxillipedes (Crisp, 1961; Anderson, 1980a; Anderson, 1981; Anderson & Southward, 1987).

Each cirrus is composed of a bi-segmented basal portion (the pedicel or protopod) topped by two rami: the exopod (anterior ramus) and the endopod (posterior ramus). Each is made up of a number of segments. The age of the barnacle and the position of the cirrus on the body determine the number of ramal segments. The detailed structure of the cirri, mouthparts and oral cone vary from species to species and in addition to the numerous acorn barnacle species described by Darwin (1854) have been described for *Boscia anglicum* (Anderson, 1978), *Lepas pectinata* (Anderson, 1980a), *Verruca recta* Aurivillius and *V. stroemia* (Anderson, 1980b), *Balanus balanoides* (Stubbings, 1975), *Balanus perforatus* (Anderson, 1981), *Tesseropora rosea* (Anderson & Anderson, 1985) and *Tetraclitella purpurascens* Wood (Anderson & Anderson, 1985). A brief description of the cirral and mouthpart morphology of many *Lepas* species, *Pollicipes cornucopia* (= *P. pollicipes*), *Lepas anatifera*, *P. elegans*, *P. spinosus* (= *Calantica spinosa*), *P. sertus*, *P. mitella* (= *Capitulum mitella*) and *P. polymerus* amongst other pedunculate cirripedes was given by Darwin (1851). Further accounts of the cirral morphology of *P. polymerus* may be found in Nussbaum (1890) and Barnes & Reese (1959).

Cirral and mouthpart morphology has been shown to correlate with diet and ecology in some species of barnacle, e.g. *Boscia anglicum* (Anderson, 1978), *Verruca recta* and *V stroemia* (Anderson, 1980b). The current study aimed to produce a more

detailed comparison of the mouthpart and cirral morphology of *Pollicipes pollicipes*, *Capitulum mitella* and *Lepas anatifera*. The cirral morphology in *P. pollicipes* of various sizes was examined to investigate the morphological evidence for any shift in diet or feeding strategy on becoming adult similar to that reported in *P. polymerus*, by Lewis (1981) and *P. pollicipes* by Hui (1983).

Materials & Methods

Figures 4.1 and 4.2 show the ventral and lateral view of *Pollicipes pollicipes* respectively, indicating the nomenclature used.

For ease of examination and preservation of limited stocks at Menai Bridge, the cirral structure could best be assessed by using the barnacle moults. Moults from *Pollicipes pollicipes* of known RC length, *Lepas anatifera* and *Capitulum mitella* were collected and preserved in 2% formaldehyde in seawater. The cirral structures were investigated through direct observation under a stereo dissecting microscope and through photomicrography.

Individual cirri from the right side of every moult were removed using fine dissecting scissors under a binocular microscope. Each cirrus was then placed on a microscope slide with the lateral (outer) side uppermost (see Fig.4.3) and was mounted in a few drops of 2% formalin. The two rami were arranged to show clearly the setae and were photographed with a Wild photo microscope. Magnifications were noted and a micrometre scale was photographed for calibration.

Cirrus length, segment length, setal length and intersetal distances were measured, on enlarged photographs (x 4) by means of vernier callipers (see Fig 4.3). The mouthparts of *P. pollicipes*, *C. mitella* and *L. anatifera* were examined, the appendages were drawn and described.

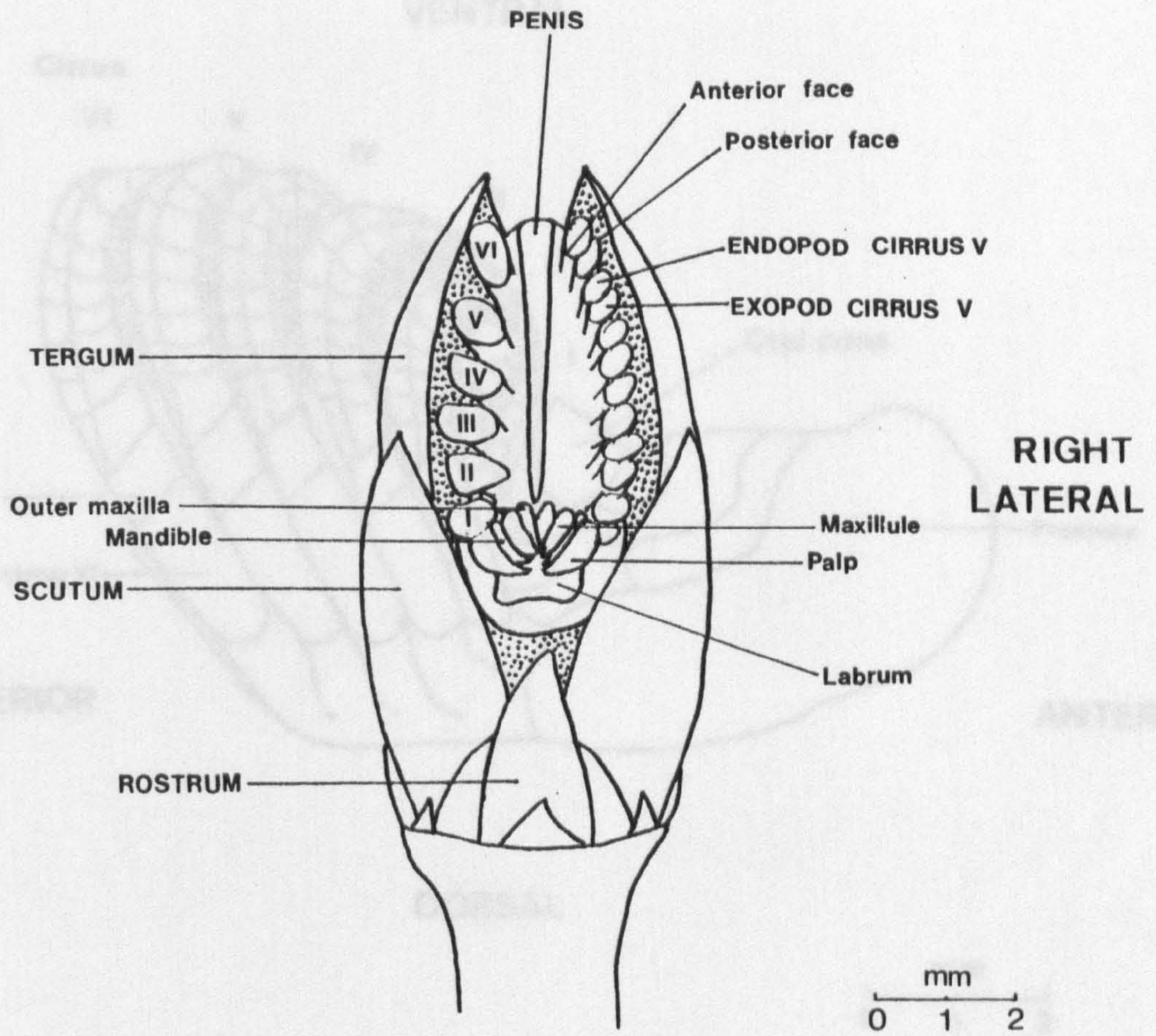


Figure 4.1. Diagram of the ventral view of *Pollicipes pollicipes* indicating the nomenclature used. The cirri on the right are cut across the rami while cirri on the left are cut across the pedicel.

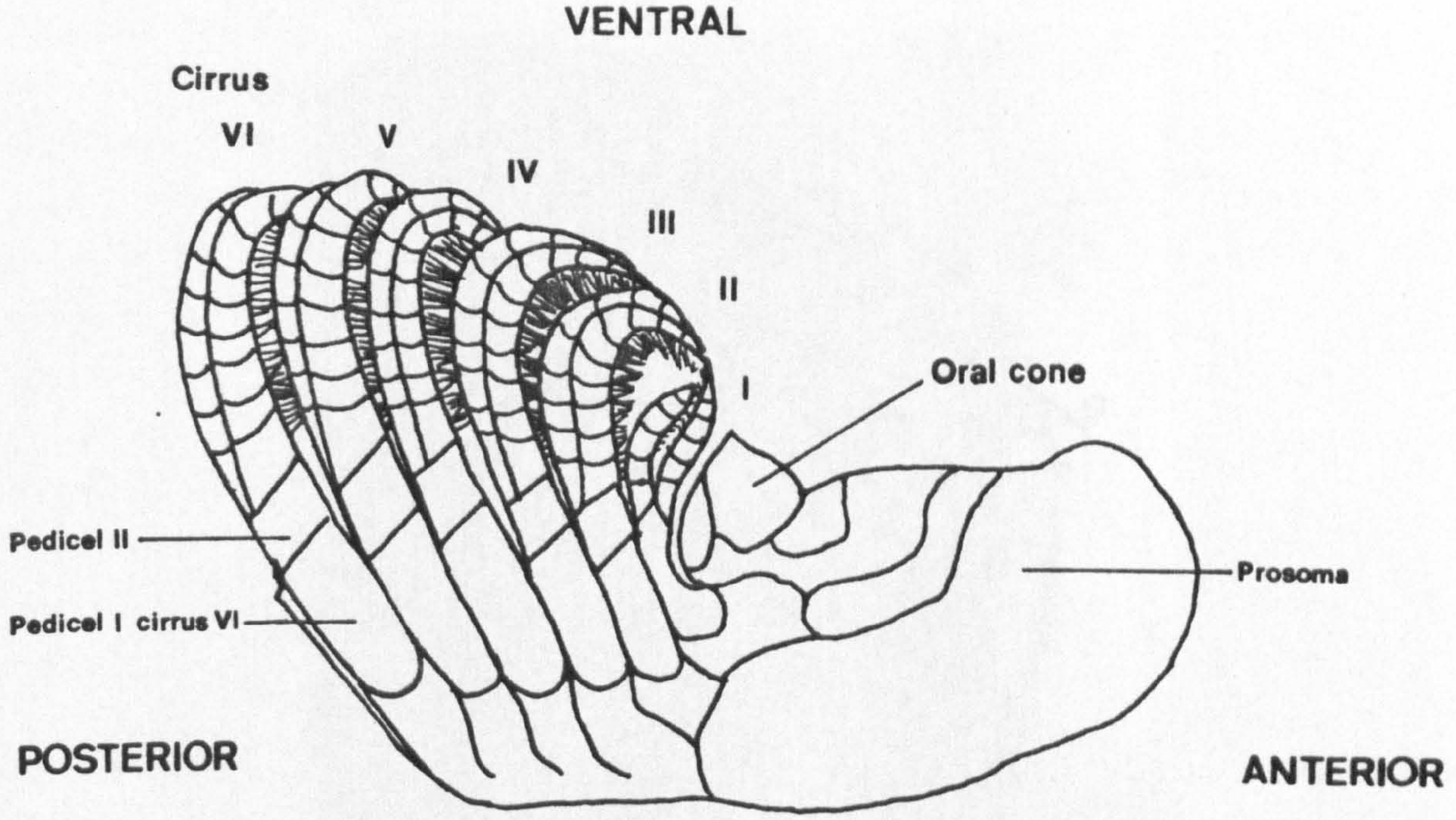
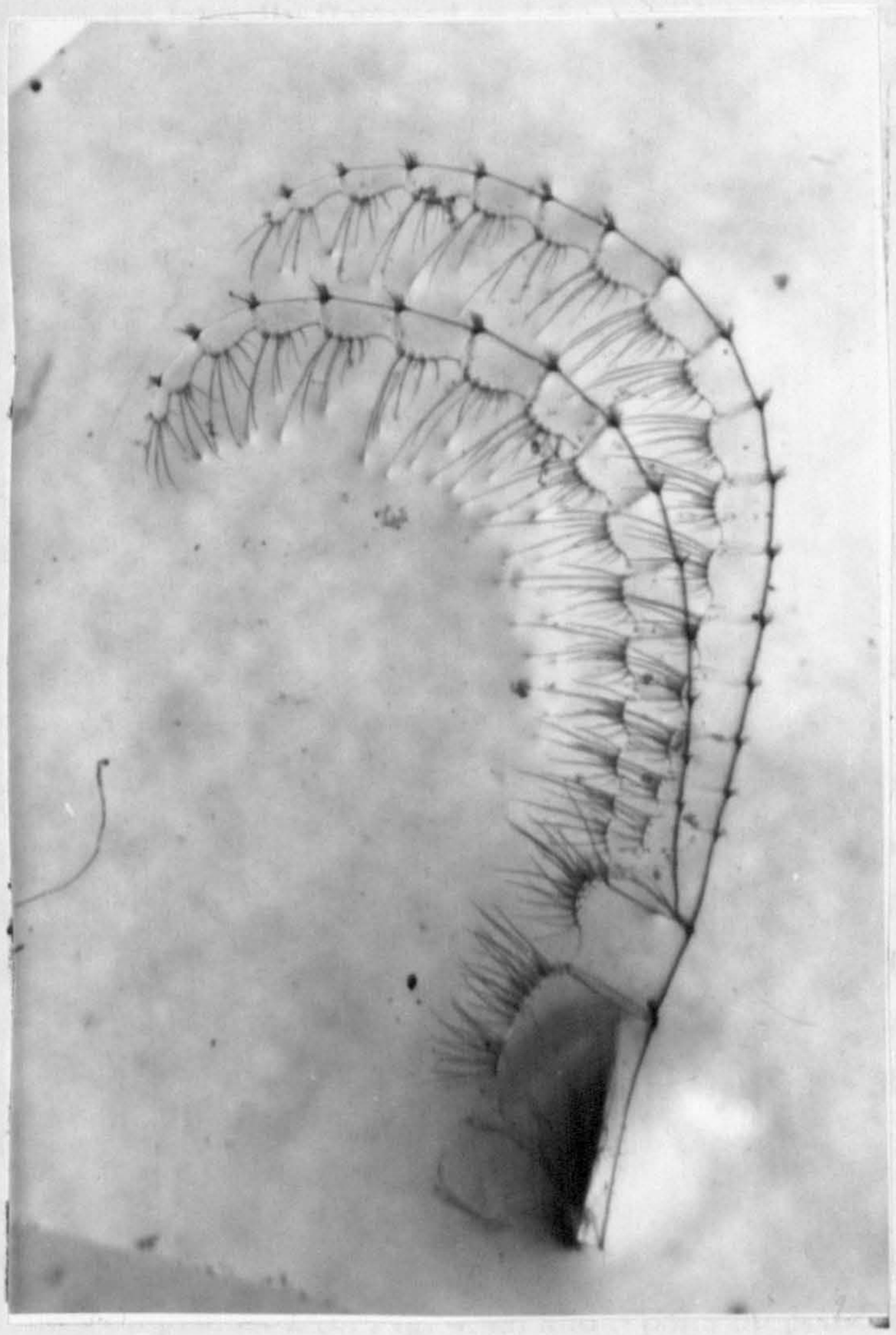


Figure 4.2. Diagram of the Lateral view of *Pollicipes pollicipes*' body indicating the nomenclature used.

E_x
E_y
S
I_L
V_p
P_1
P_2
1
2
3
4

Figure 4.3. Photograph of cirrus V of *Pollicipes pollicipes* (7.0 mm RC) indicating the measurements taken on each cirrus.



KEY:

Ex = Exopod En = Endopod P = podomeres or segments

P_1 = Pedicel 1 P_2 = Pedicel 2

L = Podomere (segment) length

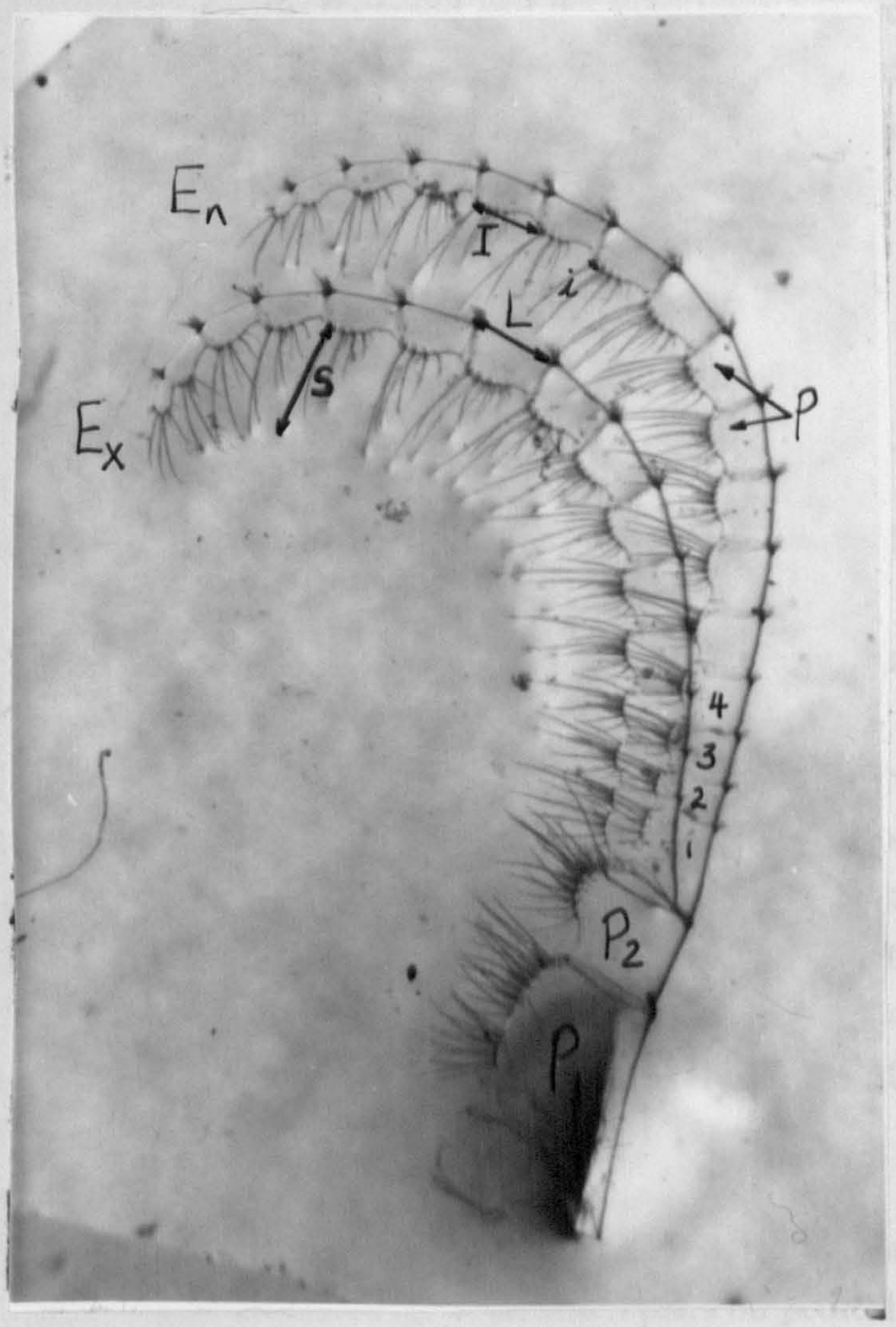
Cirrus length l = $(L_1 + L_2 \ldots\ldots + L_n)$

S = Setal length

I = Intersetal distance between corresponding setae on adjacent segments

i = Intersetal distance between setae on same segments

Figure 4.3. Photograph of cirrus V of *Pollicipes pollicipes* (7.0 mm RC) indicating the measurements taken on each cirrus.



KEY:

Ex = Exopod En = Endopod P = podomeres or segments

P_1 = Pedicel 1 P_2 = Pedicel 2

L = Podomere (segment) length

Cirrus length l = $(L_1 + L_2 \ldots\ldots + L_n)$

S = Setal length

I = Intersetal distance between corresponding setae on adjacent segments

i = Intersetal distance between setae on same segments

Results

The cirral morphology of *Pollicipes pollicipes.*

Cirrus I-VI of *Pollicipes pollicipes* can be seen in Figures 4.4 - 4.9

All cirri of *P. pollicipes* are laterally flattened and the rami taper towards the distal end. The segments have a protuberance on the anterior face on which the setae are distributed. On most of the segments the protuberance is symmetrical about the cirrus midline. However, on some cirri the protuberance is asymmetrical, being enlarged on one side. The proximal segments of most rami are broader than they are long whilst the more distal segments are longer than they are broad. Each cirrus has two pedicel segments.

Cirri IV - VI (Figs. 4.4 - 4.6)

The structure of cirri IV-VI is very similar and consists of 13-19, laterally flattened segments with small protuberances on the anterior face. The length of the cirri increases from IV - VI. The rami of each cirrus are approximately equal in length. The distribution of the setae on the segments of both rami is very similar. There are 5 pairs of anterio-laterally directed setae per segment distributed on the protuberances (a, Fig. 4.4a and a, Fig. 4.4b). The longest setae on each segment are the distal pair and there is a gradation in size to the shortest pair proximally. The short proximal spines point downwards but do not touch the distal spines on the next segment. Between the two rows of setae, on the crown of the protuberance, there is a small tuft of fine spines which point distally (b, Fig. 4.4b). There is also a group of small setae on the posterio-distal margin of all segments (b, Fig. 4.4a).

The setae on pedicels 1 and 2 are distributed in the manner seen on the ramal segments but with 7 pairs of setae on pedicel 2 (c, Fig. 4.4a) and 8 pairs on pedicel 1, although these are distributed in two groups (d, Fig. 4.4a). The cluster of smaller setae in the centre is more highly developed on pedicel 2.

Cirrus III (Fig. 4.7).

The rami of cirrus III are approximately equal in length and have 12-15 segments. The endopod, has fewer segments but, other than having more numerous anterio-lateral spines, is similar to cirri IV-VI. The distal segments of the exopod are all similar and resemble those of the posterior rami. The three proximal segments of the exopod are, however, highly modified bearing more numerous setae with the degree of setation increasing proximally (a, Fig 4.7).

Figures 4.4 a and b. Cirrus VI of *Pollicipes pollicipes* (see text for explanation of labelling).

Fig. 4.4 a. Cirrus VI from an animal 8.6 mm RC. The right cirrus in lateral view

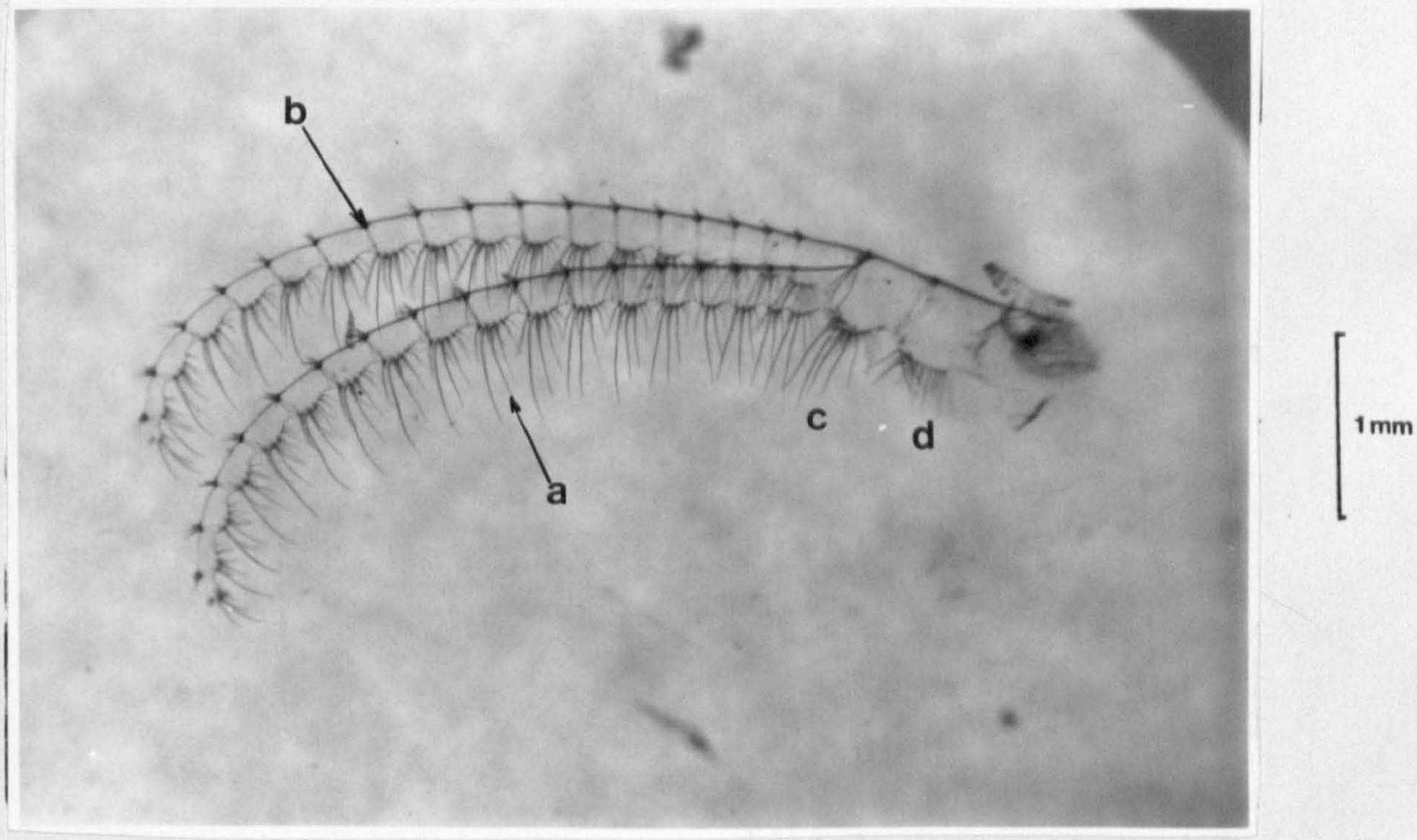
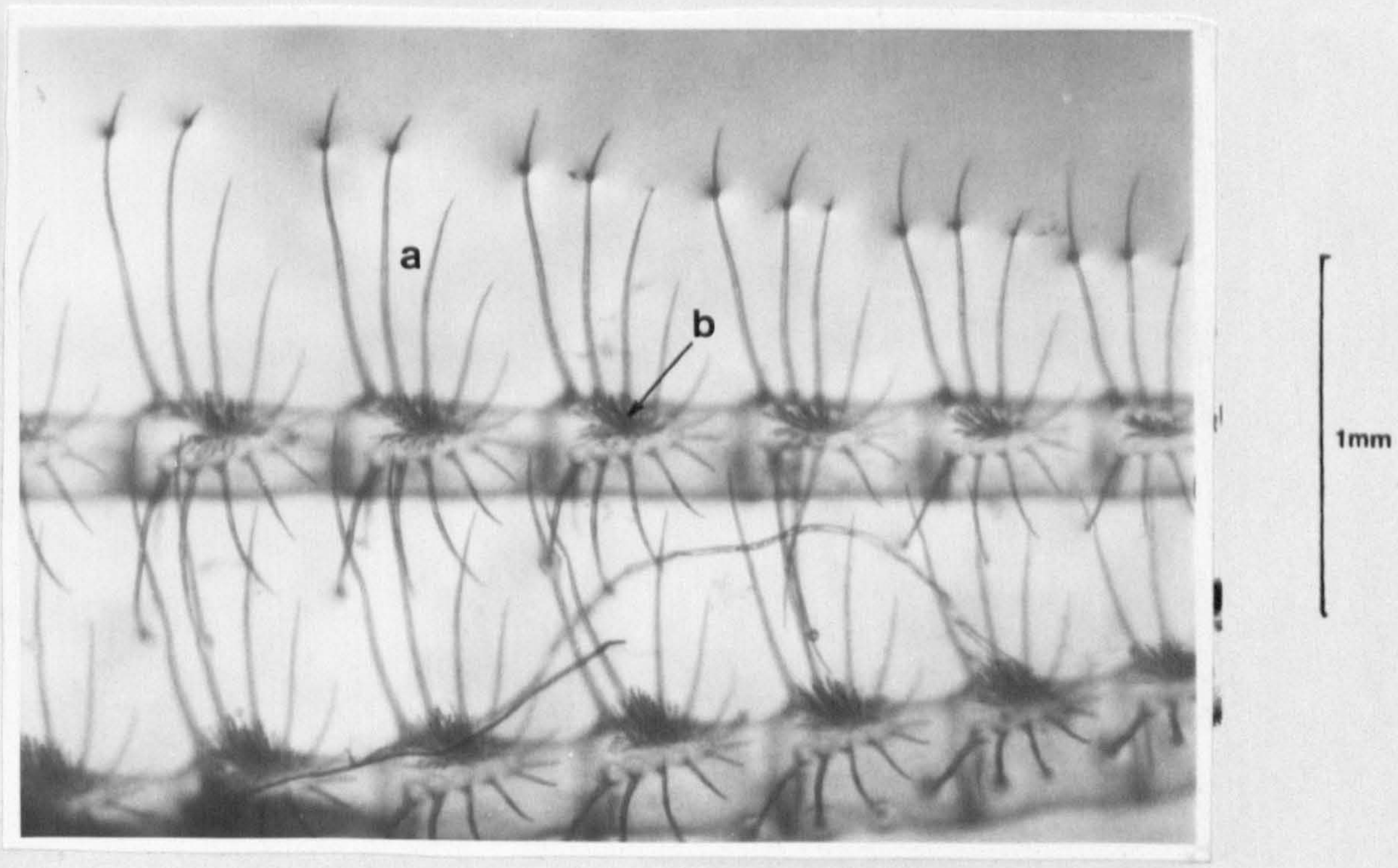


Fig 4.4b. The inside face of Cirrus VI from a *P. pollicipes* 11.15 mm in RC showing the distribution of setae common to cirri IV-VI.



Figures 4.5 and 4.6. Cirrus IV and V of *Pollicipes pollicipes*.

Fig. 4.5. Cirrus V of an animal 8.6 mm RC. The photograph shows the right cirrus in lateral view.

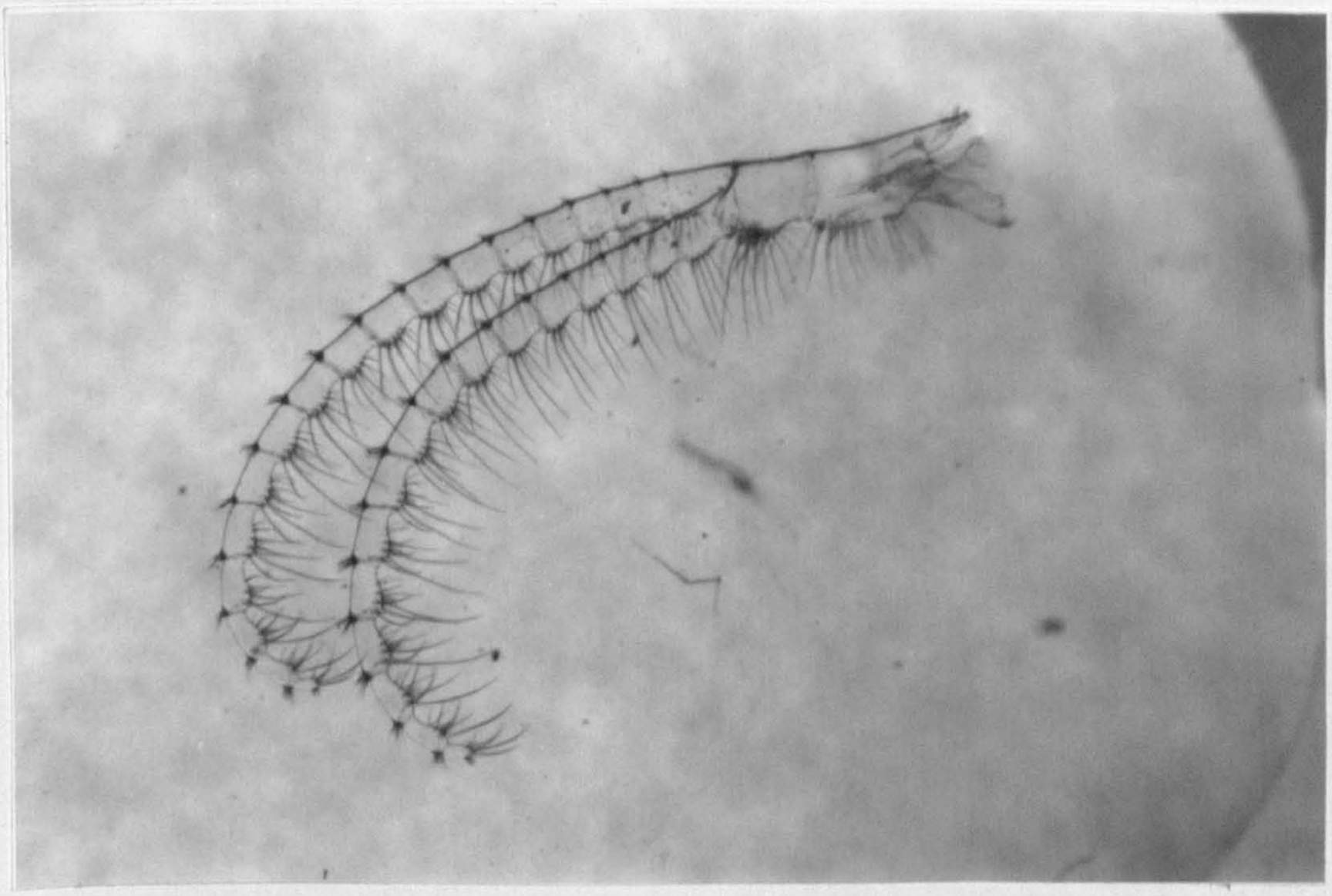
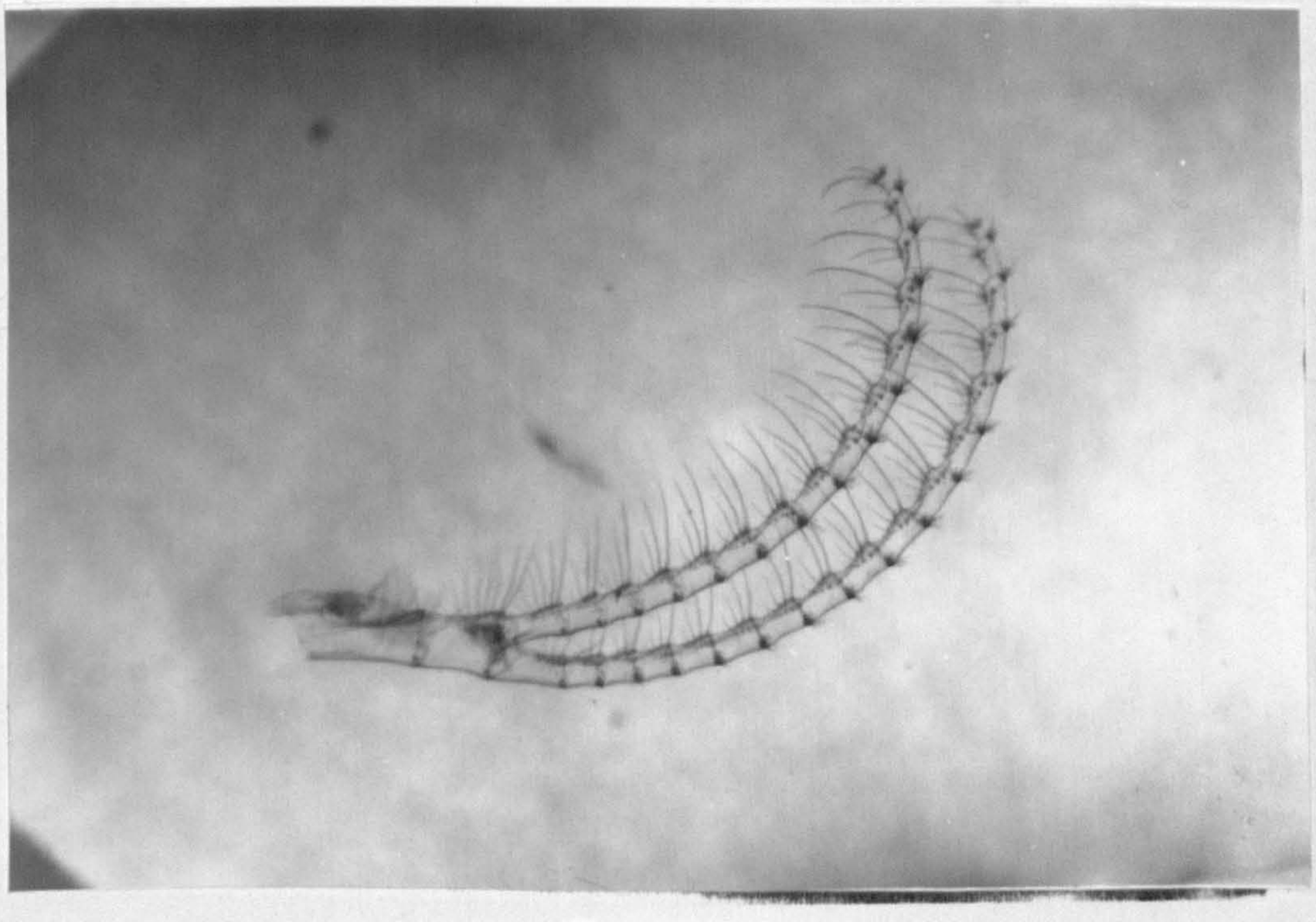


Fig. 4.6. Cirrus IV of an animal 8.6 mm RC. Left lateral view of the left cirrus.



Figures 4.7 and 4.8 a. Cirri III and II of *Pollicipes pollicipes* (see text for explanation of labelling).

Fig. 4.7. Cirrus III from an animal 4.6 mm RC, showing the anterior surface of the right cirrus.

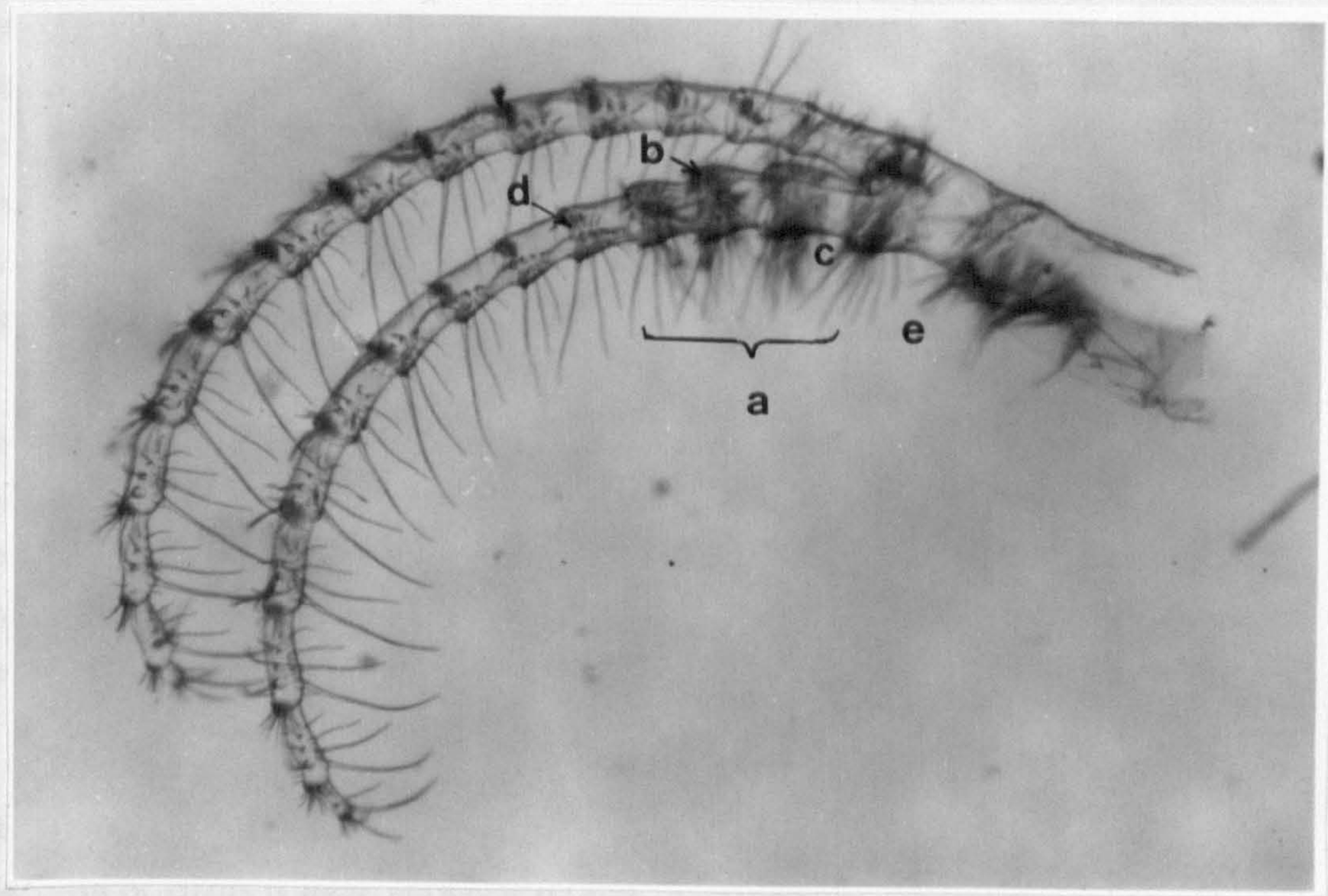
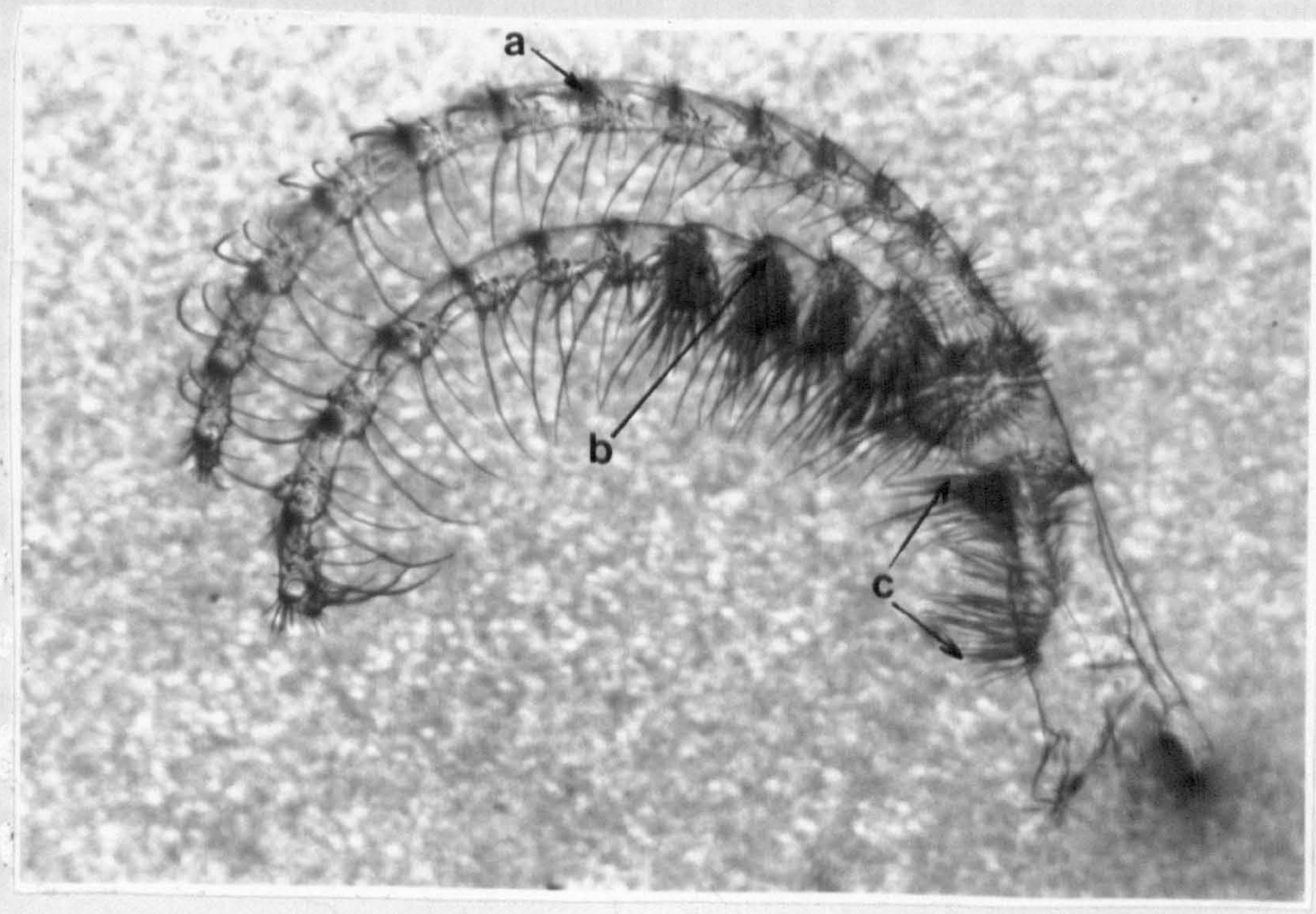


Fig. 4.8 a. Cirrus II is from an animal of 8.6 mm RC. The photograph shows the right cirrus in lateral view.



The outer, lateral, setae of segment 3 are similar to those of the distal segments with stout setae which decrease in size proximally. The setae are anteriorly directed becoming shorter, finer and more numerous towards the inner surface of the segment (b, Fig. 4.7).

The outer, lateral, setae of segments 1 and 2 are similar to those of segment 3 but are more numerous on segment 1. The distribution pattern of the rest of the setae is similar, decreasing in size from the outer to inner margins across the anterior segment surface. The size and asymmetry of the segment protuberances increases proximally (c, Fig. 4.7). On segment 4 of the exopod, there is a secondary set of very short setae running parallel to, and outside the anterio-lateral setae (d, Fig. 4.7)

Pedicels 1 and 2 both bear large, dense clumps of fine, anteriorly directed setae. On pedicel 1 these are longer proximally and distally, shortest in the middle of the pedicel. On pedicel 2 the paired setae show graduation in size, longest distally, running down the lateral edges of the anterior protuberance (e, Fig. 4.7). There are also shorter setae in the middle portion of the pedicel.

Cirrus II (Figs. 4.8a and b.)

The rami of cirrus II have between 10 and 14 segments. The exopod is longer than the endopod and the distribution of setae on the two rami is quite different. The setal distribution on the endopod and all but the proximal four segments on the exopod follows very much the same pattern as on cirri IV - VI. There are, however, only four pairs of setae per segment and additional groups of short, fine setae on the outer anterio-lateral portion of the segment (a, Fig. 4.8a). The setal distribution on segments 1-4 of the exopod is quite different from that on any other segments. The segment protuberances bear brush-like pads of finer, slightly shorter spines (b, Fig. 4.8a; a, Fig.4.8b). The outer anterio-lateral spines on each of these segments are analogous to those on cirri IV-VI but they are thicker, longer and point anteriorly, becoming shorter and finer towards the inner face. Again, the segment protuberances increase in size and asymmetry towards the proximal part of the ramus.

Pedicels 1 and 2 both bear dense tufts of fine setae on the anterior surface. On Pedicel 2 there is a dense central tuft of shorter setae surrounded by a ring of spines, longest on the outer lateral margin, shortest on the inner lateral margin. The spines are directed forward and somewhat proximally On Pedicel 1, the proximal spines are distally directed whilst the distal spines are proximally directed. The longest spines are located on the outer lateral margin, particularly proximally and distally (c, Fig. 4.8a).

Figures 4.8 b and 4.9 a. Cirri II and I of *Pollicipes pollicipes* (see text for explanation of labelling).

Fig. 4.8 b. Cirrus II of an animal of 11.15 mm RC, showing the dense setation on the protuberances of the proximal four segments of the exopod.

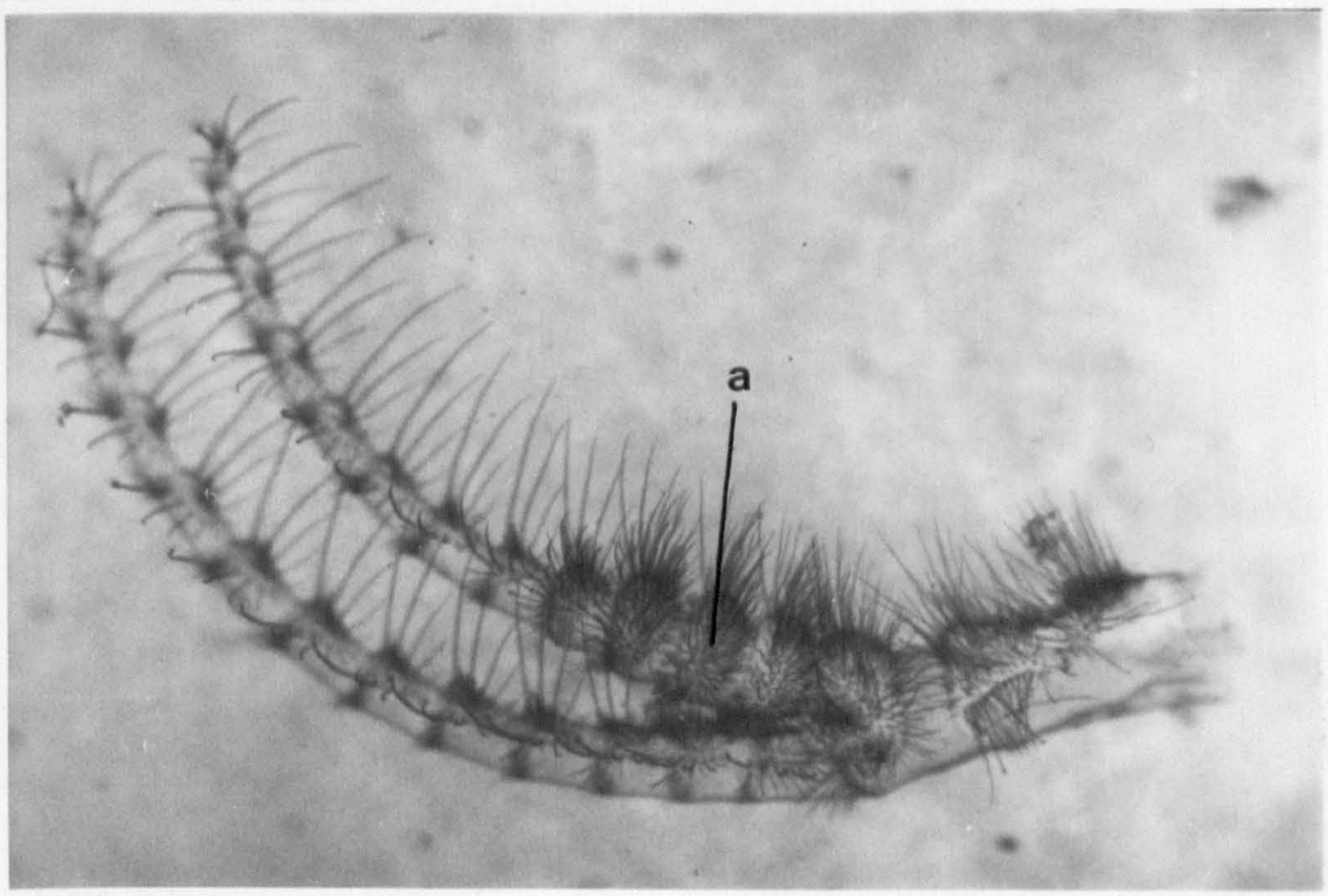
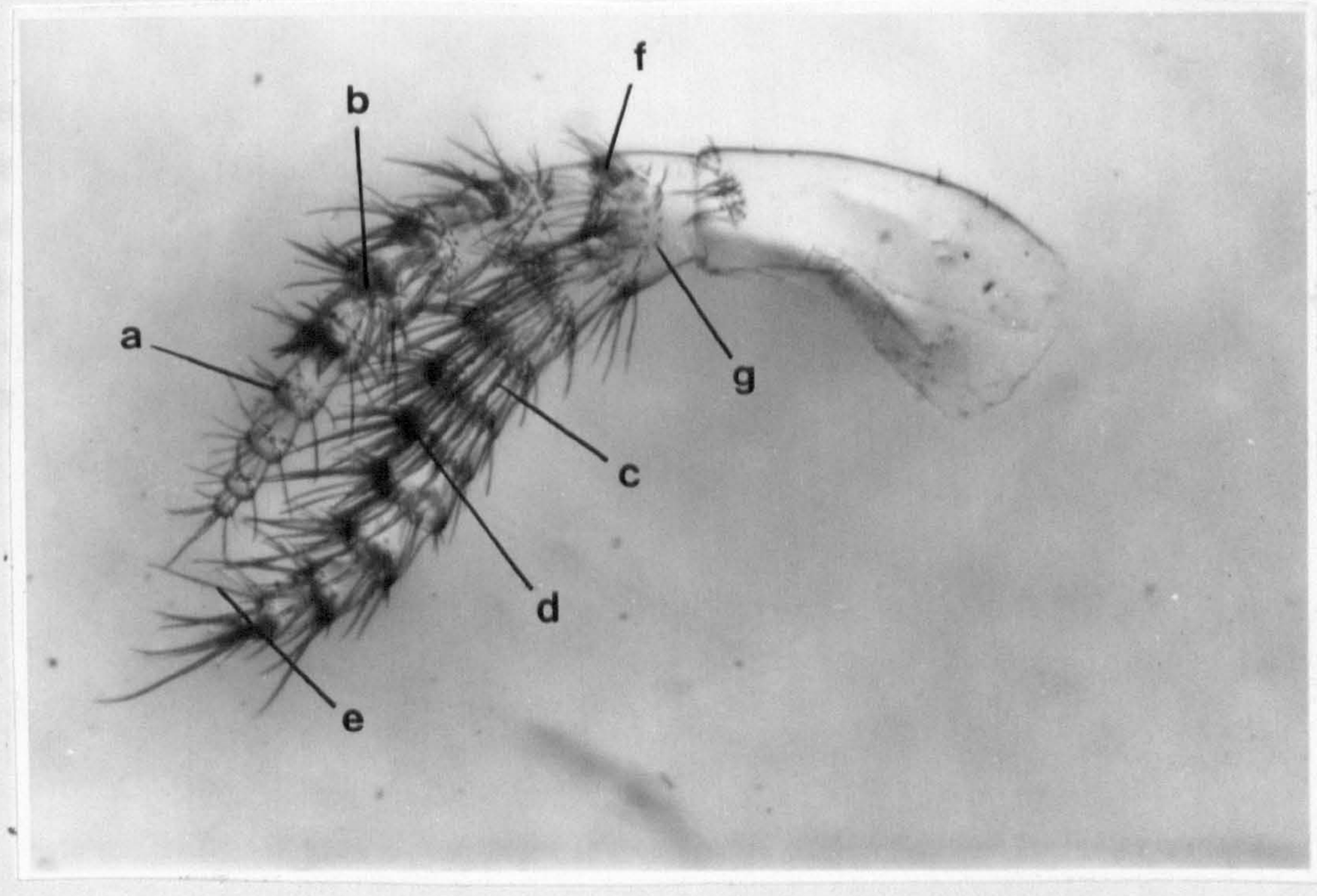


Fig. 4.9 a. Cirrus I of an animal of 7 mm RC. The photograph shows the right cirrus in median view.



Cirrus I (Fig. 4.9 a and b)

Cirrus I arises at the side of the mouth. The rami leave the pedicel at a more acute angle than do those of the other cirri. In its natural position, the anterior ramus (the exopod) points almost directly to the anterior of the animal and lies along the side of the mouth. The exopod is longer than the endopod. Most segments of both rami exhibit significant modification of the basic morphology.

The terminal 4 segments (5-8) of the endopod have fewer setae than the other segments of either ramus and are more similar to the segments of cirri IV - VI although they have more short spines on the distal median margin (a, Fig. 4.9a). The proximal 4 segments have larger numbers of finer, distally directed setae covering the anterior surface, concentrated on the distal half of the segment, on the protuberance (b, Fig. 4.9a). The 5 proximal segments of the exopod have large distally pointing spines distributed on the anterior (ventral) surface of the ramus (c, Fig. 4.9a) with a concentration of short fine spines on the ventral and inner lateral face (d, Fig. 4.9a). The 4 distal segments have fewer setae. Each has a collar of long, stout setae on the distal margin, on the ventral and inner lateral surfaces (e, Fig. 4.9a). These setae point outward and distally.

Pedicel 2 bears a group of distally directed spines on the anterior surface towards the distal margin (b, Fig. 4.9a and a, Fig.4.9b). There are also a few shorter, more widely spaced, proximally directed spines below these (g, Fig. 4.9a and b, Fig.4.9b). Pedicel 1 bears fewer, shorter, distally directed setae.

Caudal Appendages (Fig. 4.10)

Pollicipes pollicipes has a pair of short, wide caudal appendages flanking the penis posterior to Cirrus VI. Each has four segments decreasing in length and width distally. There are few setae present. The most distal segment has a crown of simple setae (s, Fig. 4.10) on the end. The caudal appendages lie flat against the body and cirrus VI.

Figures 4.9 b and 4.10. Cirrus I and the caudal appendages of *Pollicipes pollicipes*. (see text for explanation of labelling).

Fig. 4.9b. Cirrus I of an animal 11.15 mm RC, showing the dense setation typical of this appendage.

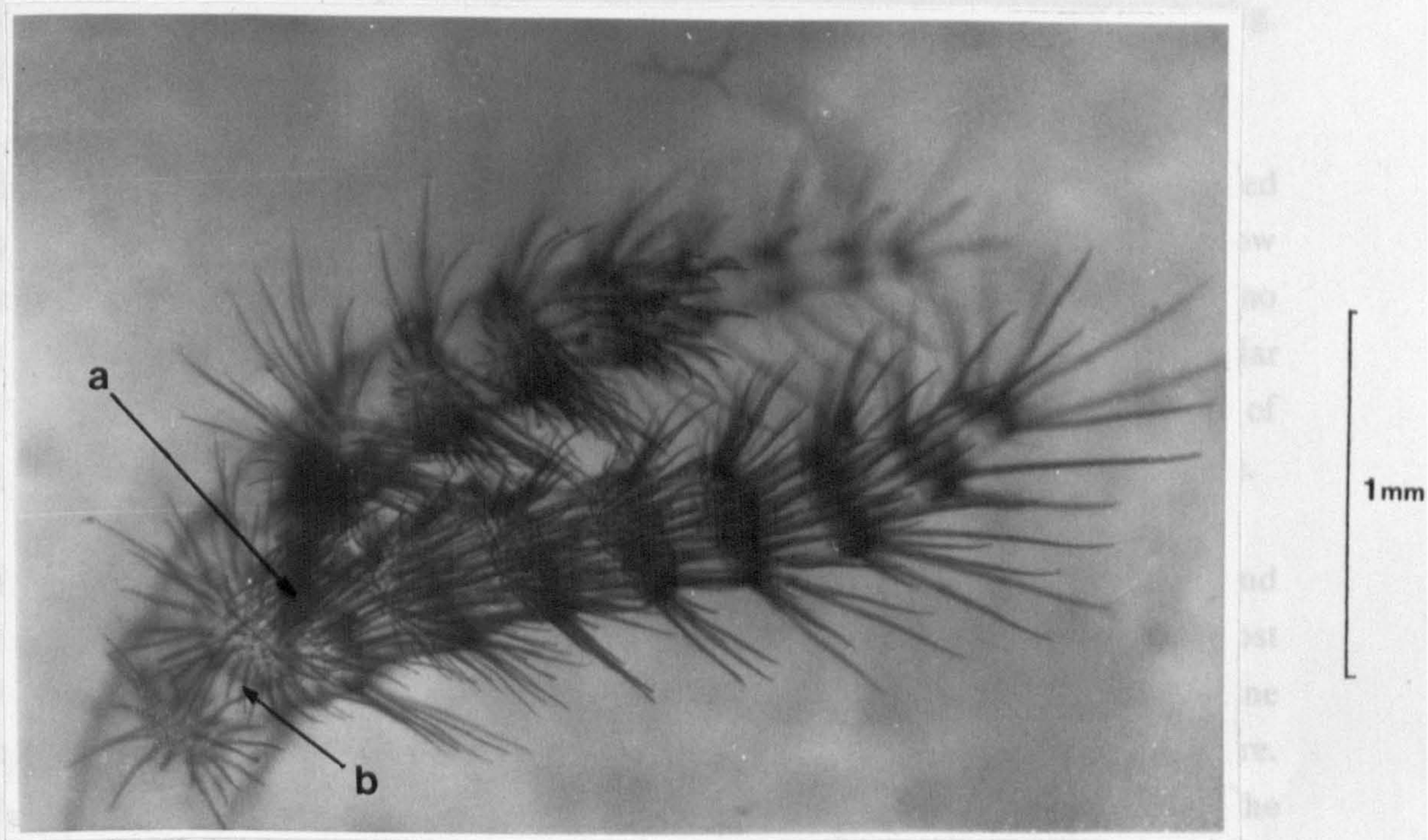
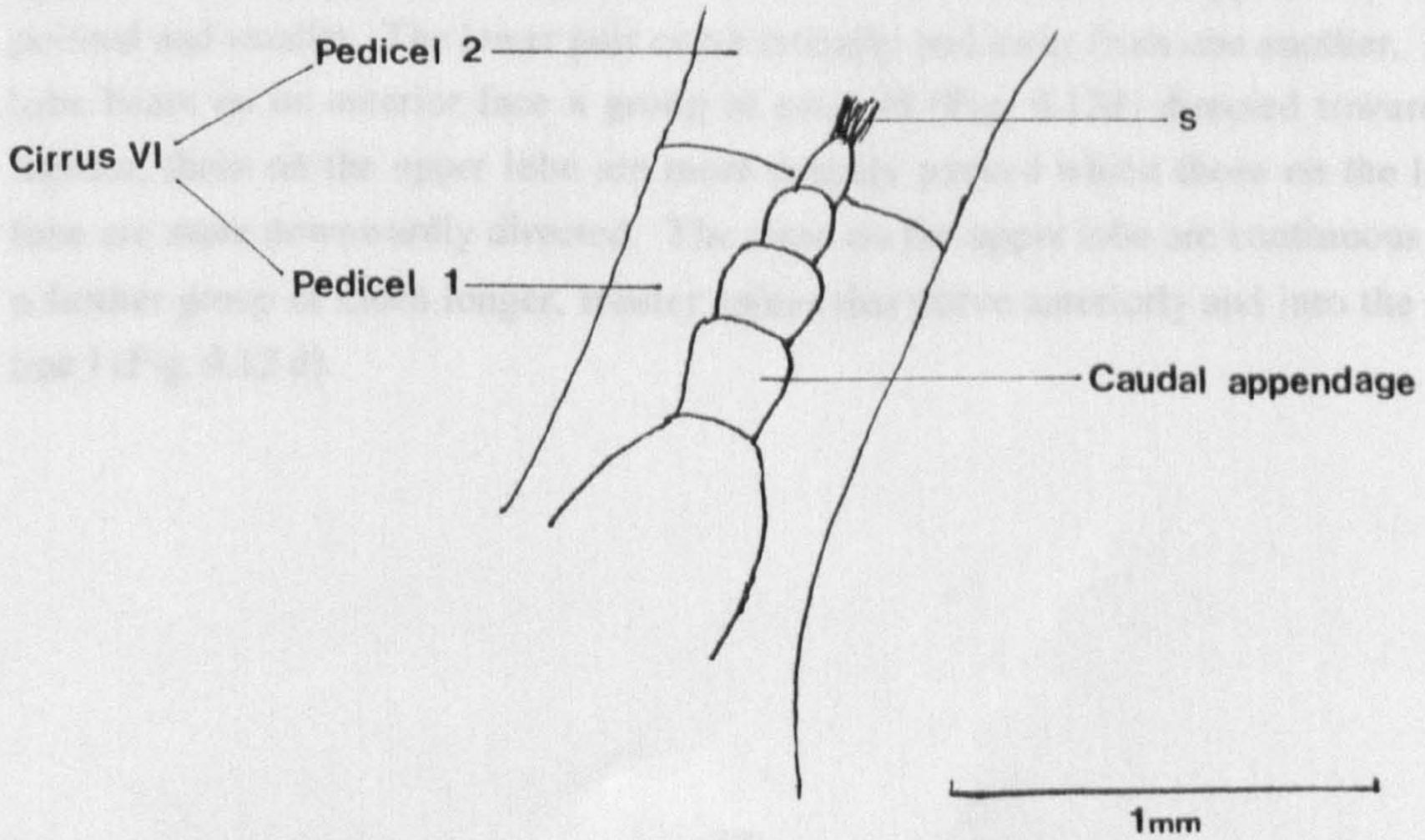


Fig. 4.10. The caudal appendages of *Pollicipes pollicipes*.



The mouthparts of *Pollicipes pollicipes*

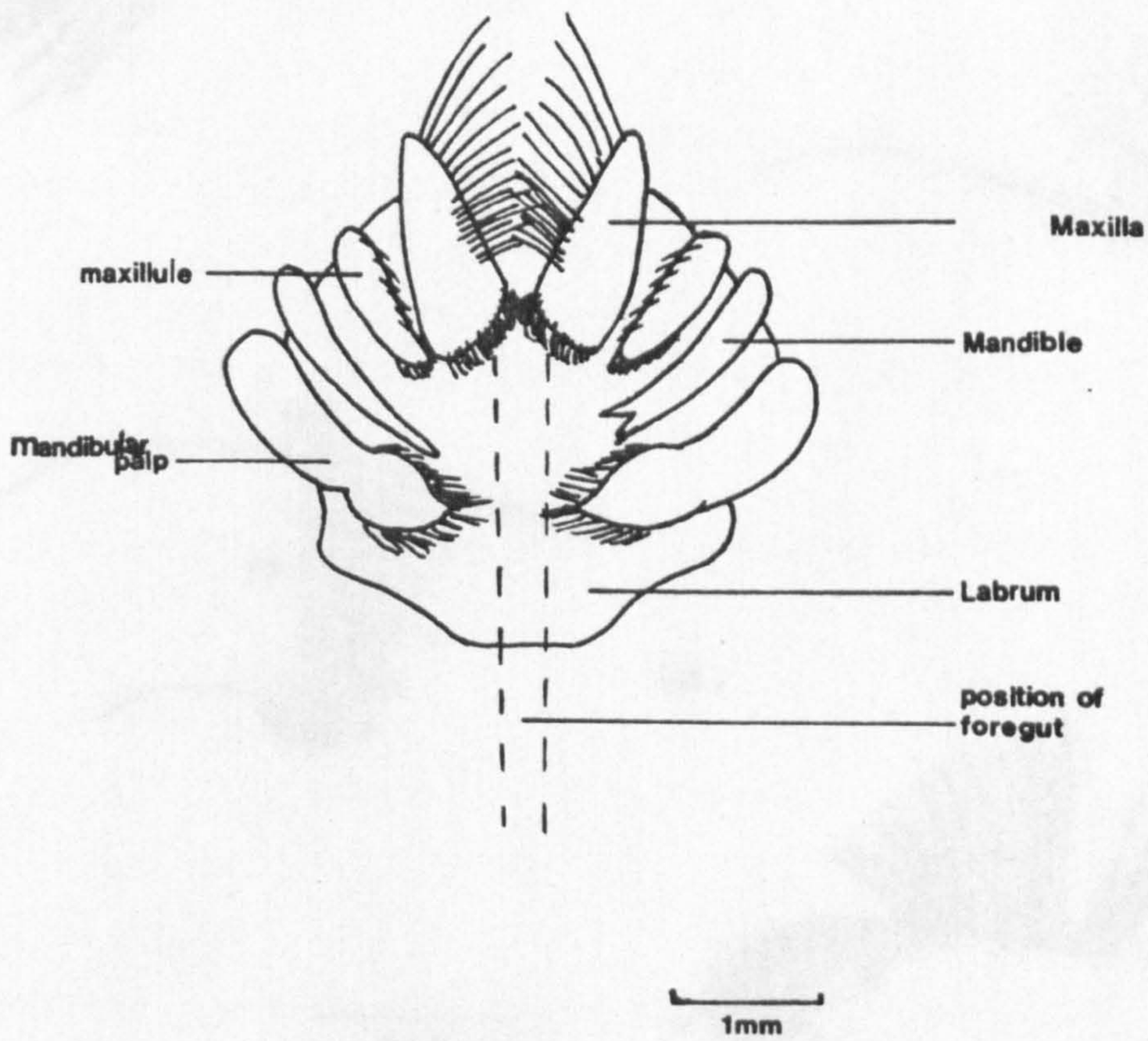
Figure 4.11. shows the arrangement of the mouthparts of *Pollicipes pollicipes*. The structure of each appendage is shown in Figure 4.12 a - d. The labrum narrows ventrally and is bulbous. The palps lie across the anterior part of the mouth to form the ventral border to the oral cone. The palps are paddle-shaped, edged with long spines that curve towards the mid-line and the mouth. The spines in position A (Fig. 4.12 a) are very fine, and are longer than those on the lower margin B (Fig 4.12 a).

Behind the palps lie the mandibles. The mandibles are broad, flat sickle-shaped appendages that are heavily chitinised on the distal edge. The mandibles are yellow and have four strong, sharply pointed teeth C (Fig. 4.12 b) on the distal edge with no secondary cusps. Below these teeth there is a blunt lobe D (Fig. 4.12 b), the molar process, which bears a number of short, stout setae directed towards the mid-line of the mouth. On the upper surface there is a small patch of fine setae DD (Fig. 4.12 b).

The maxillule (also known as maxilla I) lies above the mandible. It is flat and somewhat rectangular in shape, with a roughly trilobed leading edge. The uppermost lobe bears a pair of long stout spines E (Fig. 4.12 c). Underneath there is a tuft of fine spines which are very densely packed and graded in size with the longest in the centre. Similar groups are present on the middle and lower lobes and on the underside of the lower lobe F (Fig. 4.12 c). Between these groups of finer spines there are several pairs of shorter, stouter spines G (Fig. 4.12 c), three pairs on the second lobe and four pairs on the third. There are a few short setae on the inner surface of the appendage FF (Fig. 4.12 c). The upper surface also bears fine setae, distally distributed and directed towards the mid-line GG (Fig. 4.12 c)

The maxillae lie behind the maxillules and nearer to the mid-line. They form the uppermost margin to the mouthparts. Each has two blunt lobes, the uppermost slightly pointed and smaller. The lower pair curve laterally and away from one another. Each lobe bears on its anterior face a group of setae H (Fig. 4.12d) directed toward the labrum, those on the upper lobe are more densely packed whilst those on the lower lobe are more downwardly directed. The setae on the upper lobe are continuous with a further group of much longer, stouter spines that curve anteriorly and into the mid-line I (Fig. 4.12 d).

Figure 4.11. The arrangement of the mouthparts of *Pollicipes pollicipes*. Drawn to scale.



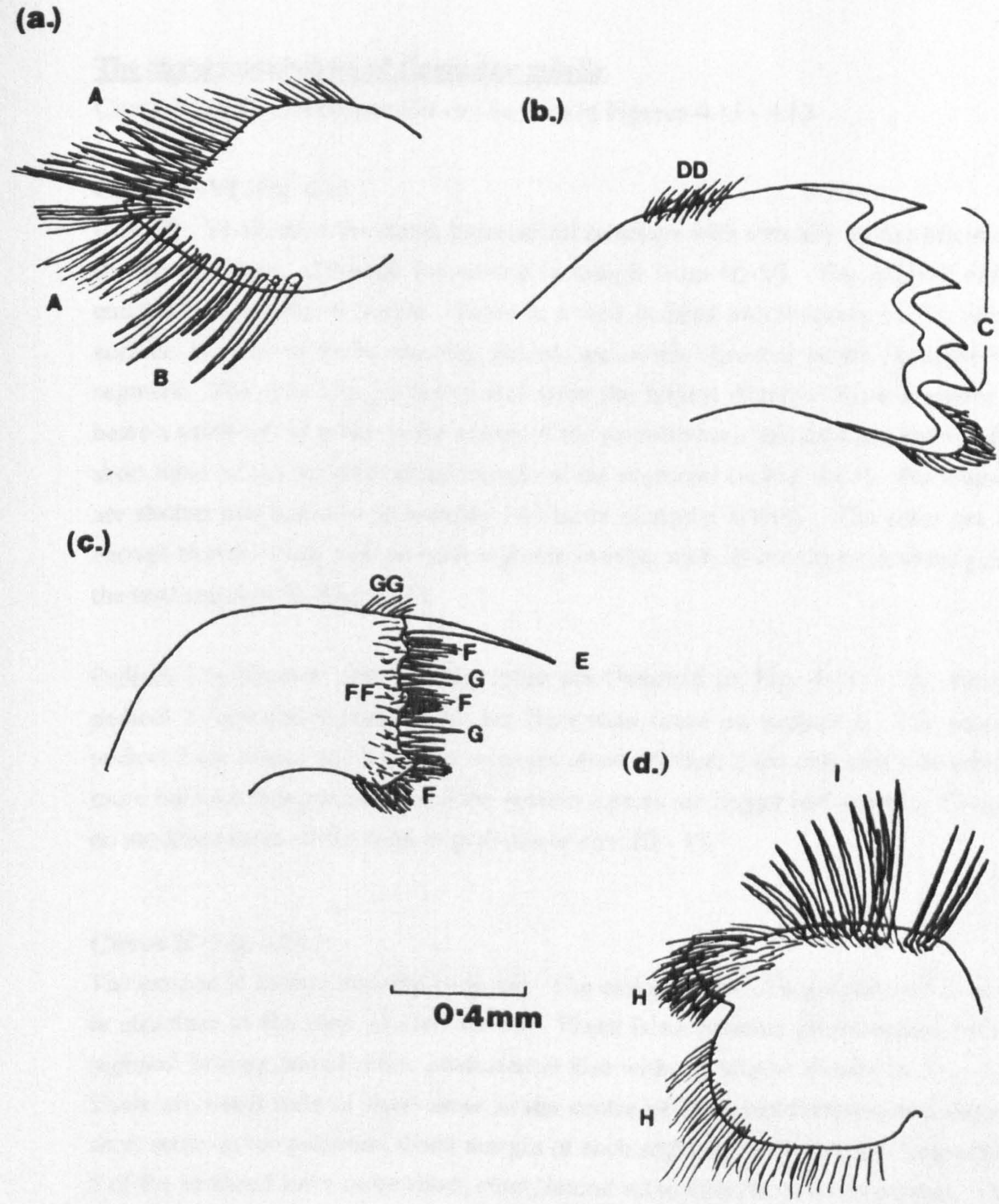


Figure 4.12. Scale drawings of the mouthparts of *Pollicipes pollicipes* taken from an animal 14.9 mm RC.

a) Inner view of left mandibular palp.

b) Inner view of right mandible.

c) Inner view of right maxillule.

d) Inner view of left maxilla.

The cirral morphology of *Capitulum mitella*

Cirri I-VI of *Capitulum mitella* can be seen in Figures 4.13 - 4.15.

Cirri III -VI (Fig. 4.13.)

Cirri III - VI all show the same, basic, cirral structure with virtually no modification to the general form, although increasing in length from III-VI. The exopod and the endopod are similar in length. There is a well-defined protuberance on the anterior surface of each segment bearing paired, anteriorly directed setae, four pairs per segment. The setae are graded in size from the largest distally. Each segment also bears a small tuft of setae on the crown of the protuberance and there are small tufts of short setae on the posterior distal margin of the segments (a, Fig. 4.13). The segments are shorter and squatter proximally and more elongate distally. The setae are long enough that the distal pair on each segment overlap with all but the most distal pair on the next segment (b, Fig. 4.13).

Pedicel 1 is bilobed, although the lobes are flattened (c, Fig. 4.13). The setae on pedicel 1, especially proximally, are finer than those on pedicel 2. The setae on pedicel 2 are longer and the distal setae are stout. Pedicel 2 has only one lobe which is more bulbous than pedicel 1 and the setae it carries are longer and stouter. There are no modified setae on the rami or pedicels or cirri III - VI.

Cirrus II (Fig. 4.14.)

The exopod is shorter than the endopod. The endopod has 15 segments and is similar in structure to the rami of cirri III-VI. There is an anterior protuberance on each segment bearing paired setae, graduated in size with the largest distally (a, Fig. 4.14). There are small tufts of short setae in the centre of each protuberance and there are short setae on the posterior, distal margin of each segment (b, Fig. 4.14). Segments 1-5 of the endopod have more short, stout, lateral setae than the distal segments. These segments are in no other way modified. The exopod has 13 segments and the proximal 4 segments are highly modified. The segment protuberance is very well developed, it is large and flattened in anterior view and very bulbous in lateral view (c, Fig. 4.14). The protuberance becomes increasingly asymmetric proximally (d, Fig. 4.14). These segments are much broader, deeper and less elongate than the more distal ones.

There is very dense covering of fine setae over the whole anterior surface of the protuberance. Although most of the setae are simple there are pectinated spines present on the anterior surface of exopodal segments 1 - 5. On segments 1 and 2 these

Figures 4.13 and 4.14. Cirrus IV and II of *Capitulum mitella* (see text for explanation of labelling).

Fig. 4.13. Cirrus IV of an 11.8 mm RC individual. The cirrus appears dark because the damaged exoskeleton still contains most of the limb.

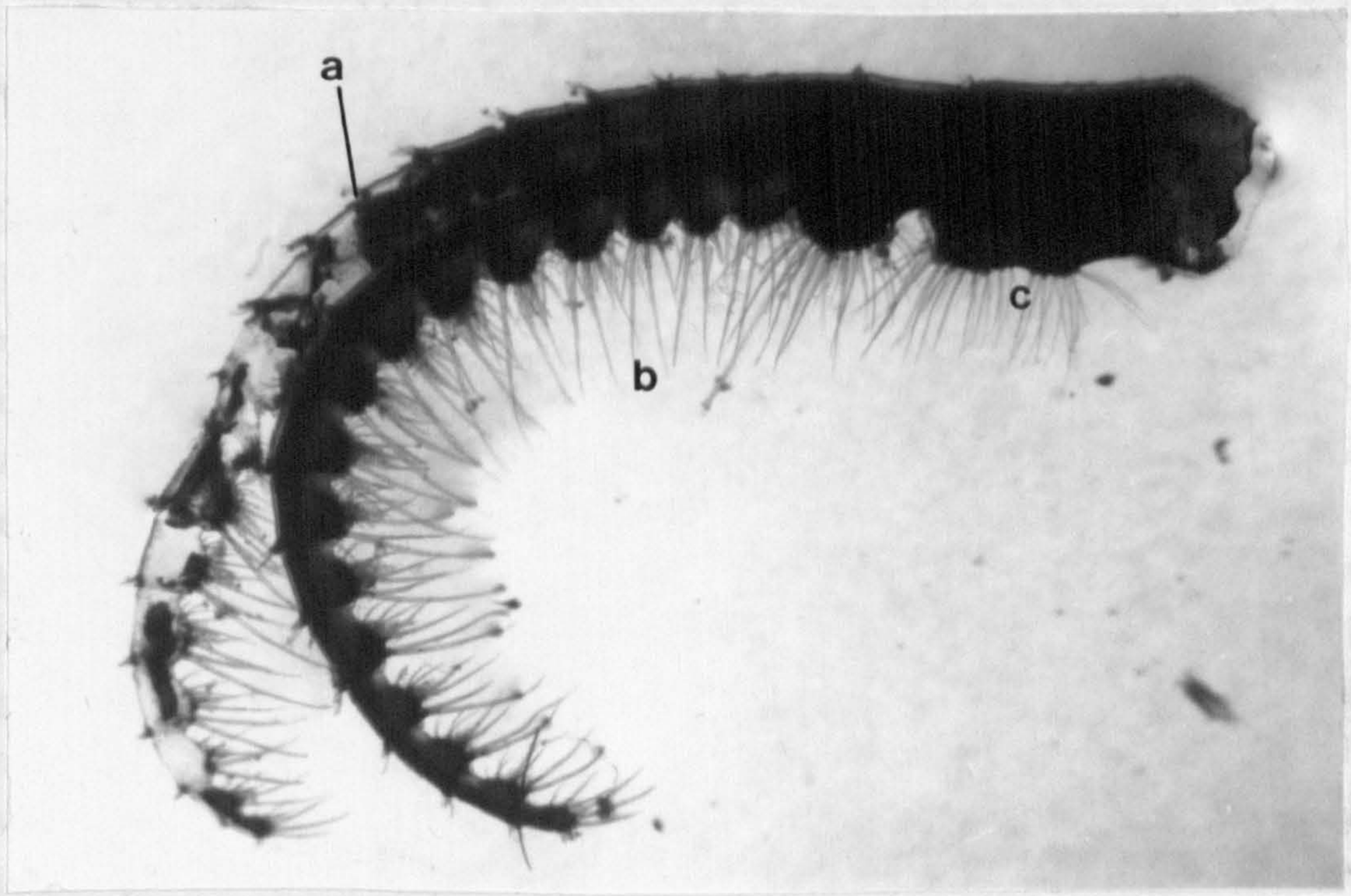
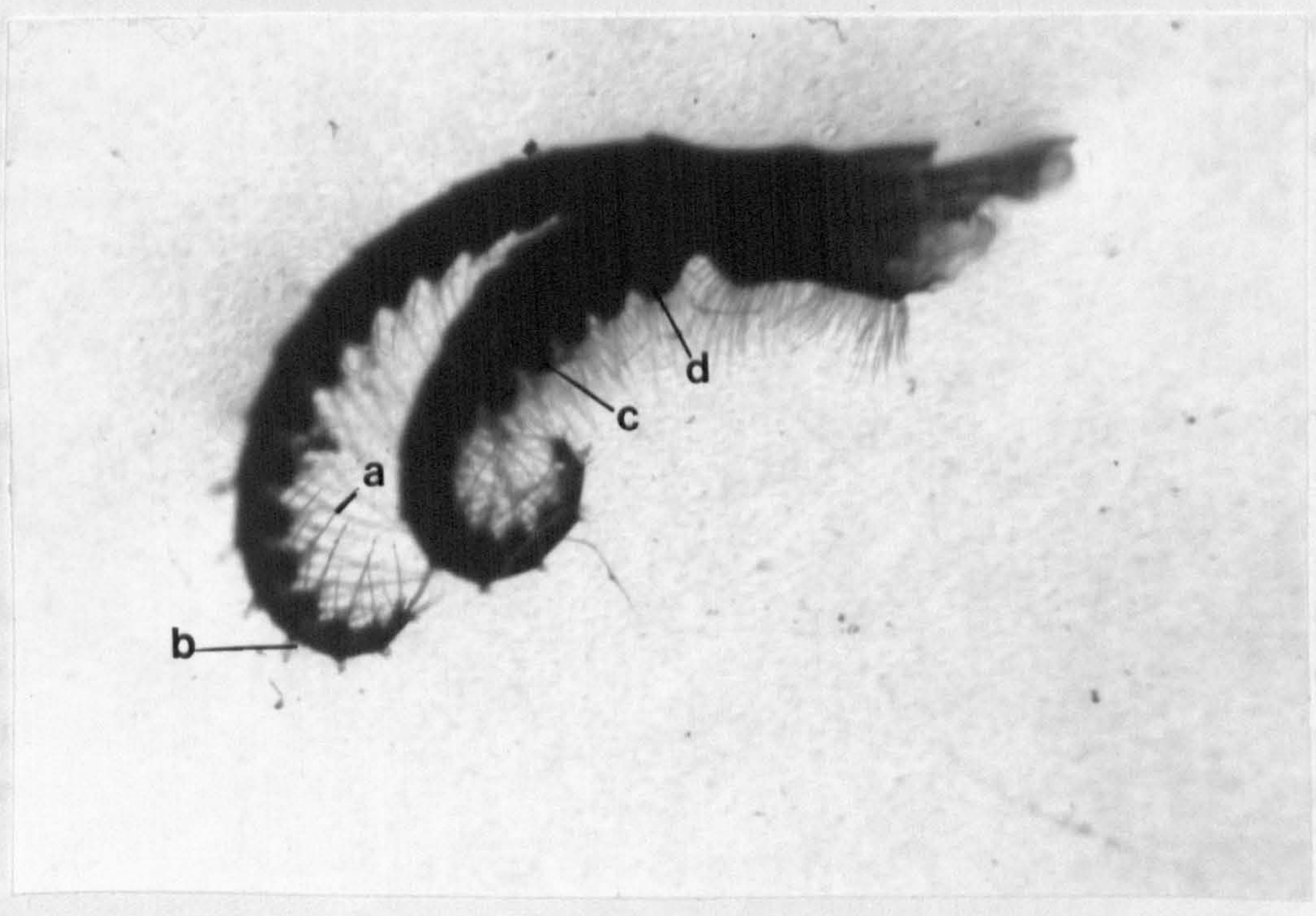


Fig. 4.14. Cirrus II of the same animal. Although this is a poor photograph it is still possible to see the swollen protuberances on the proximal 4 segments of the exopod.



1mm

spines are few, unevenly distributed and most abundant toward the inner margin of the anterior surface. Segment 5 bears the greatest density of pectinated spines.

There are no modified spines on pedicel 1. Pedicel 2 has five pectinated setae which are concentrated on the endopodal side of the anterior lobe. Pedicel 1 is very large with two large, flattened lobes bearing many fine setae which are longer on the distal lobe. Pedicel 2 is much more bulbous than 1 and has longer setae.

Cirrus I (Fig. 4.15.)

The exopod is longer, although less stout, than the endopod. There are nine segments in the exopod and eight in the endopod of an individual of 11.8 mm RC. The first segment of both rami is elongate compared to the rest (a, Fig. 4.15). Each segment has a large, broad protuberance on the anterior surface, which is densely covered with setae. There are also well developed tufts of stout setae on the latero-distal margins of the segments (b, Fig. 4.15). These setae are longer on the more distal segments. Endopodal segments 5 and 6 bear a mixture of simple and pectinated spines with the latter, most abundant on segment 5 (c, Fig. 4.15). There are no modified spines on the exopod and there are a few setae on either pedicel. The protuberance on pedicel 2 bears setae and there are small tufts of fairly stout setae on the latero-distal margin on the endopodal side of both pedicels (d, Fig. 4.15) but only pedicel 2 bears such setae on the exopodal side (e, Fig. 4.15).

Caudal Appendages (Fig. 4.16.)

The paired caudal appendages of *Capitulum mitella* are very short and stout. They have two segments mounted on a basal region situated at the base of cirrus VI. The proximal segment is very large and stout while the distal one is about a quarter of the length and half as broad. Both segments have a collar of setae around the distal margin (a, Fig. 4.16). The caudal appendages do not lie flat against the body or the cirri but stick up clear of both.

The mouthparts of *Capitulum mitella*

The arrangement of the mouthparts of *Capitulum mitella* is shown in Figure 4.17 while Figure 4.18 a to d show the structure.

The labrum is bullate and broad. The palps lie across the front of the mouth and at a more acute angle than those of *P. pollicipes*. The margins bear spines which curve towards the mid-line, the uppermost ones A (Fig 4.18 a) are finer than those on the underside B (Fig 4.18 a). The mandibles are, like those of *P. pollicipes*, sickle-shaped,

Figure 4.15 and 4.16. Cirrus I and caudal appendages of *Capitulum mitella* (see text for explanation of labelling).

Fig. 4.15. Cirrus I of an 11.8 mm RC individual.

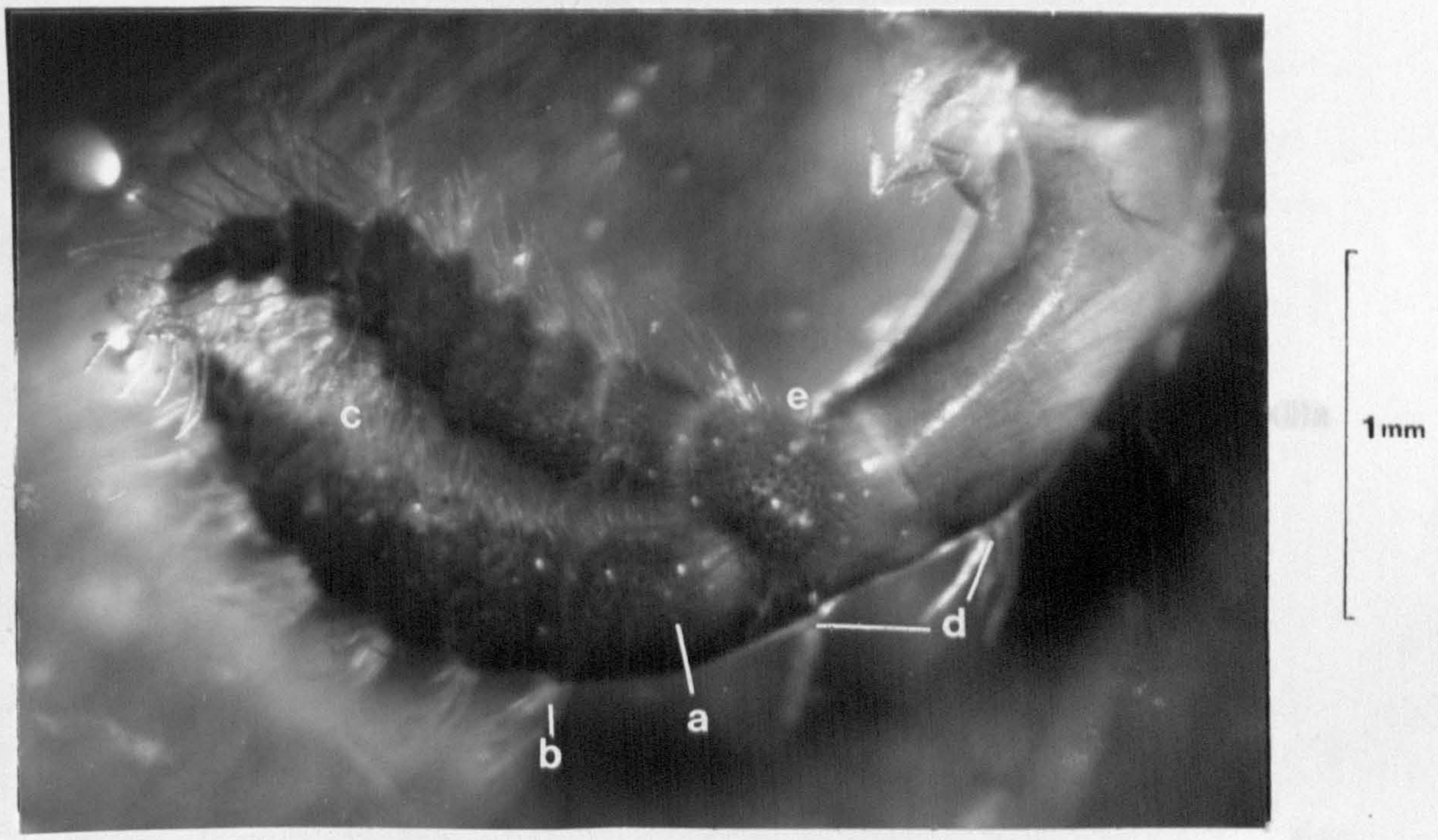


Fig. 4.16. Caudal appendage of *Capitulum mitella*.

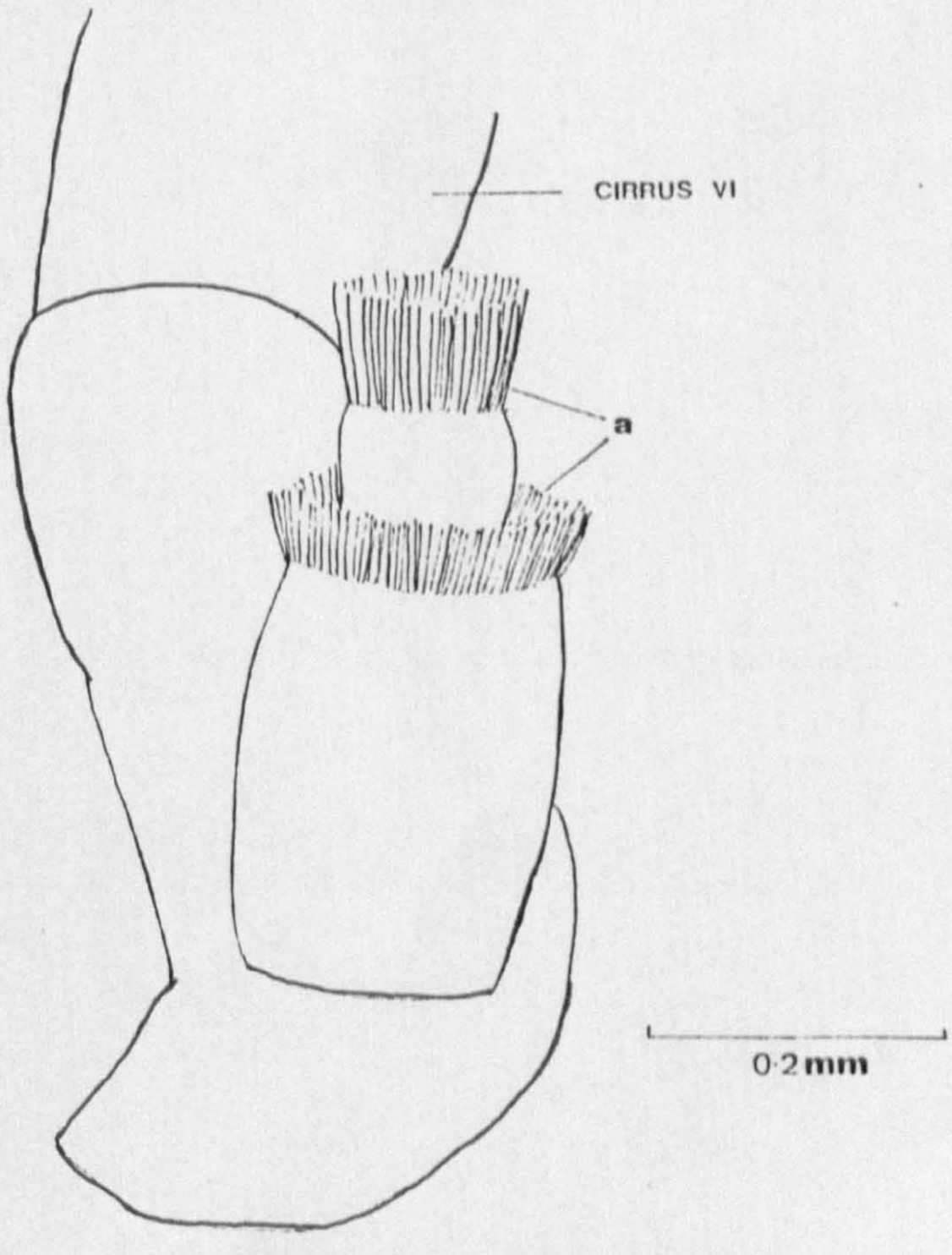
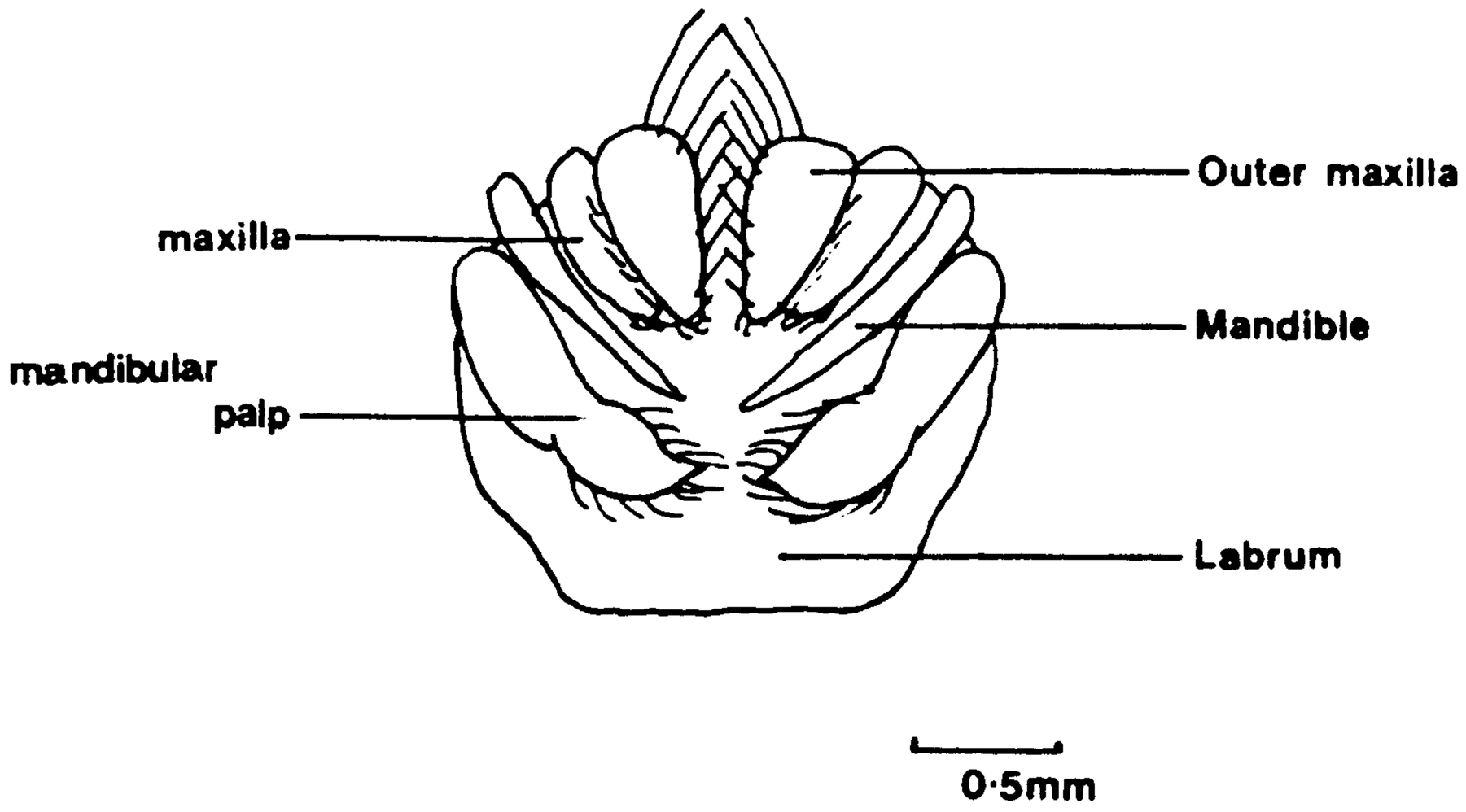


Figure 4.17. The arrangement of the mouthparts of *Capitulum mitella*. Drawn to scale.



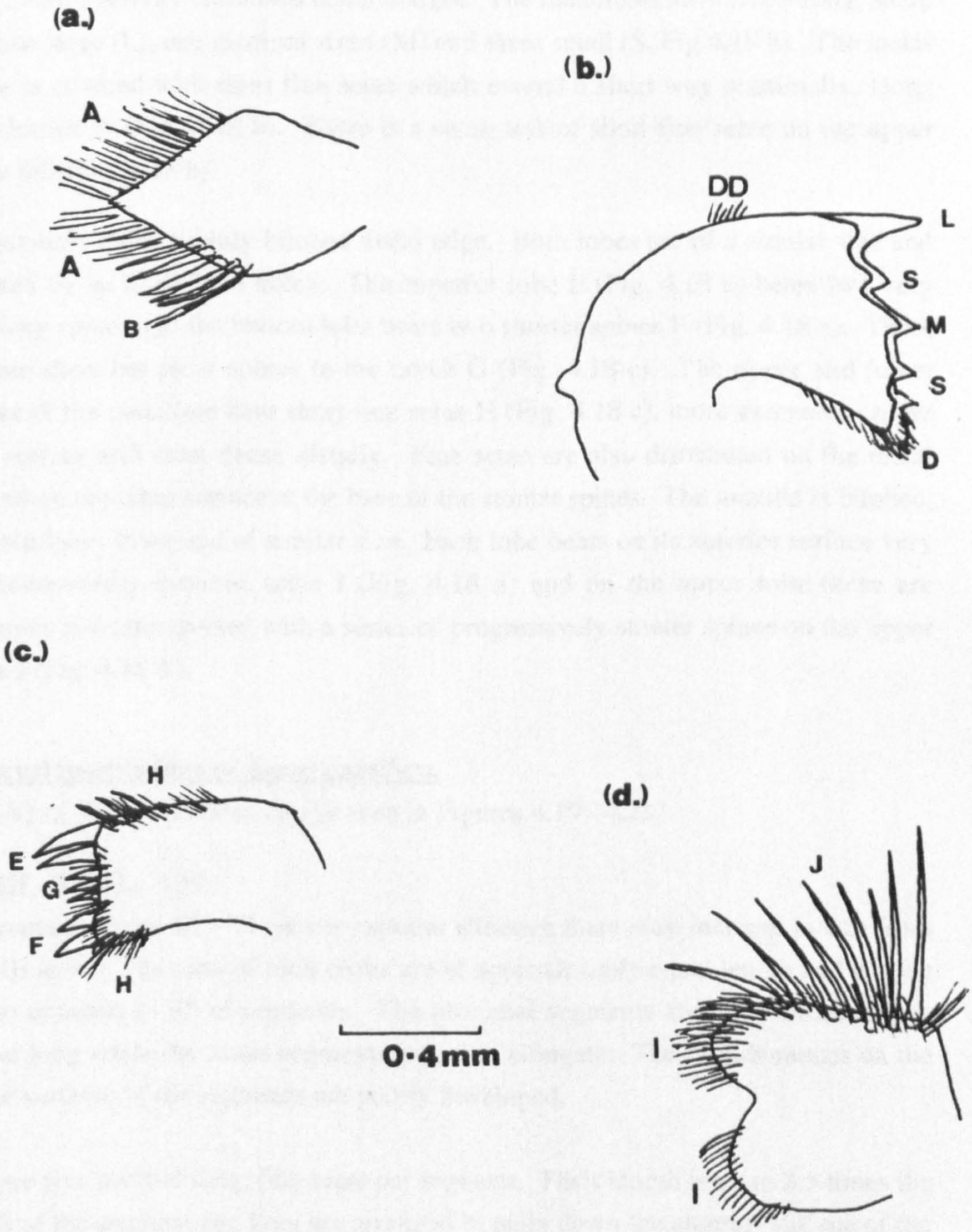


Figure 4.18. The structure of the mouthparts of *Capitulum mitella*. drawn to scale.

a) Inside view of the left mandibular palp

b) Inside view of right mandible

c) Inside view of right maxillule

d) Inside view of right maxilla.

yellow, with a heavily chitinised distal margin. The mandibles have five strong, sharp teeth, one large (L), one medium sized (M) and three small (S, Fig 4.18 b). The molar process is covered with short fine setae which extend a short way proximally, along the underside D (Fig. 4.18 b). There is a small tuft of short fine setae on the upper surface DD (Fig. 4.18 b).

The maxillule has a slightly bilobed distal edge. Both lobes are of a similar size and separated by an ill-defined notch. The superior lobe E (Fig. 4.18 c) bears two very stout, long spines and the bottom lobe bears two shorter spines F (Fig. 4.18 c). There are three short but stout spines in the notch G (Fig. 4.18 c). The upper and lower surfaces of the maxillule bear short fine setae H (Fig. 4.18 c), more extensive on the upper surface and most dense distally. Fine setae are also distributed on the distal edge and on the inner surface at the base of the stouter spines. The maxilla is bilobed, with both lobes blunt and of similar size. Each lobe bears on its anterior surface very fine, downwardly directed setae I (Fig. 4.18 d) and on the upper lobe these are continuous and interspersed with a series of progressively stouter spines on the upper surface J (Fig. 4.18 d).

The cirral morphology of *Lepas anatifera.*

Cirri I-VI of *Lepas anatifera* can be seen in Figures 4.19 - 4.21.

Cirri III - VI (Fig. 4.19.)

The structure of cirri III - VI are very similar although there is an increase in size from cirrus III to VI. The rami of each cirrus are of approximately equal length and consist of large numbers (~30) of segments. The proximal segments are much broader than they are long while the distal segments are more elongate. The protuberances on the anterior surfaces of the segments are poorly developed.

There are five pairs of long, fine setae per segment. Their length is up to 2.5 times the breadth of the segment and they are arranged in pairs down the anterior surface of the segments. The setae are graded in size, decreasing proximally. There is no obvious crown of setae on the centre of each protuberance as seen in *Capitulum mitella* and *Pollicipes pollicipes*. (a, Fig. 4.19). There are a few short setae on the posterio-distal margin of segments (b, Fig. 4.19) but none on the lateral margins.

Pedicel 1 is flattened, although roughly bilobed, and bears two patches of very fine setae. Pedicel 2 is small and has a more rounded protuberance. It bears a number of setae arranged in a manner similar to that on the ramal segments. The setae are more densely arranged than they are on pedicel 1 and there are no modified setae on any of the rami or the pedicels.

Figure 4.19 and 4.20. Cirrus IV and II of *Lepas anatifera* of unknown size (see text for explanation of labelling).

Fig. 4.19. Right cirrus IV in lateral view. The tips of the rami have been damaged.

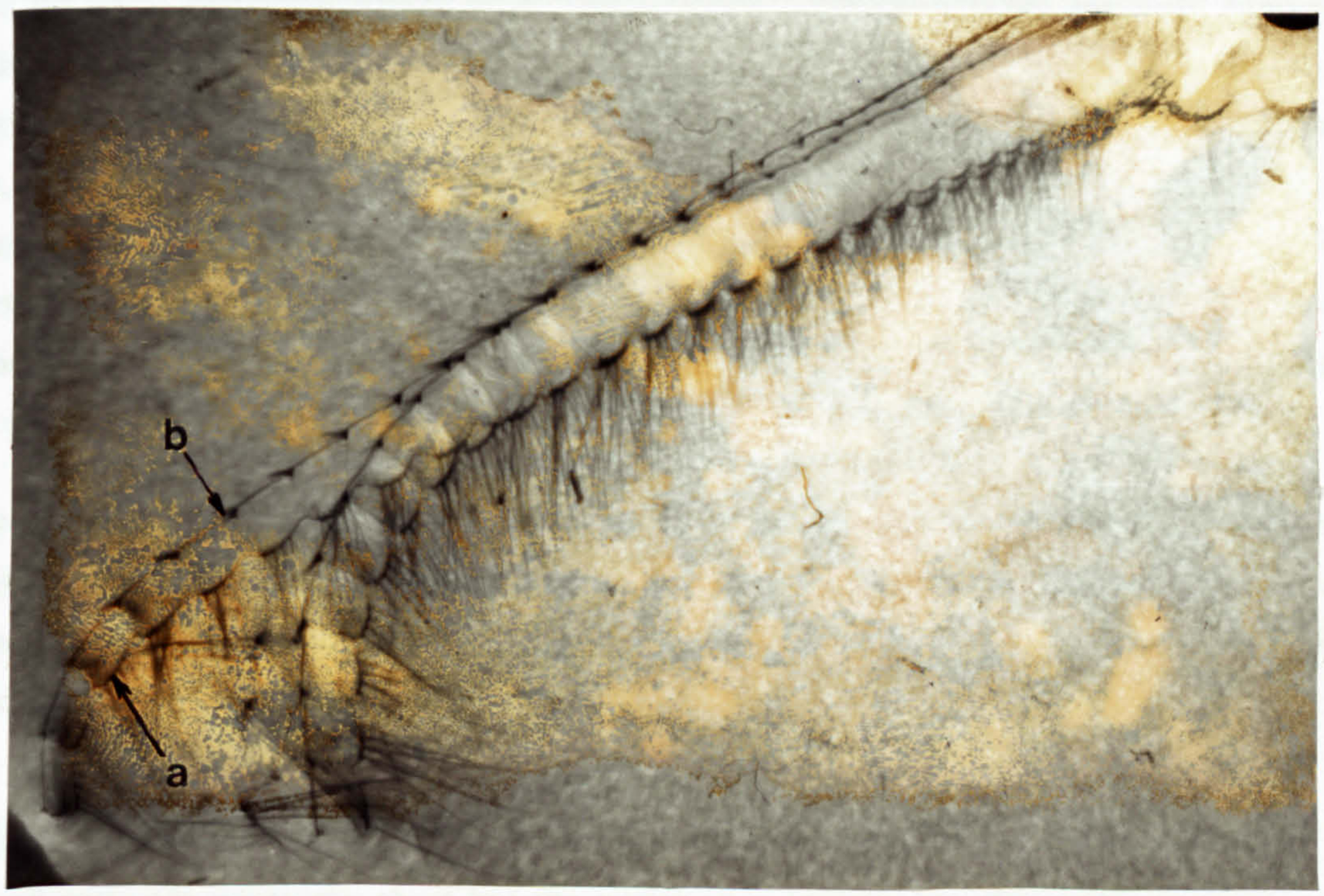


Fig. 4.20. Right cirrus II of *Lepas anatifera* in lateral view. The exopod is damaged and some setae have become dislodged.

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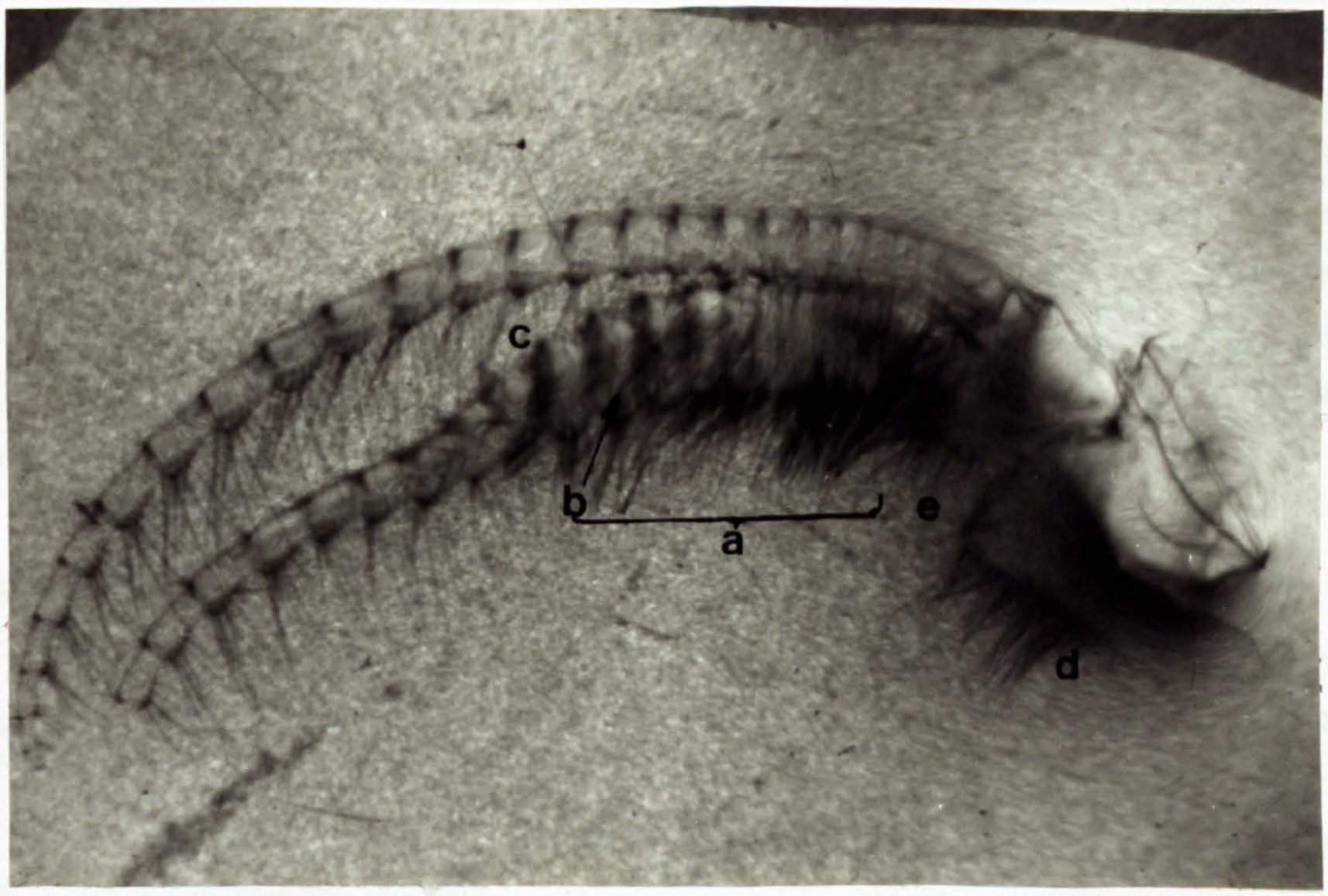


Cirrus II (Fig. 4.20.)

The exopod is longer than the endopod. Again, the proximal segments are broader than they are long and the segments become progressively longer than broad distally. The endopod is fairly narrow and not unlike the posterior cirri, with paired setae running down the anterior surface of the segments. There are 4-5 pairs of medium length setae and a few short setae, on the posterio-distal margin, on each segment. The distal segments of the exopod are similar in structure to those of the endopod. However, the first twelve exopodal segments are modified, being short and very broad (a, Fig. 4.20). The protuberance on the anterior surface is large and much more setose than that of the endopodal segments (b, Fig. 4.20). There are very dense tufts of fine setae on the surface of the protuberance and a well developed collar of fine, densely packed, short setae on the distal margin of segments (c, Fig. 4.20). The collar of setae is particularly well developed on the lateral surface of the ramus.

Pedicel 1 is larger than 2, has a flattened anterior surface and is somewhat lobed distally. It bears two patches of setae, which are fine and more densely packed proximally (d, Fig. 4.20). Pedicel 2 is very protuberant in side view, but shelf-like when viewed from the front.

Cirrus I of *L. anatifera* (Fig. 4.21.)

Both rami are relatively short but the endopod is longer than the exopod. Both rami have around 17 segments and show modification from the basic cirrus form. The first segment of both rami is elongate (a, Fig. 4.21), as are the distal 5-7 segments (b, Fig. 4.21), the rest are very short and stout. The exopod has twelve modified segments and the endopod seven. The exopodal segments are more setose than those of the endopod. The protuberance on the modified segments bears very dense tufts of fine, anterio-distally directed setae (c, Fig. 4.21). On the lateral margins of the segments of both rami, there are dense tufts of fine setae, also upwardly directed (d, Fig. 4.21). The less modified distal segments also bear the lateral setae but have fewer setae on the anterior surface. The lateral setae are longer and stouter on distal segments. There are far fewer setae on pedicel 1 than pedicel 2. All the setae are very fine. There are no modified setae on any segments of rami or pedicels.

Caudal Appendages of *L. anatifera* (Fig. 4.22.)

The caudal appendages of *Lepas anatifera* are short, unsegmented and without setae. They are twice as long as they are broad and taper slightly distally. They sit between cirri VI rather than behind them, and although not lying flat like those of *Pollicipes pollicipes*, they are not as prominent as those of *Capitulum mitella*. In profile they curve slightly towards the anterior.

Figure 4.21 and 4.22. Cirrus I and caudal appendage of *Lepas anatifera* (see text for explanation of labelling).

Fig. 4.21. Left cirrus I in lateral view. Large numbers of setae can be seen in the surrounding water. The setae of *Lepas* are so long and brittle that the application of the coverslip caused considerable damage.

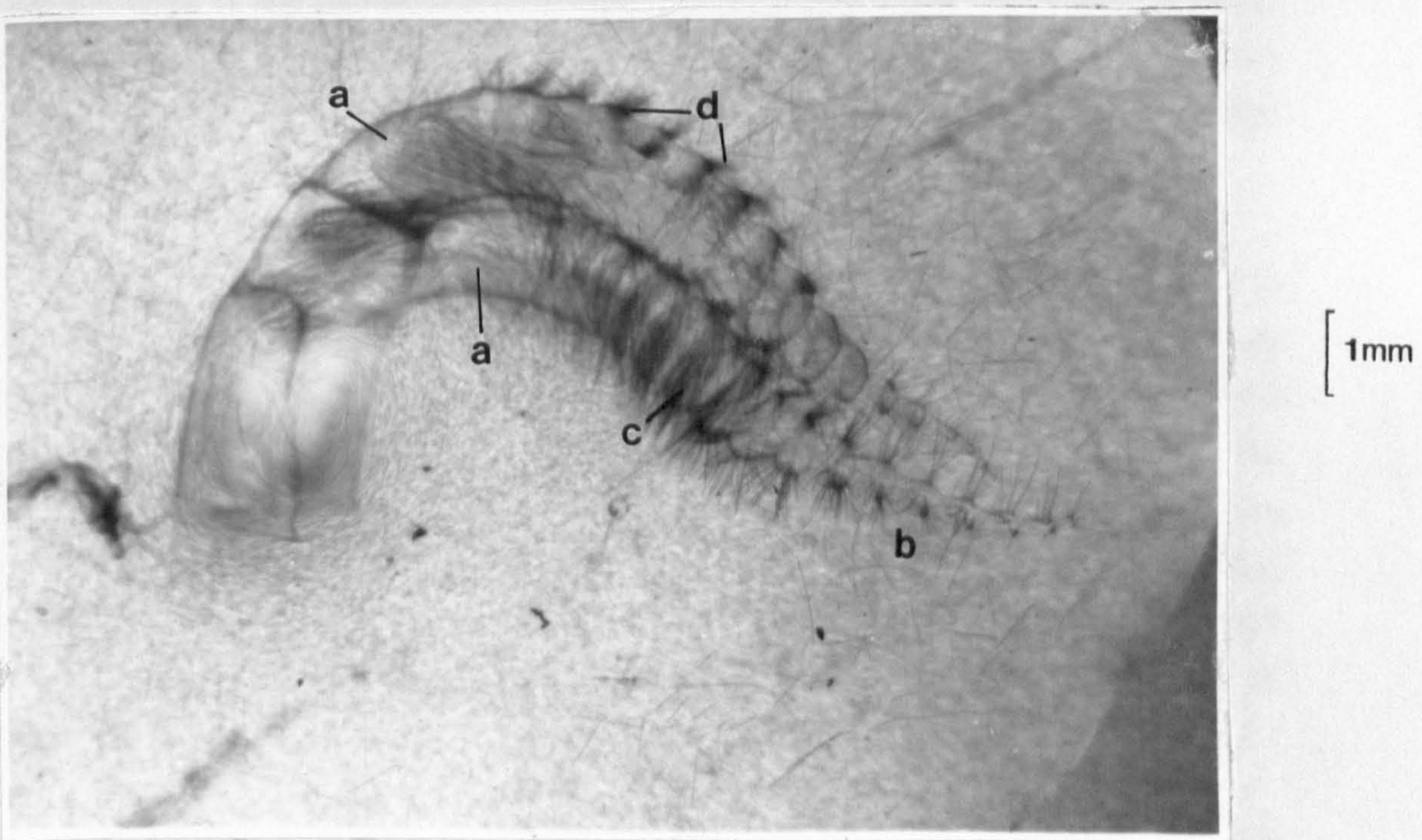
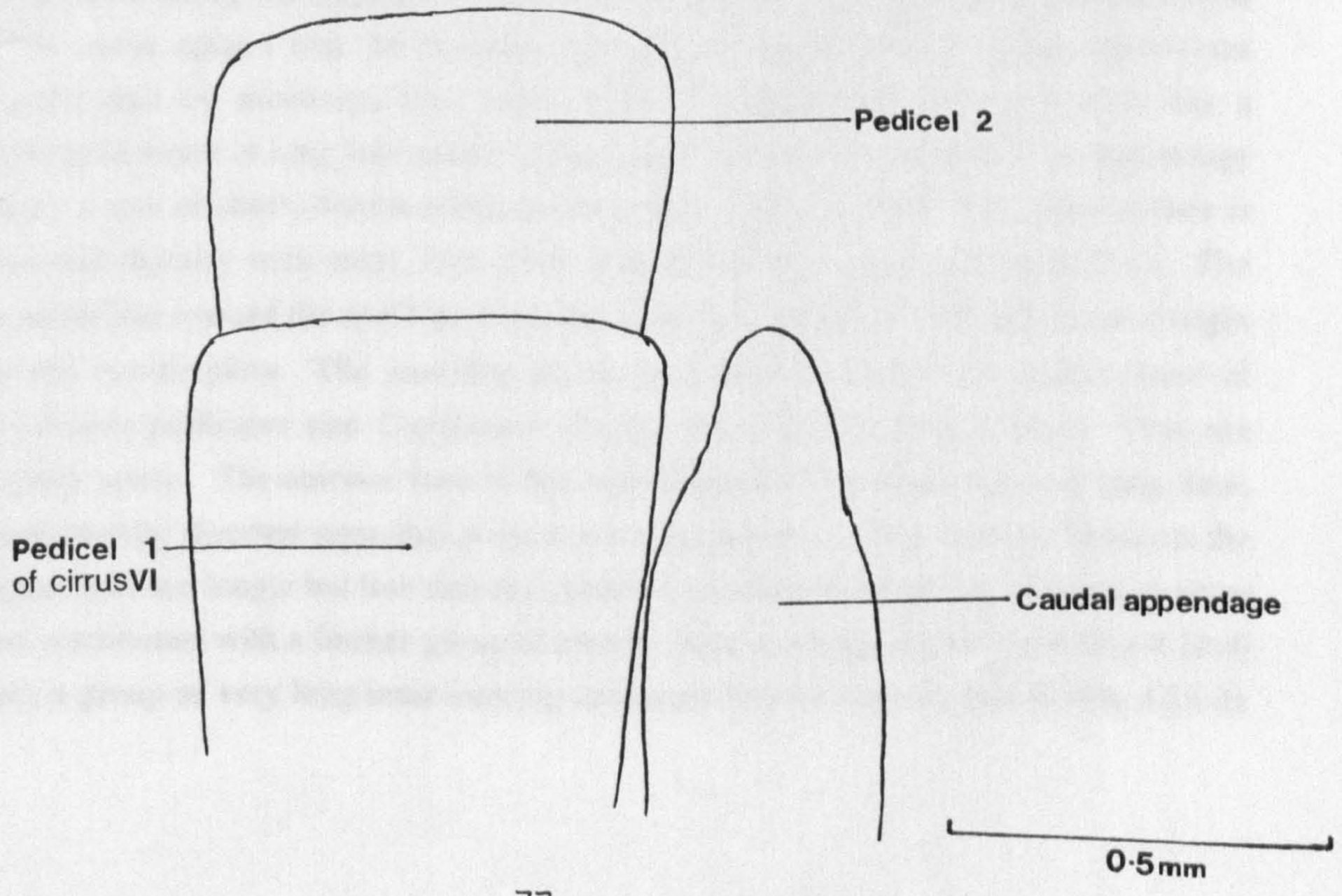


Fig. 4.22. Caudal appendage of *Lepas anatifera*.



The mouthparts of *Lepas anatifera.*

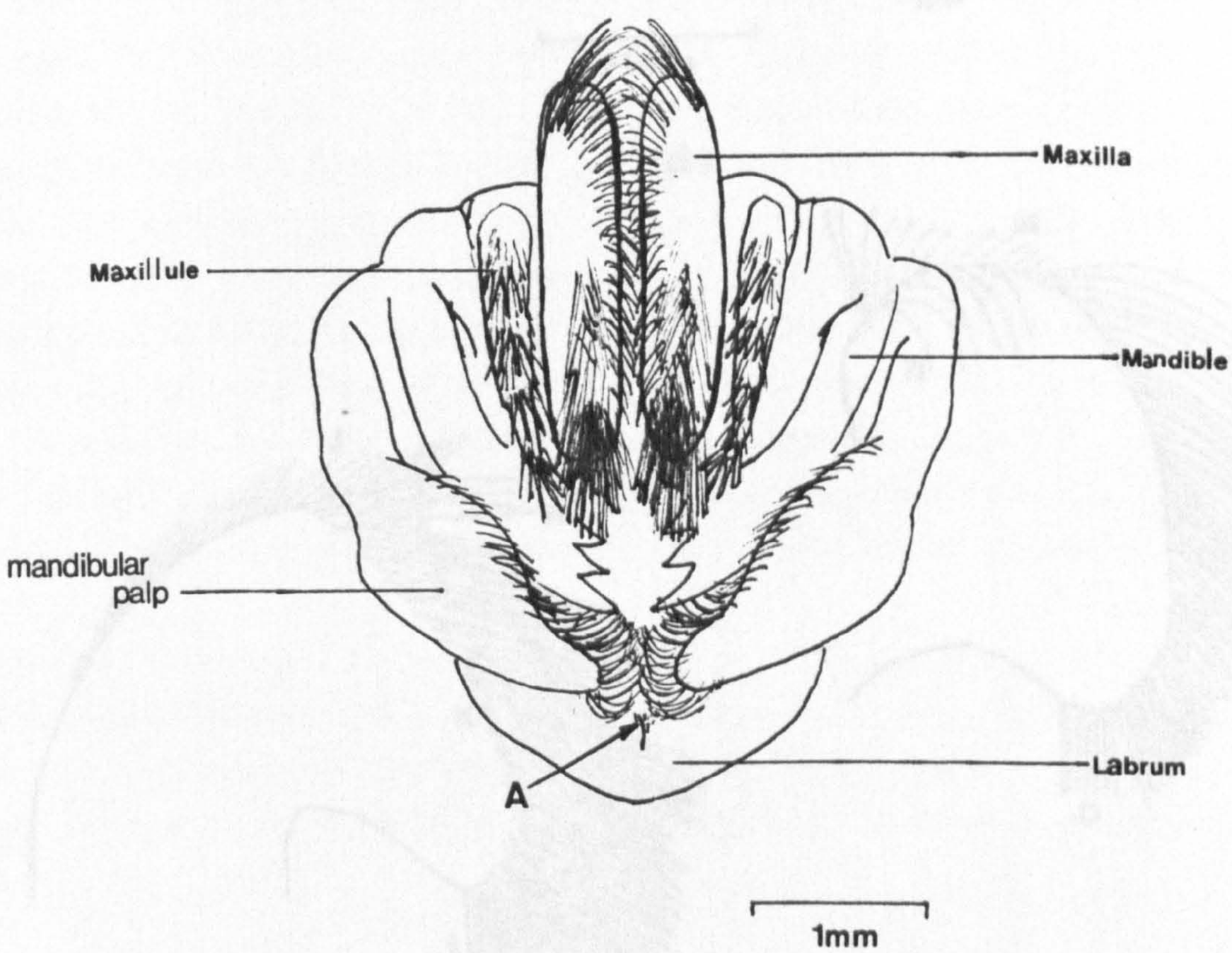
The arrangement of the mouthparts of *Lepas anatifera* is shown in Figure 4.23 and Figure. 4.24. a) to d) show the structure of the mouthparts.

The labrum is protuberant in side view and tapers ventrally. It bears short, fine setae on the notch A (Fig. 4.23 a) which runs down the mid-line. The mandibular palps lie across the front of the mouth and form the ventral border to the oral cone. They are paddle shaped and highly setate. Long fine setae along the distal margin A (Fig. 4.24 a) curve towards the mid-line and towards the mouth. Shorter setae on the upper surface B (Fig. 4.24 a) are, proximally, directed towards the mouth.

Behind each palp lies a mandible. The mandibles are broad, flat and sickle-shaped with a heavily chitinised distal edge. The mandibles have five strong, sharply pointed teeth with no secondary cusps C (Fig 4.24 b). Below these teeth there is a pointed molar process which bears a row of short, stout, downwardly directed setae D (Fig 4.24 b). There are two groups of finer short setae proximal to the molar process the first of which point toward the mid-line E (Fig 4.24 b) while the second group point proximally EE (Fig 4.24 b). The distal third of the inside surface of the appendage bears short, fine setae all directed toward the mid-line F (Fig 4.24 b). A dense row of short fine setae extends along the upper surface of the mandible G (Fig 4.24 b).

Above the mandible lies the maxillule which flanks the maxilla. It is flat and somewhat sickle-shaped (Fig 4.24 c), with has four angular lobes of approximately equal size on the distal edge. Each lobe bears a group of downwardly directed setae and a collection of stout spines. The top lobe bears the four stoutest spines, the uppermost being the longest H (Fig 4.24 c). The second and third lobes each bear eight stout spines and the bottom lobe has 13 stout spines. These spines are surrounded by numerous fine setae. The underside of the bottom lobe has a regimented row of long fine spines I (Fig 4.24 c) and the top margin of the appendage bears a row of short, downwardly directed setae J (Fig 4.24 c). The inner surface is covered distally with short, fine, downwardly directed setae K (Fig 4.24 c). The maxilla lies toward the mid-line from the maxillule and forms the uppermost margin to the mouth parts. The maxillae are roughly oval in shape and, unlike those of *Pollicipes pollicipes* and *Capitulum mitella*, are not lobed (Fig 4.24 d). They are highly setate. The anterior face of the appendage bears a dense mass of long, fine, downwardly directed setae that point toward the labrum L (Fig 4.24 d). Those on the upper half are longer but less densely packed than those lower down. The upper setae are continuous with a further group of shorter setae pointing anteriorly M (Fig 4.24 d) and a group of very long setae curving anteriorly toward the mid-line N (Fig 4.24 d).

Figure 4.23. The arrangement of the mouthparts of *Lepas anatifera*.



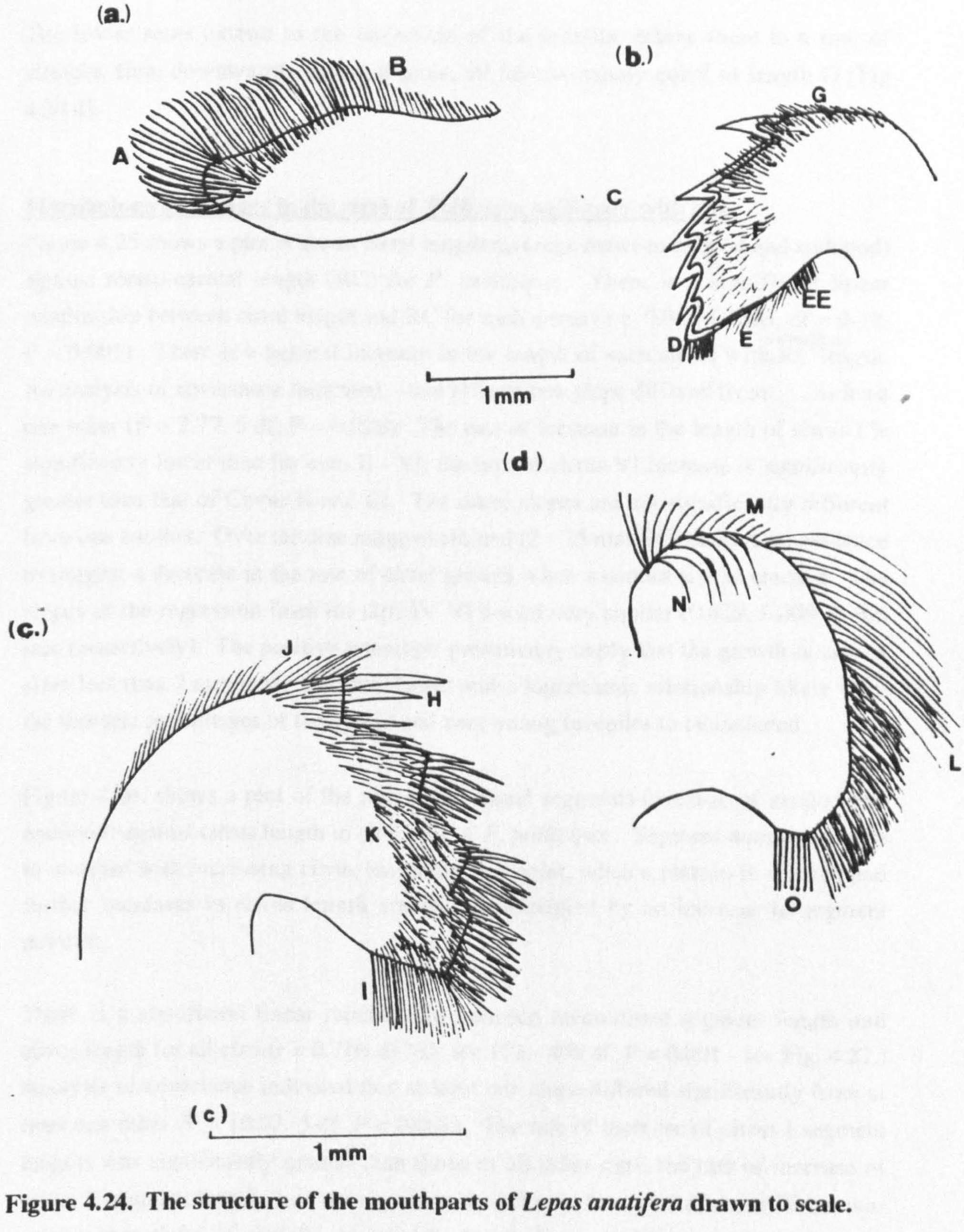


Figure 4.24. The structure of the mouthparts of *Lepas anatifera* drawn to scale.

a) Inside view of the left mandibular palp

b) Inside view of right mandible

c) Inside view of right maxillule

d) Inside view of right maxilla.

The lower setae extend to the underside of the maxilla, where there is a row of straight, fine, downwardly directed setae, all approximately equal in length O (Fig 4.24 d).

Morphological changes in the cirri of *Pollicipes pollicipes* with size.

Figure 4.25 shows a plot of mean cirral length (average between exopod and endopod) against rostro-carinal length (RC) for *P. pollicipes*. There is a significant linear relationship between cirral length and RC for each cirrus (r = 0.815 - 0.881, df = 9-10, $P < 0.001$). There is a general increase in the length of each cirrus with increasing RC length. An analysis of covariance indicated that at least one slope differed from at least one other ($F = 2.77$, 5 df, $P = 0.026$). The rate of increase in the length of cirrus I is significantly lower than for cirri II - VI, the rate of cirrus VI increase is significantly greater than that of Cirrus II and III. The other slopes are not significantly different from one another. Over the size range examined (2 - 15 mm RC) there is no evidence to suggest a decrease in the rate of cirral growth when a certain size is reached. The slopes of the regression lines for cirri IV-VI are all very similar (0.418, 0.409, 0.466 mm respectively). The positive intercepts presumably imply that the growth of cirri at sizes less than 2 mm is significantly faster and a logarithmic relationship likely were the thoracic appendages of the cyprid and very young juveniles to be included.

Figure 4.26. shows a plot of the number of ramal segments (average of exopod and endopod) against cirrus length in cirri I-VI of *P. pollicipes*. Segment number appears to increase with increasing cirrus length up to a point, when a plateau is reached and further increases in cirrus length are not accompanied by an increase in segment number.

There is a significant linear relationship between mean cirral segment length and cirrus length for all cirri (r = 0.716 -0.783, for 172 - 408 df, $P < 0.001$ - see Fig. 4.27.) Analysis of covariance indicated that at least one slope differed significantly from at least one other ($F = 10.03$, 5 df, $P < 0.001$). The rate of increase of cirrus I segment lengths was significantly greater than those of all other cirri, the rate of increase of cirrus II segment lengths was not significantly different from that of cirrus III but was greater than those of cirri IV, V and VI. Similarly cirrus III segments increase in length at a greater rate than those of cirrus IV, V, and VI. There were no significant differences between cirrus IV, V and VI.

A plot of mean intersetal distance, between the corresponding setae on adjacent segments (I, Fig. 4.3) against cirral length is given in figure 4.28. Intersetal distance increases with increasing cirral length (r values 0.168 - 0.699, df 55 - 70, $P < 0.001$)

Figure 4.25. Plot of cirral length (average of exopod and endopod ± SE) against rostro-carinal length RC for *P. pollicipes*. Lines fitted by least squares regression.

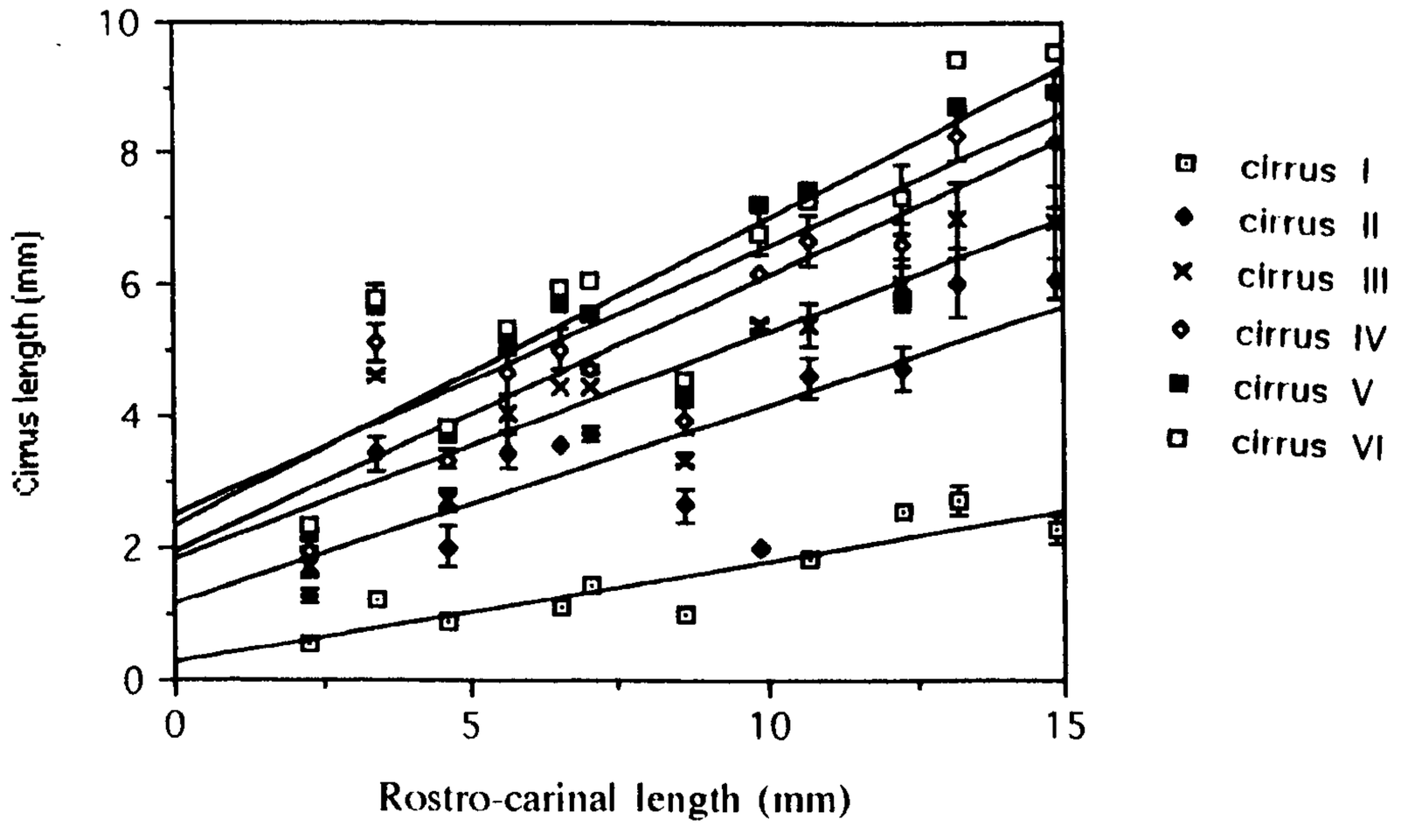


Table 4.1. Regression analysis and analysis of covariance of RC length vs. cirrus length in *P. pollicipes*.

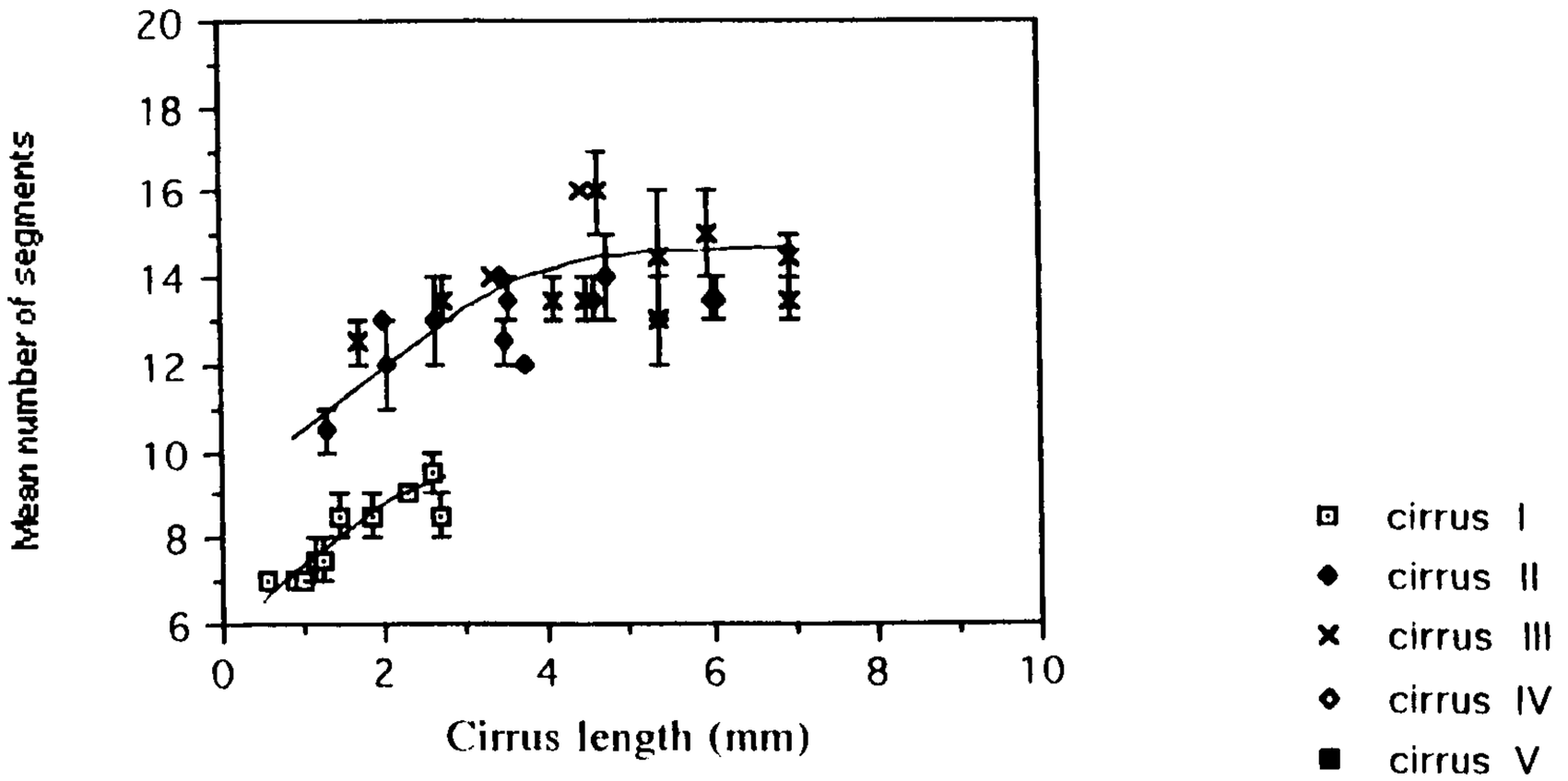
Cirrus	r	df.	P	Intercept	Slope	SE of slope
I	0.880	9	<0.001	0.281	0.1557	0.0604
II	0.878	9	<0.001	1.18	0.3202	0.0598
III	0.861	10	<0.001	1.81	0.3460	0.0594
IV	0.881	10	<0.001	1.93	0.4184	0.0594
V	0.815	10	<0.001	2.48	0.4092	0.0594
VI	0.876	10	<0.001	2.34	0.4656	0.0594

Analysis of covariance

Source	DF	Seq.SS	Adj SS	Adj MS	F	P
RC	1	128.396	131.914	131.914	174.82	<0.001
Cirrus number	5	167.653	6.879	1.379	1.82	0.122
Interaction	5	10.459	10.459	2.092	2.77	0.026
Error	58	43.766	43.766	0.755		
Total	69	350.274				

Figure 4.26. Plot of number of segments per cirrus (average for exopod and endopod ± SE) against cirrus length for *P. pollicipes*, a) cirri I - III and b) cirri IV - VI. Lines fitted by eye.

a)



b)

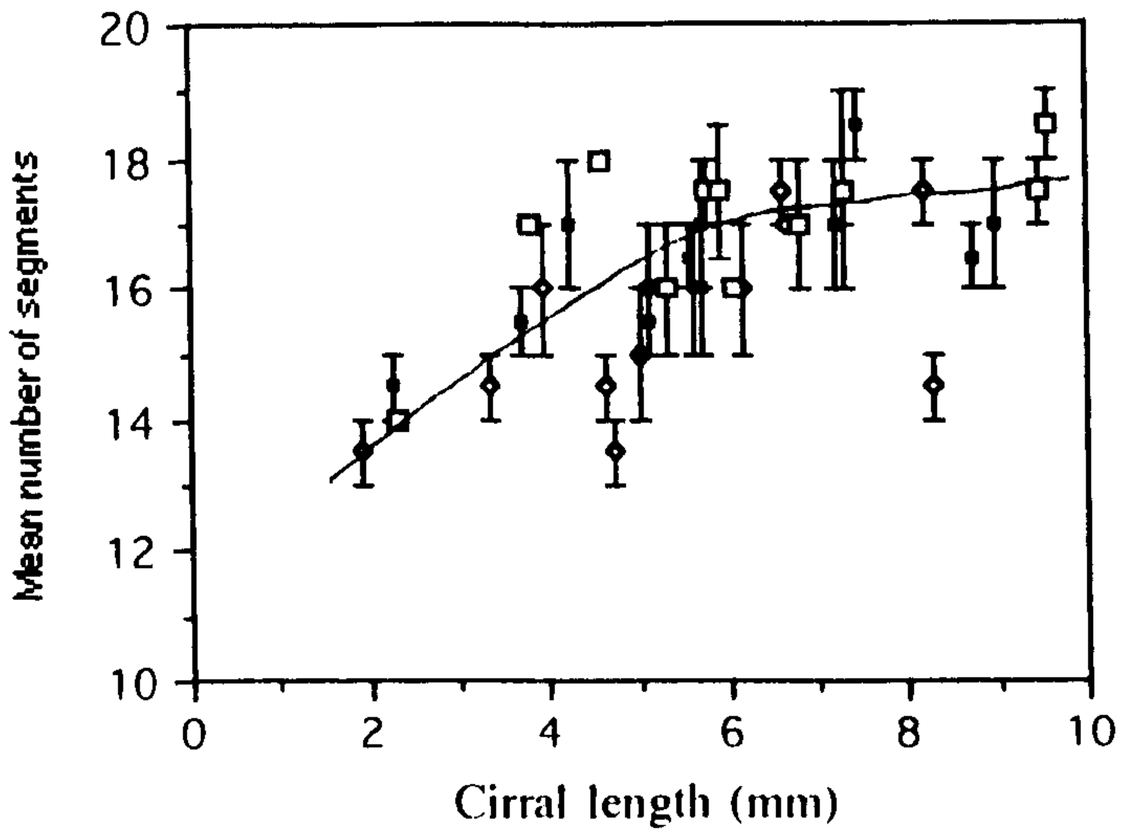


Table 4.2. Pearson correlation coefficients for the relationship between number of segments and cirrus length for *P. pollicipes*.

Cirrus	r	df.	P
I	0.889	9	<0.001
II	0.664	10	0.02
III	0.237	10	0.50
IV	0.548	10	0.10
V	0.587	10	<0.05
VI	0.625	10	<0.05

Figure 4.27. The mean length of cirral segments in cirri I-VI of different sizes in *Pollicipes pollicipes*. The standard error of the means are indicated. Lines fitted by least squares regression.

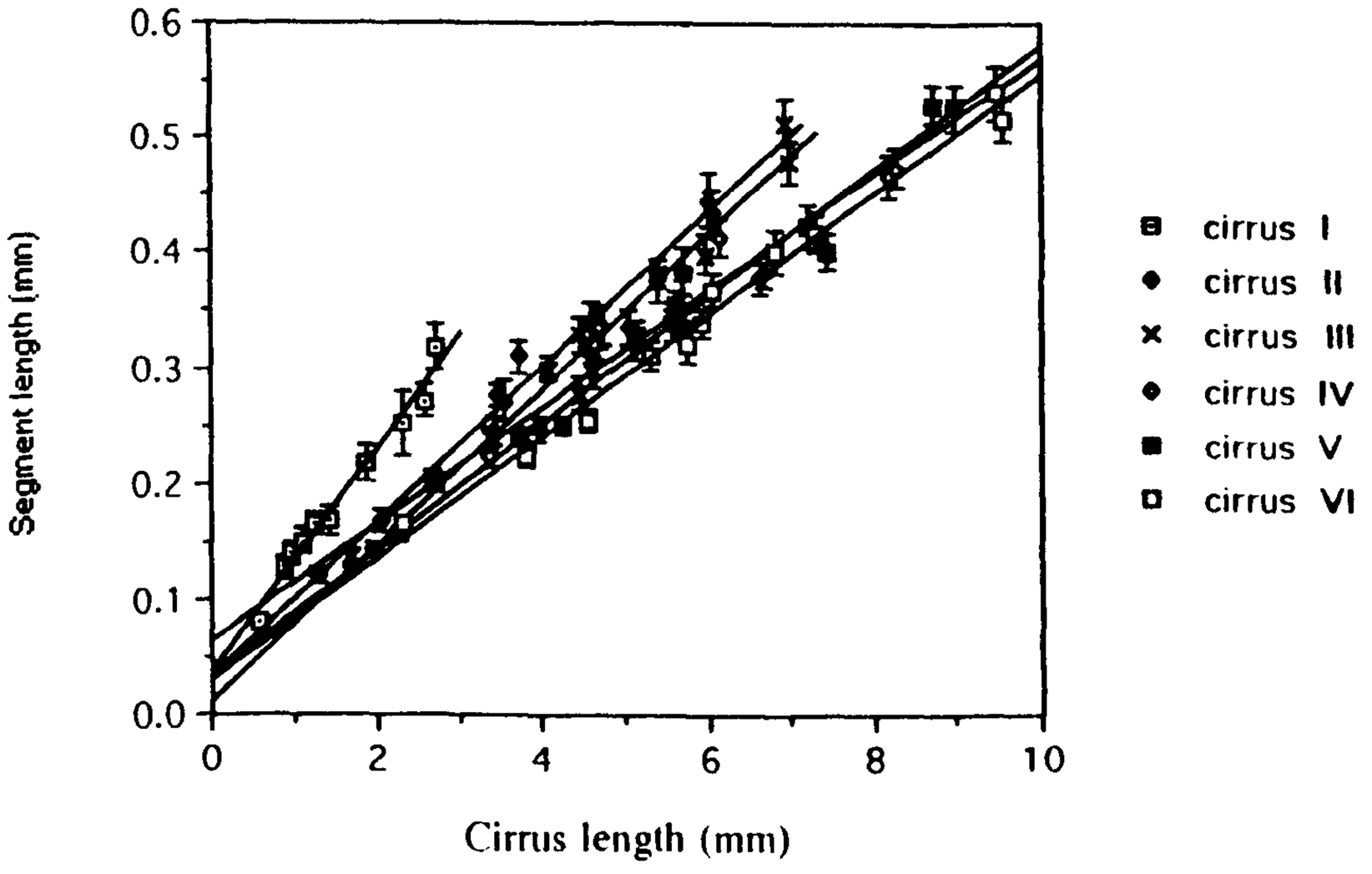


Table 4.3. Regression analysis and analysis of covariance of cirral segment length vs. cirrus length in *P. pollicipes*. Analysis performed on all data.

Cirrus	r	df.	P	Intercept	Slope	SE of slope
I	0.716	172	<0.001	0.0371	0.0975	0.0078
II	0.783	269	<0.001	0.0341	0.0673	0.0034
III	0.734	310	<0.001	0.0247	0.0654	0.0033
IV	0.756	346	<0.001	0.0678	0.0494	0.0027
V	0.760	391	<0.001	0.0337	0.0549	0.0026
VI	0.760	408	<0.001	0.0344	0.0522	0.0025

Analysis of variance

Source	DF	Seq. SS	Adj SS.	Adj.MS	F	P
Cirrus length	1	20.2300	8.3829	8.3829	1210.56	<0.001
Cirrus number	5	1.2040	0.0379	0.0076	1.09	0.362
Interaction	5	0.3472	0.3472	0.0694	10.03	<0.001
Error	1896	13.1294	13.1294	0.0069		
Total	1907	34.9106				

Figure 4.28. The intersetal distance between corresponding setae on adjacent segments (I) against cirrus length for *Pollicipes pollicipes*.

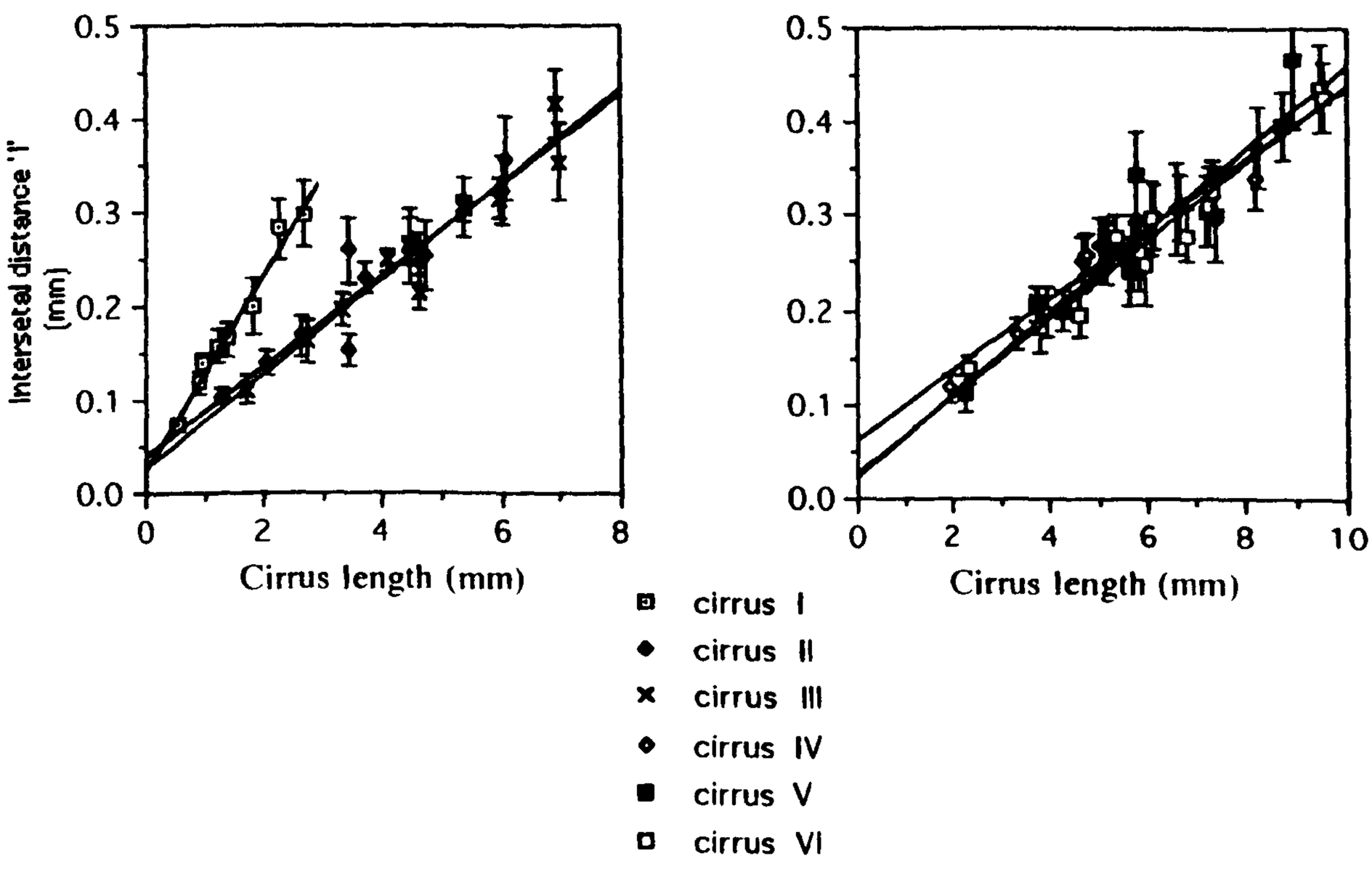


Table 4.4. Regression analysis and analysis of covariance of intersetal distance (I) vs cirrus length in *P. pollicipes*. Analysis performed on all data.

Cirrus	r	df.	P	Intercept	Slope	SE of slope
I	0.570	58	<0.001	0.0360	0.1055	0.0163
II	0.592	55	<0.001	0.0665	0.0470	0.0079
III	0.645	57	<0.001	0.0463	0.0498	0.0073
IV	0.657	66	<0.001	0.0666	0.0370	0.0070
V	0.699	70	<0.001	0.0236	0.0439	0.0064
VI	0.468	70	<0.001	0.0880	0.0340	0.0062

Analysis of variance

Source	Df	Seq.SS	Adj.SS	Adj.MS	F	P
Cirrus length	1	2.19856	1.48831	1.48831	160.00	<0.001
Cirrus number	5	0.24959	0.01991	0.00398	0.43	0.829
Interaction	5	0.12407	0.12407	0.02481	2.67	0.022
Error	386	3.59061	3.59061	0.00930		
Total	397	6.16284				

and the plot strongly resembles that of segment length (Fig. 4.27), although the variability is greater. It appears that any increase in the intersetal distance between segments (I) with increasing cirral length is a function of increasing segment length. Again, an analysis of covariance indicated that at least one slope differed from at least one other ($F = 2.67$, 5 df, $P = 0.022$). Like segment length, the rate of increase in intersetal distance, I, of cirrus I was significantly higher than those of CII - VI while that of cirrus III was higher than that of cirrus VI. All other rates of increase were not significantly different.

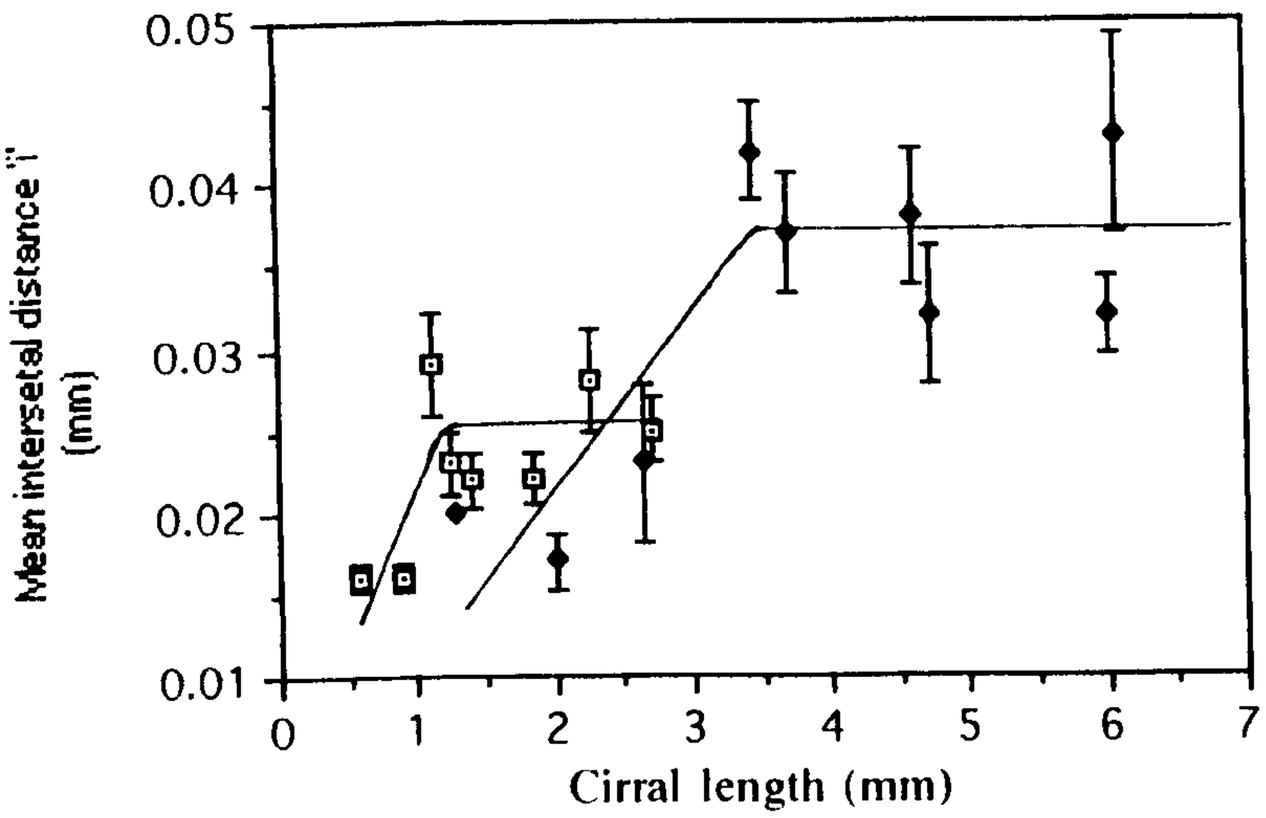
Figure 4.29 shows the relationship between cirral length and the mean intersetal distance between adjacent setae within a segment (i, Fig. 4.3). Although there is quite high individual variability, i increases with increasing cirral length but only to a point, after which, the size of the intersetal distance remains fairly constant, however long the cirrus becomes. The maximal i for each cirrus are as follows:

Cirrus	i (mm)	Cirrus length (mm)	RC (mm, from Fig 4.25)
I	0.025	1.0	5.0
II	0.036	3.2	6.5
III	0.042	4.5	8.0
IV	0.042	4.5	5.0
V	0.046	5.0	7.3
VI	0.046	5.0	5.5

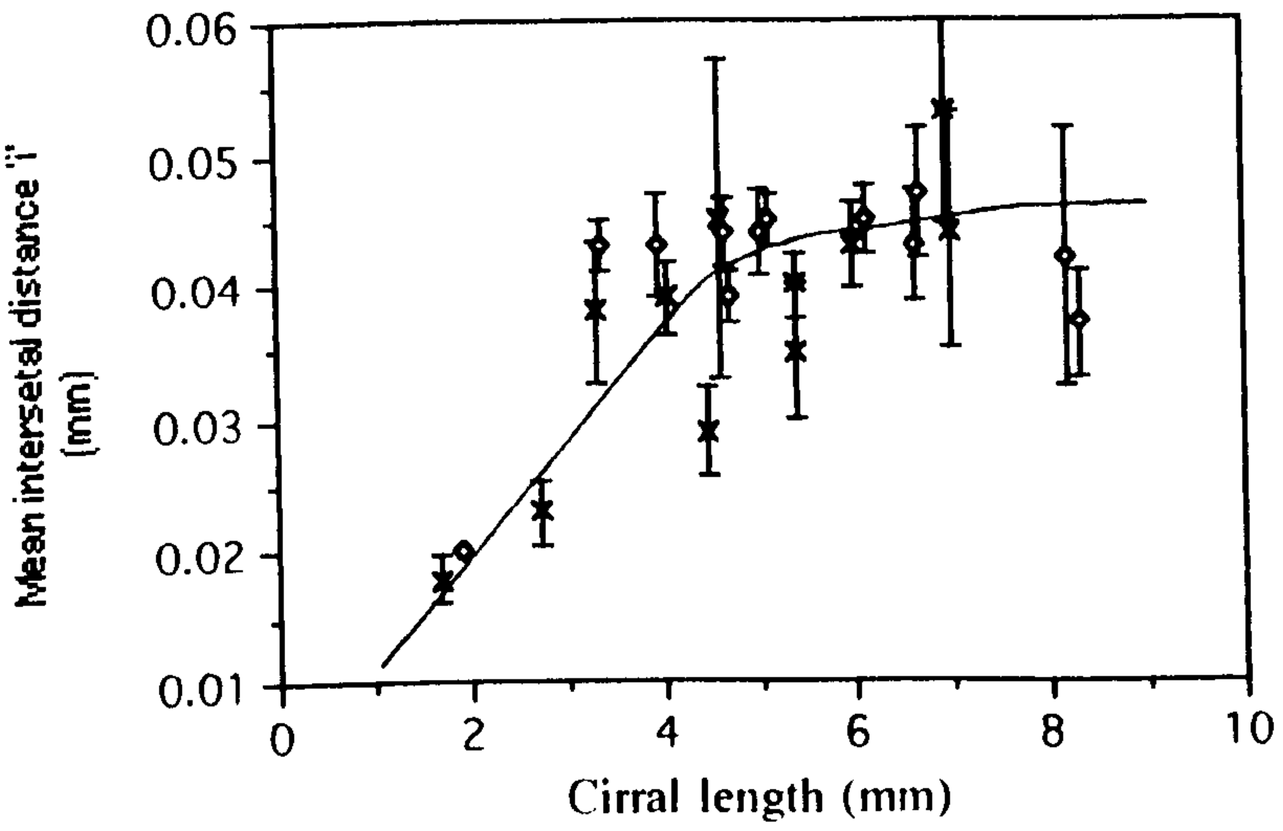
The changes in setal length with increasing cirral length are shown in Figure 4.30 a and b. All cirri show a significant positive correlation between setal length and cirrus length but in all cases, the association appears curvilinear. The rate of increase in setal length is slower in larger barnacles. The smallest increase in setal length with increasing cirrus length is shown by cirrus I (Fig. 4.30 a.) and the largest by cirri II and III (Fig. 4.30 a) with cirri IV- VI showing intermediate values (see Fig. 4.30 b).

Figure 4.29. Mean distance between setae on the same segment 'i' (Fig 4.3), against cirral length for *Pollicipes pollicipes* a) cirri I & II, b) cirrus III & IV and c) cirrus V - VI. Standard errors for each mean are given. Lines fitted by eye.

a)



b)



c)

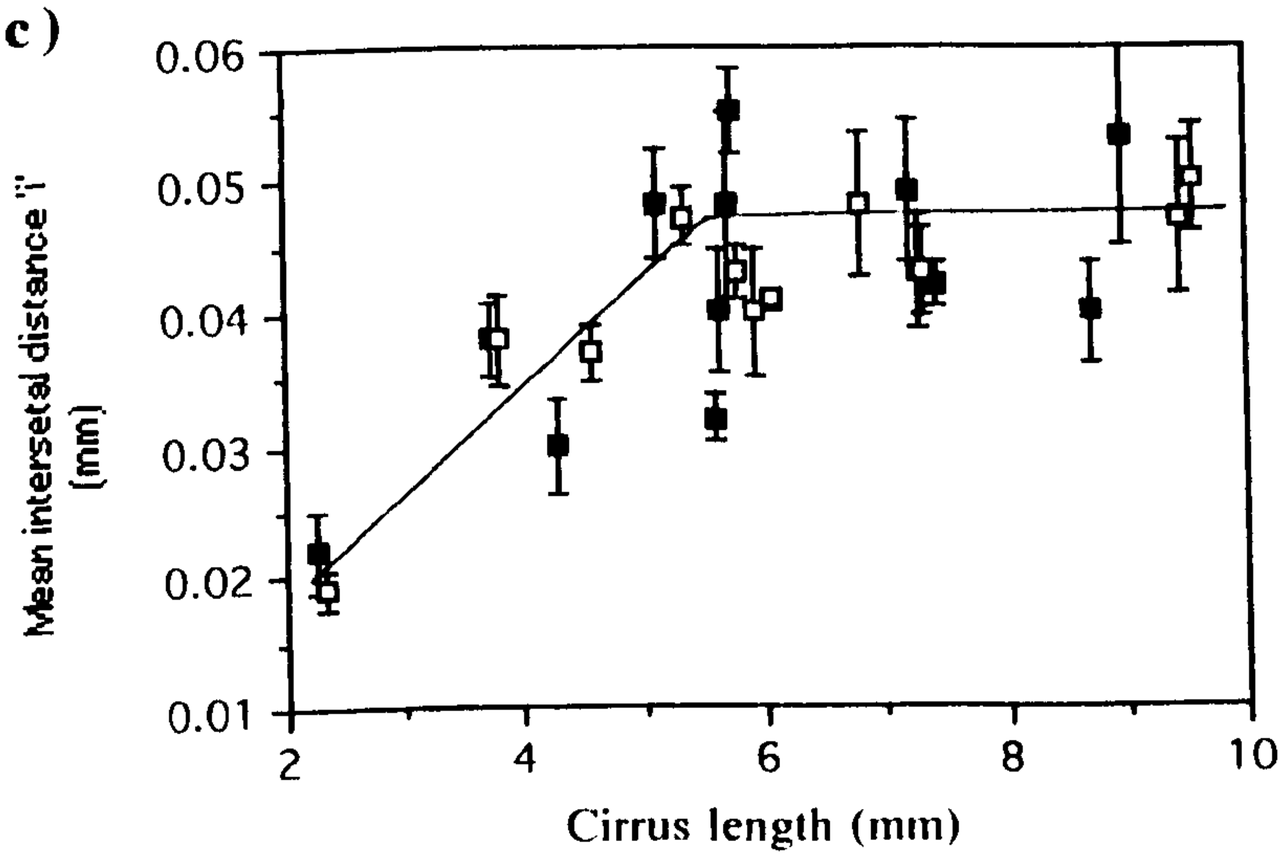


Table 4.5. Correlation coefficients for intersetal distance (i) vs cirrus length.

Cirrus	r	df.	P
I	0.197	51	0.200
II	0.51	52	<0.001
III	0.518	61	<0.001
IV	0.185	65	0.200
V	0.417	70	<0.001
VI	0.553	70	<0.001

Figure 4.30. Mean (±SE) length of setae on a) cirrus I-III and b) cirrus IV-VI of *Pollicipes pollicipes* cirri of different lengths

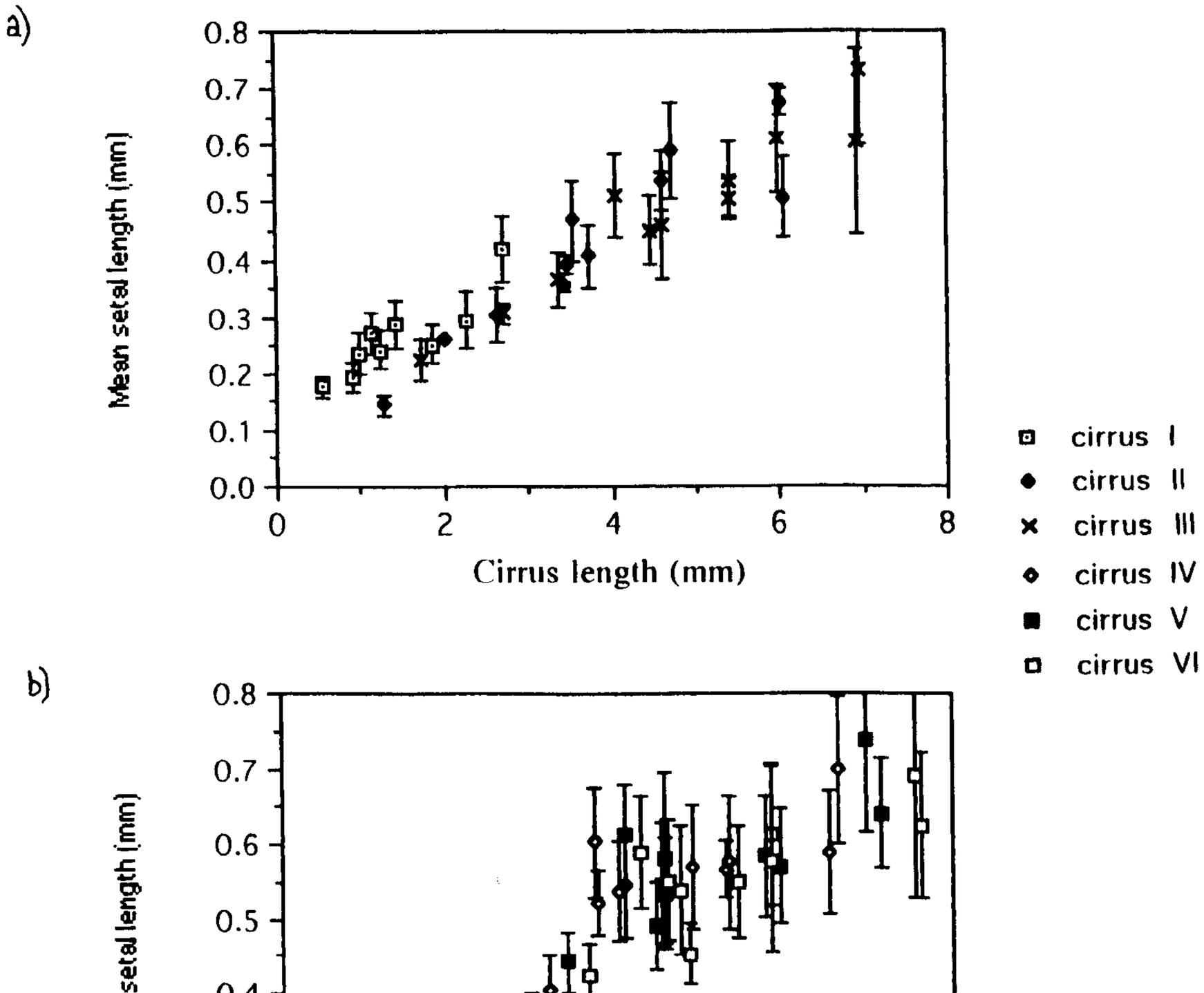


Table 4.6. Pearson correlation coefficients for mean setal length vs. cirrus length.

Cirrus	r	df.	P
I	0.540	58	<0.001
II	0.737	53	<0.001
III	0.621	62	<0.001
IV	0.431	65	<0.001
V	0.491	70	<0.001
VI	0.467	70	<0.001

Discussion

The cirri and mouthparts of all described pedunculate and acorn barnacles have marked similarities and the conservative nature of the morphology suggests a consistency of function. Those of the members of the genus *Pollicipes* are very similar to one another (Darwin, 1851) and it has been noted that *P. pollicipes* and *P. polymerus* have mouthparts that are almost identical (Høeg *et al.*, 1994). However, there are distinct and consistent variations in limb morphology that characterise each species (Barnes & Reese, 1959). The differences may, in some species of barnacle, be correlated with their diet and ecology.

The segments of the captorial cirri (IV - VI, and III in some species) bear a series of paired setae running down the anterio-lateral surface, graded in size with the largest distally. Closely related species generally have similar numbers of setae. Omnivorous barnacles which exploit a wide variety of prey e.g. *Verruca stroemia*, 4 pairs/segment (Anderson, 1981) and *Tetraclitella purpurascens* 3 pairs (Anderson & Anderson, 1985) or *Balanus balanoides*, 6-10 (Stubbings, 1975) generally have greater numbers of setae per segment, therefore a finer cirral mesh, than those species more reliant on large prey of animal origin e.g. *V. recta*, 3 pairs/segment (Anderson, 1981), *Tesseropora rosea*, 2 pairs (Anderson & Anderson, 1985) and *Boscia anglicum*, 2 pairs (Anderson, 1978). On the segments of the captorial cirri *P. pollicipes*, *C. mitella* and *Lepas anatifera* have 4, 4, and 5 pairs of setae respectively (although these three species and *P. polymerus* were found to have 6 pairs by Darwin, 1851).

The greater the degree of carnivory the larger the effective setal mesh size. *P. pollicipes* had fewer short setae on the crown of the protuberance than *C. mitella* or *P. polymerus*, but the degree of setal overlap is much greater in *L. anatifera*, *P. polymerus* (Barnes & Reese, 1959) and *C. mitella*. The effective mesh size of *P. polymerus* is smaller than that of *P. pollicipes* indicating that smaller prey may be captured, although there is perhaps evidence that all the species looked at are equipped for an omnivorous existence.

The maxillipedes (cirri I and II in all species and III in some) are generally responsible for microfiltration of small particles and for the transfer of all captured food to the mouth (Southward, 1955a). Omnivorous barnacles have more setate maxillipedes with longer and more numerous setae than those of more carnivorous animals. The mouthparts of omnivorous barnacles are endowed with teeth, sharp cutting blades and heavily chitinised mandibular teeth that can macerate large prey, but also have numerous fine setae that retain and transport micro-particulate material. Carnivorous

barnacles have far fewer, shorter setae and more developed teeth (see Anderson, 1978; Anderson & Anderson, 1985).

The maxillipedes of *Lepas* are much more highly setate than *P. pollicipes* and *P. polymerus* and the mouthparts, although more toothed (particularly the mandibles) than those of *P. pollicipes*, *P. polymerus* and *C. mitella* (see also Høeg *et al.*, 1994) are covered with more numerous, fine spines and setae, suggesting that *L. anatifera* can efficiently capture, macerate and transfer to the mouth, both large animal prey and microalgae.

P. pollicipes, *C. mitella* and *L. anatifera* appear to have the limbs and mouthparts of omnivores, but *Lepas* is likely to be able to capture and ingest a wider variety of prey of more disparate sizes and with greater efficiency. The cirral net of *L. anatifera* is also proportionally larger than that of *P. pollicipes*. and the cirri are very long and fine. *Lepas* are not subjected to the high water flows experienced by the scalpellids which have much shorter and sturdier cirri, perhaps shorter to reduce drag and thicker to give them the strength they require to withstand the potential wave battering they receive.

The structure of the cirral net is likely to represent a trade-off: the smaller the effective mesh size of the cirral net, the smaller the minimum prey size captured and therefore the greater the amount of material that can and will be removed from the water column. However, the mesh size has to be sufficiently coarse to allow water penetration. A very fine mesh in fast water flow will not allow water to filter through it but will push the water aside. Barnacle cypris larvae are able to detect and respond to flow (Moore, 1933; Crisp, 1955; Eckman *et al.*, 1990) but it is unclear whether cyprids only settle in regions where water flow will be sufficiently low to penetrate the cirral nets of the juvenile and adult barnacle, or whether settlement often occurs in areas of unsuitable high flow that effectively starves the metamorphosed barnacles to death. Either way the cirral mesh size should correlate with the water conditions prevailing where the species are found.

Pollicipes pollicipes and *Capitulum mitella* both live on exposed, rocky coasts in regions of very high water movement, whereas *Lepas anatifera* is generally found in the open ocean, attached to drifting flotsam. *L. anatifera* usually drift *with* the direction of the water flow, unless, for example, the wind was driving their 'float' against the tide. *L. anatifera* will generally be subject to low speeds of water movement through the cirral filter. It has a much longer and more mobile peduncle than *P. pollicipes* or *C. mitella* and can feed more actively. The lower water flow

permits *L. anatifera* to have long and quite closely spaced setae which will allow the capture of a wide variety of food items (including small particles). Efficient food capture is essential for life in a relatively unproductive environment; there is an average of ~100 mg C m^{-2} d^{-1} primary production and 15 mg C m^{-2} d^{-1} secondary production in the open ocean compared to 1000 and 50 mg C m^{-2} d^{-1} respectively in coastal areas (Parsons, 1980). *Lepas* survives the 4 - 10 fold diminution in productivity by virtue of a large cirral net and a setal arrangement capable of capturing particulate matter over a wide size range from <10 μm phytoplankton to >2 cm zooplankton.

The scalpellids all have a smaller cirral net with larger effective mesh size, (shorter cirri, shorter setae and reduced degree of setal overlap, both between adjacent rami and between segments) than *Lepas*. The high water movement to which these species are subjected permits passive filtering of the overlying water. Coastal waters are also environments of plentiful and diverse food (Parsons, 1980; Woodwell, 1980) so *Pollicipes* and *C. mitella* can have a coarser cirral mesh (than *L. anatifera*) which allows fast flowing water to penetrate at the expense of capturing smaller particles which tend to be phytoplankton.

Pollicipes pollicipes lives mid to low on the shore in Europe and *Capitulum mitella* mid to high in the intertidal zone in Asia (Nakamura & Tanaka, 1989). However, living lower on the shore than *C. mitella*, *P. pollicipes* has longer to feed so it is not critical that the degree of setal overlap is less and the setae are more widely spaced. The need for a larger mesh size (presumably dictated by the prevailing hydrodynamic conditions) must outweigh the necessity to feed maximally during periods of immersion. Animals exposed by the tide for long periods may well be better adapted for a more carnivorous diet. Although weight for weight algal and zooplankton food have a similar energy content (e.g. *Rhinomonas* 23.7 and *Artemia* 23 J mg^{-1}) it takes less time to ingest sufficient energy for survival when feeding on larger, animal prey. Whether the lack of fine spines on cirrus III of *Capitulum mitella* is merely a generic difference or whether it is indicative of a more carnivorous diet is not clear. *Capitulum mitella* has no fine setation on cirrus III but has a greater number of mandibular teeth, shorter setae on the mandibular palps, a less setate outer maxilla and more toothed maxillule, than *P. pollicipes*, perhaps favouring a more carnivorous diet.

The caudal appendages in adult barnacles originate from the caudal appendages of cypris larvae. In the cyprid, their function is thought to be sensory, perhaps aiding substratum selection prior to settlement (Crisp & Barnes, 1954). The structure of the adult caudal appendages can vary enormously within a genus. *Verruca recta* has a

pair of short caudal appendages with five segments and a basal region (Anderson, 1981). The caudal appendages of *V. stroemia* are, in contrast, very long and antenniform (Stone & Barnes, 1973) with 25 segments. Anderson (1981) suggested that they are used as a regulator of cirral extension, detecting by contact with the substratum, the stage at which full extension is obtained.

The caudal appendages of the pedunculate barnacles examined appeared similar to the descriptions given by Darwin (1851), any differences in length and segment number are probably attributable to the age and size of the individuals examined. All three species had relatively small caudal appendages: *Lepas* had the shortest and the only unsegmented appendages whilst *Pollicipes* had the longest with the most segments. The caudal appendages of the pedunculate cirripedes are further from the substratum than they are in the acorn barnacles and therefore could not function as regulators of extension, especially in *Lepas*, whose peduncle is particularly long. The function of the caudal appendages in lepadomorph barnacles is unclear but may be sensory.

Changes in cirral morphology occurred with increasing 'size' in *P. pollicipes* . Although the sample size was very small and variability high, some significant trends were seen. Cirral length increases with rostro-carinal length (RC); the captorial cirri increase in length at a greater rate over a given range of RC than the maxillipedes (cirri I - III). Cirrus I increases in length most slowly. In small animals the increase manifests itself in increasing numbers of segments as well as an increase in the length of segments. There is some evidence to suggest that adult *P. pollicipes* (those >9 mm RC) increase cirral length primarily by increasing segment length with little, or no, further rise in segment number. A similar pattern was shown in *Balanus balanoides* by Stubbings (1975).

As the overall dimensions of the cirral net increase, so the intersetal distances (I) increase. It could be expected, therefore that the minimum size of particle retained by the filter would also increase with size and the efficiency of capturing small phytoplankton and small zooplankton would decrease. It appears that any increase in 'I' with increasing cirral length is a function of increasing segment length.

The distance between adjacent setae within cirral segments (i, Fig. 4.3) did not show a steady increase with increasing animal size but reached a maximum value. These setae are located on the segment protuberances. If segment length increases primarily by elongation of the region under the protuberances, the rate at which 'i' will increase will be slower than 'I' and may stop altogether. The situation is however, more complex as the effectiveness of the cirral net must rely on the way in which the setae

of adjacent cirri interlock and overlap. No simple measurements can adequately quantify the situation. The barnacle size at which the plateau in 'i' was reached varied from cirrus I - VI (3.5 - 8.0 mm RC) but did not coincide with the onset of sexual maturity. The maximum 'i' values for cirri I, II and III (those cirri used for algal capture) were reached at 5, 6.5 and 8.0 mm RC respectively. Hence only very small juvenile *P. pollicipes* are better equipped than adults for microparticulate feeding although the smaller cirral net area, will allow less water to be filtered. The changes in cirral structure with increasing size add little support to the theory of a juvenile to adult switch in feeding behaviour accompanying the proposed switch in diet from micro- to macroparticulate feeding reported for *P. polymerus* (Lewis, 1981) and for *P. pollicipes* (Hui, 1983). Even small juvenile *P. pollicipes* do not have maxillipedes that are well designed for algal feeding.

Ideally, measurements similar to those made for *P. pollicipes* (cirral length, intersetal distance, setal length etc.) would have been made for a range of *C. mitella* and *L. anatifera*, but the reluctance of *C. mitella* to moult in captivity and the difficulty in isolating *Lepas* individuals to collect moults, precluded such an investigation. However, such information would have helped clarify the relationship between cirral morphology and ecology.

In summary, all three species of pedunculate barnacle that were examined possess cirri and mouthparts that are typical of omnivorous barnacles. *Pollicipes pollicipes* and *Capitulum mitella* appear to be less well equipped for capturing microalgae than *Lepas anatifera*. *L. anatifera* has limbs well adapted for feeding efficiently on a wide range of food, from microalgae to large animal prey.

Chapter 5

Cirral activity in adult and juvenile *Pollicipes pollicipes*

Introduction

Cirral activity has long been known to be involved in feeding (Darwin, 1854), but has since been shown to have several functions. Cirral activity permits the capture of large particles from the water column. It also generates a current through the mantle cavity which not only has a respiratory function but is now thought also to be filtered by the maxillipedes, removing small particles (Southward, 1955a; Barnes, 1959; Anderson & Southward, 1987).

Types of cirral activity are quite variable and depend on barnacle phylogeny, ecology and physiology. Six distinct categories of cirral activity have been described for balanomorph barnacles (Crisp, 1961; Southward & Crisp, 1965; Anderson, 1981; Anderson & Southward, 1987), and are designated as 'testing', 'pumping beat', 'strong pumping beat', 'normal beat', 'fast beat' and 'prolonged extension'.

The work of Crisp (1961) and Anderson (1981) led the latter author to generalise that verrucomorph and lepadomorph barnacles use prolonged cirral extension as their primary type of cirral activity. Prolonged extension involves the valves being open, with the cirri protruded, opened into a fan and held in this extended position. Primitive balanomorph barnacles also seem to favour extension, cirral beating being confined to the phylogenetically more 'advanced' balanomorphs. All the lepadomorph species studied (see Anderson & Southward, 1987) exhibit 'prolonged extension', none show 'strong pumping' beat, 'normal' beat or 'fast' beat, and only *Octolasmis mulleri* (Crisp, 1961; Walker, 1974; Lang, 1976) and *Lepas anatifera* (Southward, 1957; Howard & Scott, 1959; Patel, 1959; Crisp, 1961; Rainbow & Walker, 1978; Anderson & Southward, 1987) exhibit any 'testing' behaviour. 'Pumping' was rarely seen in *Ibla quadrivalvis* (Anderson & Southward, 1987) and *I. idiotica* (Batham, 1945) and occurred occasionally in between cirral extensions in *Calantica spinosa* (Batham, 1945) and *C. villosa* (Anderson, 1983). 'Pumping' with extension between beats was very common in adult *Lepas anatifera* (Southward, 1957; Howard & Scott, 1959; Patel, 1959; Crisp, 1961; Rainbow & Walker, 1978; Anderson & Southward, 1987), *L. anserifera* (Bieri, 1966; Jones, 1968; Anderson, 1980a; Anderson & Southward,

1987), *L. fascicularis* (Bieri, 1966; Crisp, 1967; Anderson & Southward, 1987) and in juvenile *Pollicipes polymerus* (Lewis, 1981) and *P. pollicipes* (Hui, 1983).

No rhythmic extension and retraction of the cirral net was exhibited by either *Pollicipes polymerus* (Barnes & Reese, 1959) or *Calantica spinosa* (formerly known as *Pollicipes spinosus*) (Batham, 1945). Batham noted that, on immersion, the cirri of *C. spinosa* are extended, occasionally twitching and curling inwards. She suggested that the cirral net was acting only as a passive filter rather than actively capturing prey, i.e. acting like a drift net rather than a seine net or trawl. Many barnacles exhibit prolonged extension of the cirral net and thus exploit external water currents. It is generally thought that where there are strong prevailing currents, it is energetically less expensive to present the extended cirral net into the flow and passively filter the water than to actively sweep the water with the cirral net.

Southward (1955b; 1957; 1962; 1964; 1967) looked at the range of temperatures over which the cirri were active by measuring beat frequency at different temperatures in fifteen species of barnacles which showed some rhythmic activity. The warmer water species were active at higher temperatures and with higher beat rates than the northerly species. The two barnacles with the widest geographic distribution, *Balanus improvisus* and *Elminius modestus*, showed activity over the widest temperature range. The temperature range over which *Lepas anatifera* were showing any cirral activity was felt by Southward (1957) to be too restricted for its world-wide distribution, perhaps suggesting the existence of different physiological races adapted to their location (or perhaps just acclimated to their environment). The deep-sea barnacle *Hexelasma hirsutum* was active over a very restricted temperature range coincident with the virtually constant temperature encountered in the deep sea (Southward, 1957).

It has been demonstrated that, while temperature had little effect on the cirral activity of *Balanus balanoides* between 7.5 and 10°C, beat rate increased with temperature between 10 and 20°C, and decreased again above 20°C (Newell & Northcroft, 1965). Testing behaviour and accompanying rate of oxygen consumption was unaffected by temperature changes between 14 and 22.5°C but between 7.5 and 14°C the rate of oxygen consumption increased with temperature. The converse was true for normal beat. This difference was accounted for by the different rates of oxygen consumption of the muscles (the adductor during testing and retractors when beating) with cirral activity and the effect of temperature on oxygen consumption. The cirral beat frequency of juvenile *Pollicipes polymerus* was found to increase with temperature between 10 and 15° C (Lewis, 1981).

Specific and generic differences in cirral activity often correlate with habitat differences, particularly the prevailing water movement. A comparative study of the feeding and ecology of *Verruca recta* and *V. stroemia* showed that the deep water *V. recta* feeds by means of prolonged cirral extension and shows no rhythmic cirral activity (Anderson, 1980a). Cirral and mouthpart morphology are consistent with carnivorous captorial feeding, which is also reflected in the gut contents. *Verruca stroemia* is found in shallow water and exhibits prolonged cirral extension in moving water but rhythmic cirral extension and retraction in still water. The structure of the cirri and mouthparts suggests microphagous feeding by the maxillipedes in addition to carnivorous captorial feeding. Presumably the tidal influences experienced by *V. stroemia* expose it to extended periods of still or retrograde flow conditions which necessitate beating to feed. *V. recta*, on the other hand, presumably lives in current flows which are relatively consistent and predictable at settlement.

Barnes & Reese (1959) showed that the cirral activity of *Pollicipes polymerus* is related to water movement. As the tide rose and the animals were first covered, the cirri were seen to extend and slowly retract as each wave washed over them. On the rising tide and with prolonged wave action, the cirri were not completely withdrawn between successive waves but were allowed to hang limply from the mantle aperture. Maximal wave action stimulated full cirral extension with the net held wide open and the body raised within the mantle cavity even between successive waves. When water flow was moderate, or during continual submersion, a single cirrus or the whole net could be rapidly contracted without retraction into the mantle cavity. In still water, the cirral net was extended and contracted, but movement was slow and clearly arrhythmic (Barnes & Reese, 1959).

Balanomorph barnacles vary their cirral activity depending on the current speed (Southward, 1965). Both *Balanus balanoides* and *Elminius modestus* occasionally use cirral extension when exposed to strong currents (Southward, 1965). In still water most barnacle species stabilise into a steady rhythm of cirral activity consisting of short bursts of beating interspersed with periods of inactivity (Southward, 1965; Anderson & Southward, 1987).

The position of an animal on the shore also influences cirral activity. One study suggests that animals from the low shore have a more rapid cirral beat than high shore conspecifics (Southward, 1955c). At a site sheltered from currents and wave action, nearly all the individuals from the high shore exhibited fast beat while low shore animals showed normal beat. Southward (1955c) suggested that fast beat was an adaptation to assist feeding and respiration in still water and that the difference in

cirral activity between shore levels was as likely to be related to differences in general metabolism and growth rate as to temperature adaptation.

Cirral activity has been shown to be a function of the age and size of an individual (Southward, 1955c; 1957; Newell & Northcroft, 1965; Lewis, 1981; Hui, 1983), with older barnacles showing a slower cirral beat and exhibiting activity less frequently than younger individuals. Lewis (1981) found evidence for a shift in feeding strategies from juvenile to adult *Pollicipes polymerus*. Although cirral beating was previously thought to be absent in the genus *Pollicipes* (Barnes, 1959), she found that the juveniles exhibited cirral beating similar to sessile barnacles in still water, but in fast flow they extended their cirri like the adults. Studies made of the cirral activity of *Pollicipes pollicipes* and *Capitulum mitella* showed that the cirral behaviour of juvenile *P. pollicipes* was broadly similar to that of juvenile *P. polymerus*, whereas *C. mitella* showed no rhythmic cirral beating behaviour at all (Hui, 1983).

The presence of suitable food in the water surrounding barnacles influences the cirral activity of some balanomorph barnacles (Crisp, 1964). Feeding is not indiscriminate so nutritious food particles stimulate greater cirral activity than do inert particles. There is evidence that the cirri of barnacles can show a chemotactic response (Anderson & Southward, 1987). Crisp (1967) used *Lepas anatifera* and *L. fascicularis* to determine whether a solely chemical stimulus could elicit a feeding response in barnacles. Milk, bacto-peptone, animal coelomic fluids and tissue extracts stimulated strong feeding responses. Protein solutions caused rather weak reactions, which could be further reduced by dialysis. Several amino acids and related compounds promoted a feeding response. Solutions of larger amino acids, sugars and polypeptides elicited little feeding response and organic substances gave no reaction.

The cirri and mouthparts of *Lepas* were stimulated by trimethylamine, choline or cations such as potassium. These cations and amino acids also influence feeding in many other carnivorous Crustacea, polychaetes and molluscs (see Crisp, 1967). The response of *Lepas* to both potassium ions and amino acids at very low concentrations may allow the recognition of prey organisms once they are punctured by the cirral setae. *Balanus hameri* (Allison & Dorsett, 1977) and *Pollicipes pollicipes* (Hicks, 1993) were found to exhibit feeding activity in response to a similar range of chemicals to those found for *Lepas* by Crisp (1967). Positive feeding responses in *P. pollicipes* were also stimulated by boiled *Artemia* extract. Negative responses were exhibited when *P. pollicipes* was exposed to unboiled *Artemia* extract, ammonia, and many amino acids at high concentrations.

Howard & Scott (1959) observed that both *Lepas anatifera* and *Pollicipes* (*Mitella*) *polymerus* had similar mouthparts (but see Chapter 4) and were relatively unselective omnivores, capable of both macrophagous carnivory and microphagous herbivory/detritivory. Individuals immersed in a zooplankton suspension were seen to grasp the zooplankters, enclosing them with their cirri. The six pairs of cirri could either move independently or co-operate, as previously described for *Calantica spinosa* (Batham, 1945), *Lepas*, *Scalpellum* and *Verruca* (see Howard & Scott, 1959). If an *Artemia* touched the cirri of *Lepas*, all the cirri contracted in a grasping movement, pushing the prey towards the mouth. The functions of individual cirri were seen more readily when the animals fed on small organisms. The anterior three cirri, armed with basal, spiny 'pushing brushes' guided the food to the mouthparts where it was gripped and compacted. Other organisms were simultaneously captured by individual rami of the last three cirri which bent around them, retaining them for future ingestion. The cirri and mouthparts of *Pollicipes* were found to be anatomically and functionally similar to those of *Lepas*, although shorter, thicker and less active. The cirri responded rapidly when food was contacted (Howard & Scott, 1959).

Differences between the feeding behaviour of *Lepas* and *Pollicipes polymerus* were found (by Howard & Scott, 1959) to relate to their ecology. *P. polymerus* occur on fixed substrata, exposed to high wave action, orientated to receive the down wash of waves. Their cirri are extended for prolonged periods to filter passively the water that washes over them. The cirri are relatively short and inactive with prolonged extension the only observed feeding behaviour - ideal for filtering from the water flow. *Lepas* is found on drifting wood and are generally exposed to low water flow. The peduncle of this species is more flexible and mobile, and the cirri more active than those of *Pollicipes* (Howard & Scott, 1959) possibly enabling more efficient foraging, in the less favourable hydrodynamic conditions.

The aim of the current study is to investigate the cirral activity displayed by adult and juvenile *Pollicipes pollicipes*, furthering the preliminary work done by Hui (1983) and to compare the results with previous studies of closely related barnacles, such as *P. polymerus* (Howard & Scott, 1959; Barnes & Reese, 1959, 1960; Lewis, 1981), *Calantica spinosa* (Batham, 1945) and *Capitulum mitella* (Hui, 1983). The so-called juvenile to adult shift in feeding strategy (Lewis, 1981; Hui, 1983) will also be investigated. The factors that influence the type of cirral activity in *P. pollicipes* will be investigated as an aid to interpreting the function of the activities.

Materials and Methods

The cirral activity of lepadomorph barnacles is more restricted than that of balanomorphs and all actions are much slower. The cirral activity may be observed quite adequately without the aid of cine or video techniques used by others (Crisp and Southward, 1961; Anderson, 1981; 1983; Anderson & Southward, 1987) to study the faster-moving cirri of balanomorph barnacles.

Ten *Pollicipes pollicipes* of various sizes, ranging from 3 to 12 mm in rostro-carinal length, were observed under different conditions of water movement and in the presence and absence of food for a total of three hours and the cirral activity exhibited was described.

Investigations of cirral beating were not performed at any particular time of day or night as previous investigation had shown no evidence for periodicity in feeding activity over an 80 hour period (Hicks, 1993).

Ten juvenile *Pollicipes pollicipes* of approximately the same size (2.96 ± 0.13 SD* mm RC) were selected. These were attached to a slate which was placed in a crystallising dish of still UVFSW. The animals were observed for up to five hours. The time taken for the cirri to emerge and that for which they were held extended was measured. The frequency of cirral movement was assessed by recording the number of extensions of the cirri during a ten minute period and expressed in extensions per minute which would be roughly equivalent to beats per minute if the activity was considered to be slow rhythmic beating. The observations were made in water at 16.5°C in which the animals had been left overnight in the dark. The temperature was maintained using a water bath.

Six *Capitulum mitella* of 5.15 - 12.45 mm RC were placed in still UVFSW. After half an hour, observation of cirral activity commenced and continued for the succeeding five hours.

The effect of barnacle size on cirral extension frequency

Sixty-one *Pollicipes pollicipes* of various sizes, from 1 to 12 mm R-C were attached to slate and were placed in still UVFSW at 16°C. After ten minutes, the number of cirral extensions per ten minute period was recorded for each animal.

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* all numbers quoted in this form will be a mean ± a standard deviation (SD), unless specified

Due to shortage of animals, the same individuals were used in many of the following sets of observations. However, observations were made at weekly intervals allowing sufficient time to minimise any disturbance effects.

The effect of temperature on cirral extension frequency

The above experimental procedure was repeated using ten juvenile *P. pollicipes* (2.96 ± 0.13 mm RC). Seawater was preheated to 30°C and was mixed with seawater at 15°C to obtain the desired temperature. The frequency of cirral extension was measured for each animal at various temperatures between 16 to 24°C. This temperature range was chosen as it was around the range that would be experienced in the wild but not high enough to induce a depression in beat rate (Southward, 1955a). A water bath was not used as it made observation of cirral activity very difficult, with vibrations, in particular, disturbing normal cirral activity. The temperature did not vary more than ± 0.4 °C over the 10 minute experimental period.

The effect of aeration and the presence of food on the extension frequency

Ten *P. pollicipes* (2.96 ± 0.13 mm RC) were placed in still UVFSW. After a ten minute acclimation period, the number of cirral extensions during a ten minute period was measured for each animal. Air was then bubbled into the water. After ten minutes, extension frequencies were remeasured. Aeration was then removed and several hundred *Artemia* nauplii were added to the water. After a further ten minutes, the extension frequencies were again measured. Finally the water was again aerated and the extension frequency measured. The experimental protocol was then repeated using a suspension of the diatom *Skeletonema* (100 cells μl^{-1}) as the food source.

The effect of *Skeletonema costatum* abundance on cirral extension frequency.

Ten *P. pollicipes* (RC 2.96 ± 0.13 mm) were placed in clean seawater or one of 15 diatom suspensions, varying in concentration from 8 to 130 cells μl^{-1} (assessed by counting on a haemocytometer), with aeration. After ten minutes in each suspension (20°C) the beat frequency of each barnacle was measured over a ten minute period.

The effect of previous starvation and feeding on cirral extension frequency

Twenty-two juvenile *P. pollicipes* of approximately the same size were selected. Eleven (3.09 ± 0.18 mm RC) were kept in clean UVFSW without food overnight while the other eleven (3.10 ± 0.16 mm RC) were fed on *Artemia* nauplii overnight. The animals were then placed in clean UVFSW at 19.5°C for ten minutes to commence beating. The number of extensions in ten minutes was counted for each

animal. The procedure was then repeated after *Artemia* nauplii (10 ml^{-1}) had been introduced.

The effect of previous emersion and immersion on cirral extension frequency

In order to investigate whether *P. pollicipes* compensate for the time they spend uncovered by the tide (and therefore not feeding) with increased rates of cirral beating, ten *P. pollicipes* juveniles of similar size (2.96 ± 0.13 mm RC) were starved overnight to ensure all were in a similar condition. The animals were kept emersed for 2 hours (the time animals are likely to be exposed for at low tide) before being placed back into UVFSW at 19.5°C and left for ten minutes before the cirral beat rates were measured. *Artemia* nauplii (10 ml^{-1}) were added and the counts repeated. The experiment was repeated on another occasion with the same animals after they had been immersed in clean seawater for 2 hours. *Artemia* nauplii were added and the counts repeated.

The effect of flow on the cirral extension frequency of adults and juveniles in the presence and absence of food.

Five adult *P. pollicipes* (11.51 ± 1.45 mm RC) and five juveniles (3.00 ± 0.16 mm RC) were starved overnight. Larger sample sizes would have been preferable but shortage of animals precluded them. All were attached to slates in a glass crystallising dish containing 1.5 l of UVFSW at 19.5°C. This was placed on a magnetic stirrer (Janke & Kunkel IKAMAG model RH) which generated a gentle water flow around the dish. The number of cirral extensions in still water conditions was counted and the counts repeated under conditions of low (6.7 ± 0.11 SE cm s^{-1}), medium (9.74 ± 0.08 SE cm s^{-1}) and high (18.78 ± 0.15 SE cm s^{-1})water flow. The flow rates were measured using a Novonic Streamflo impeller flow meter. The whole procedure was then repeated after *Artemia* nauplii had been added to the water.

Effect of water flow on frequency of cirral extension of *P. pollicipes* in a flume

These observations were undertaken in a seawater flume, in which the water flow could be controlled. The water temperature was 21°C. A Novonic Streamflo flow meter was used to measure the flow at the same height as the barnacles' capitula but downstream and out of their wake. Twelve *Pollicipes* (3.22 ± 0.15 mm RC) were attached to a piece of slate 4 mm deep and this was placed on the floor of the flume, in a region designed to produce laminar flow (see Vogel, 1981). The number of cirral extensions in ten minutes made by each barnacle was counted, first in still water and then at 14 flow rates from 2.3 cm s^{-1} to 14.8 cm s^{-1}.

P. pollicipes of various sizes (3.3 mm - 12.55 mm RC) were subjected to a series of flow rates between 18.5 cm s^{-1} and 47.6 cm s^{-1} (the highest that could be generated) and the cirral activity recorded.

Investigation of water flow through the mantle cavity of *Pollicipes pollicipes* during cirral extension and retraction.

Two *Pollicipes pollicipes* (4.45 and 4.35 mm RC) were placed in a 1 litre crystallising dish containing UVFSW. The animals were left in still conditions for 30 minutes until slow rhythmic cirral beating had become established. Skimmed milk was used as a dye as it is non-toxic, more dense than sea water and rapidly sinks out of suspension, making water currents in and around the barnacle easy to see. A Pasteur pipette was used to dispense small quantities of milk into the water in various locations around the barnacle at different phases of the beat and observations were made.

Results

The following behaviour patterns which relate to cirral activity, were clearly observed in *Pollicipes pollicipes*:

1) None; shell valves tightly closed
2) Shell valves parted, cirri curled up within the mantle cavity
3) The tips of all cirri protruding motionless from the open valves
4) The cirri protrude from the mantle cavity, curled and forming a fist
5) The cirri fully extended but held together, not open
6) Cirral net is fully extended and open, forming a fan
7) Cirral net is extended and a few cirri are incurled towards the mouth
8) All cirri arrhythmically incurled towards the mouth then re-extended
9) Slow rhythmic withdrawal and extension of cirral net with cirri fully extended
10) As 9 but with incomplete extension.

Both juvenile and adult *P. pollicipes* exhibited slow, rhythmic cirral beating (9 and 10 above) in all still water observations. *Capitulum mitella*, on the other hand, showed little activity in still water. Most individuals kept their shell valves tightly shut while others allowed the valves to gape open either with the cirri remaining inside the mantle cavity or with the tips protruding. There was no evidence of any short term rhythmic cirral behaviour. Both adults and juveniles of both *Capitulum mitella* and *Pollicipes pollicipes* exhibited prolonged cirral extension when exposed to water flow,

with occasional incurling of individual, several, or all of the cirri towards the mouth (6 - 10 above).

Description of cirral beat of *Pollicipes pollicipes*

The precise nature of the 'beat' was very variable between animals. Some animals exhibited full extension of the cirral net followed by complete retraction within the mantle cavity coupled with movement of the prosoma, while others uncurled their cirri to a variable extent and keeping them in the same position moved them in and out of the aperture by means of the movement of the prosoma (see Figs 5.1 (a-f) and 5.2 (a-f)). It was unclear whether this was one variable activity or two different activities. However, individuals consistently showed only one type of activity in still water. The extension phase of the beat took 79% of the time for a complete beat. A 'beat' in the classical sense does not involve cirral withdrawal but the movement backwards and forwards of a fully extended cirral net with a slight feathering on the recovery stroke. The beats I observed appeared physically and functionally different from the beating of balanomorphs and it might perhaps be more appropriate to refer to this behaviour as slow rhythmical cirral extension and withdrawal rather than beating. However, as similar activity in both *P. polymerus* (Lewis, 1981) and *P. pollicipes* (Hui, 1983) was termed 'beating' I will use this term also.

Rhythmical cirral extension as a function of rostro-carinal length in *Pollicipes pollicipes.*

Fig. 5.3 shows the scatter plot of cirral extension frequency against rostro-carinal length (RC). Smaller animals clearly have faster frequencies in general but the result is particularly variable for the very small animals. There is a general trend for reducing frequency with size to about 5 - 8 mm RC and then the extension frequency seems independent of size at around one extension per minute. Some cirral beating was seen in most of the animals at some point.

The Effect of temperature on the rate of rhythmical cirral retraction and extension.

The rate of cirral beat increases with temperature and the variance increases in proportion with the increase in beat rate (Table 5.1).

Table 5.1: Mean cirral beat rates of juvenile *P. pollicipes* at different temperatures. N is the number of individuals.

Temperature (°C)	Mean beat frequency (beats/min)	S.D.	N
16	1.25	0.65	10
17	1.40	0.97	10
18	2.13	0.96	9
19	2.37	1.35	10
20	2.63	1.83	10
21	3.34	2.10	9
22	3.22	2.08	9
23	3.81	2.67	9
24	4.03	2.85	9

The large variance at higher temperatures results from certain animals (animals 2, 4, 5 and 10) which display 'activity' that can only occur at one frequency while another group of animals (animals 1, 3, 6, 7, 8 and 9) display an activity that is strongly affected by temperature (see Figure 5.4a). There is a Q_{10} of 5.1 for this latter group. If these data were then grouped according to whether the activity is temperature dependent a plot such as Fig.5.4b is obtained with the temperature dependent beating being associated with particular animals. It seems possible that the difference between animals relates to differences between the type of activity displayed (i.e. full extension or pumping of furled cirri), although such was not obvious at the time of measurement.

Figure 5.1. Diagrammatic representation of cirral beat type I.

a) and f) The cirri are fully extended into the full feeding position. The cirri withdraw b) and remain fully withdrawn momentarily c). The cirri are re-extended d) and e).

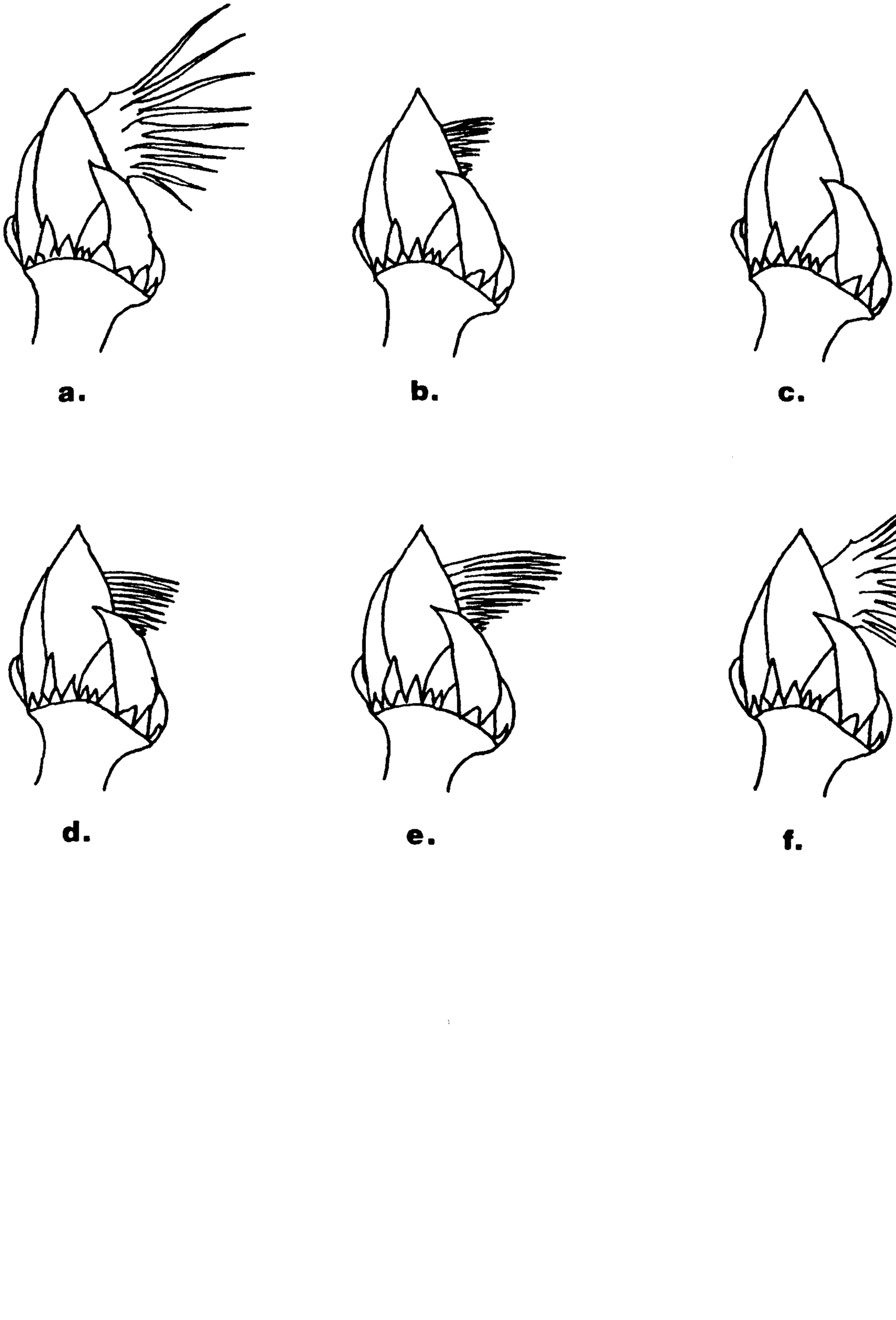


Figure 5.2. Diagrammatic representation of cirral beat type II

a) Cirri are extended by the protrusion of the prosoma but are kept partially curled. b) Cirri are withdrawn by the retraction of the prosoma c) and remain in the mantle for seconds before being re-extended by the protrusion of the prosoma d - f).

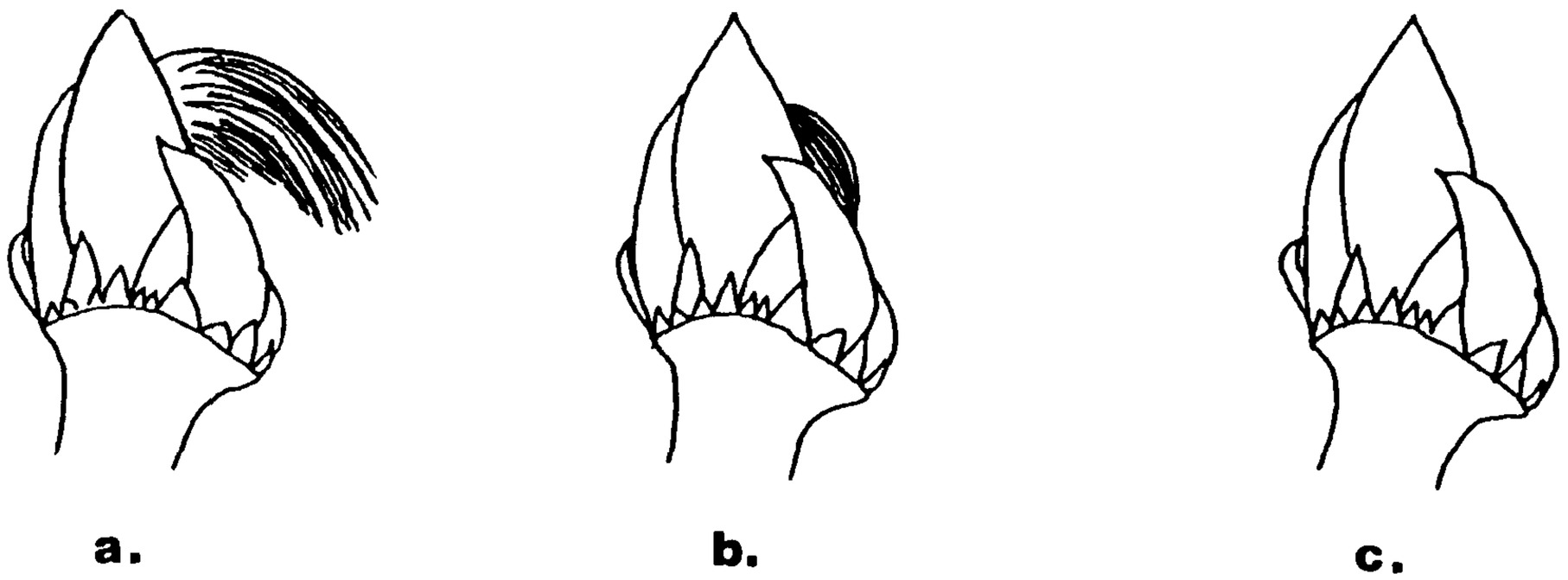


Figure 5.3. Cirral extension frequency against rostro-carinal length of *P. pollicipes* at a constant temperature of 16°C. Lines fitted by eye.

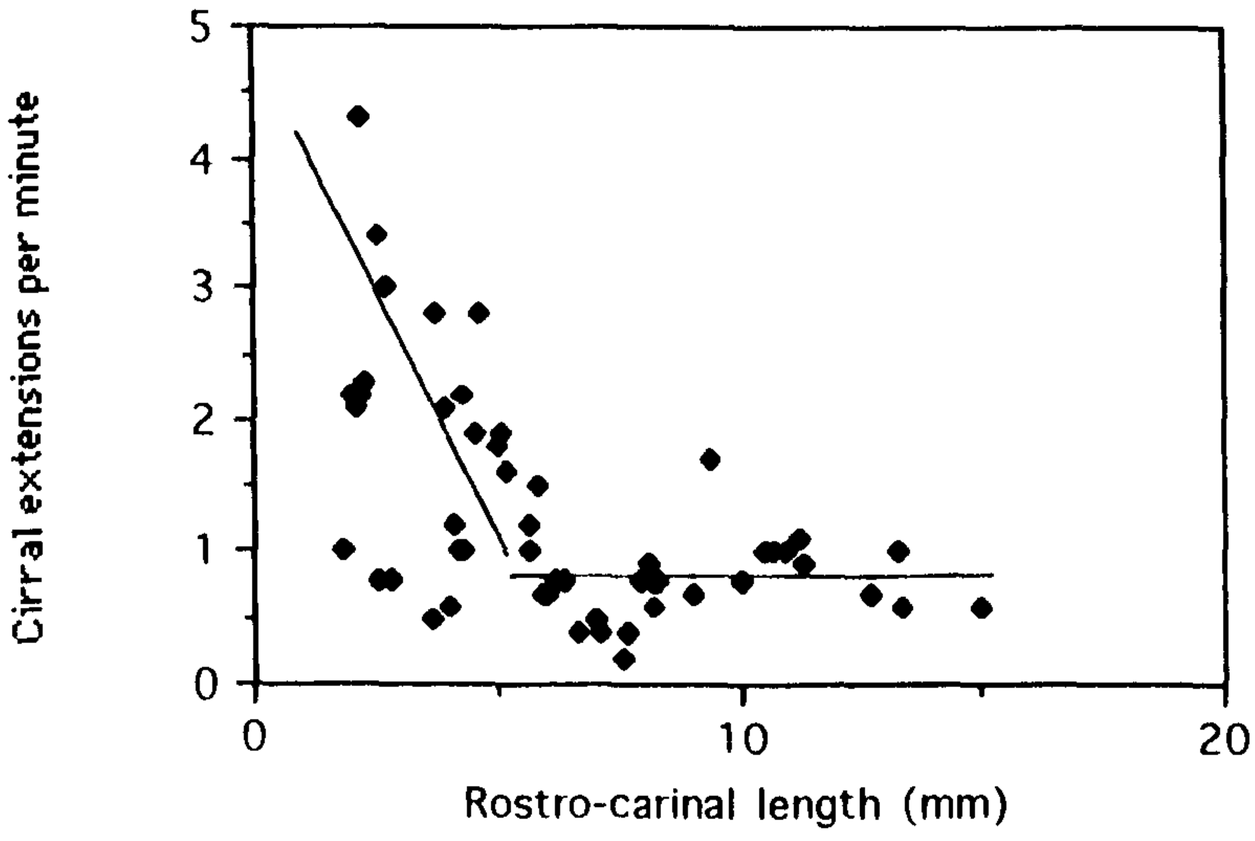


Figure 5.4a. The effect of temperature on the cirral beat rate of individual juvenile *P. pollicipes* (RC 2.96 ± 0.13 mm).

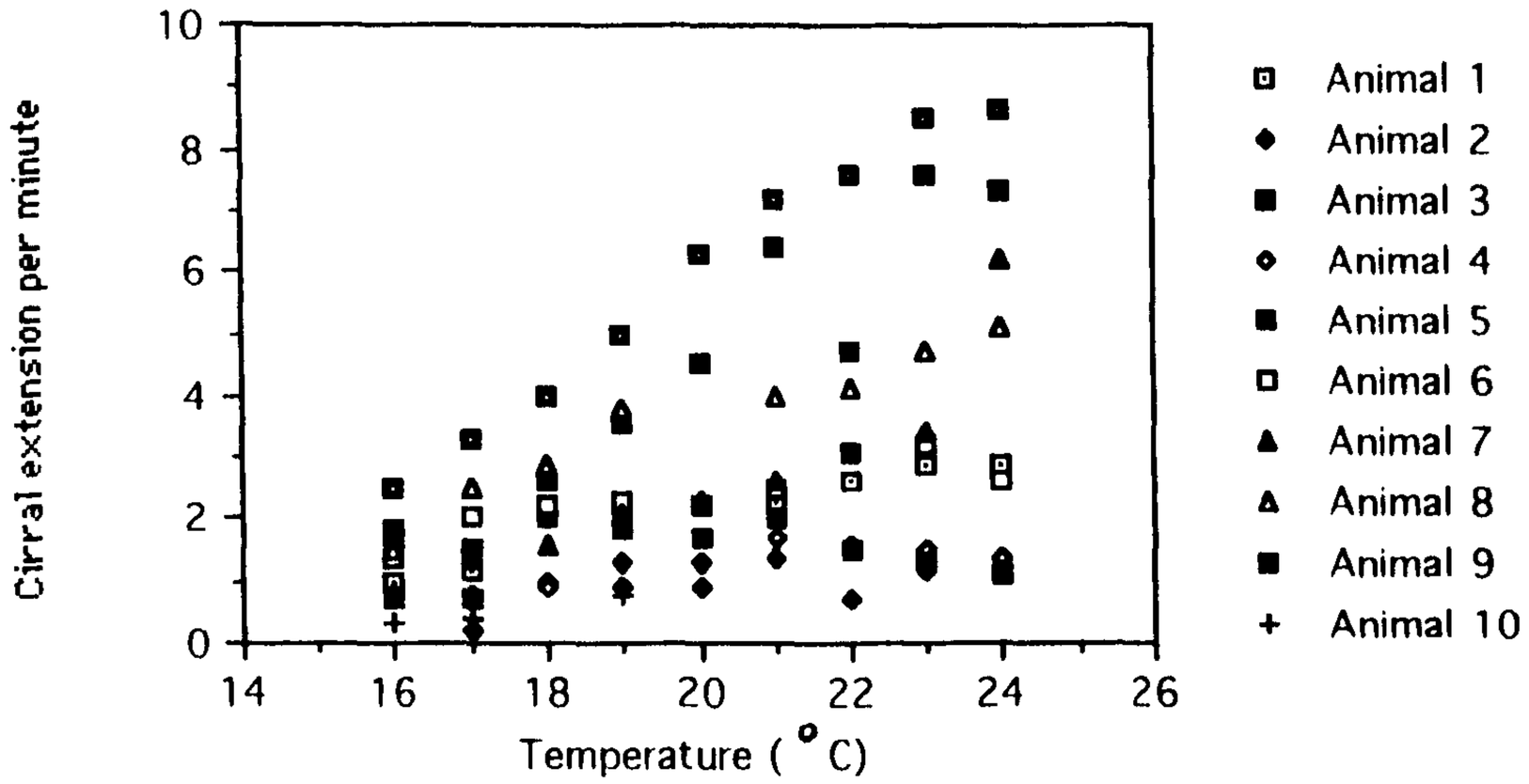
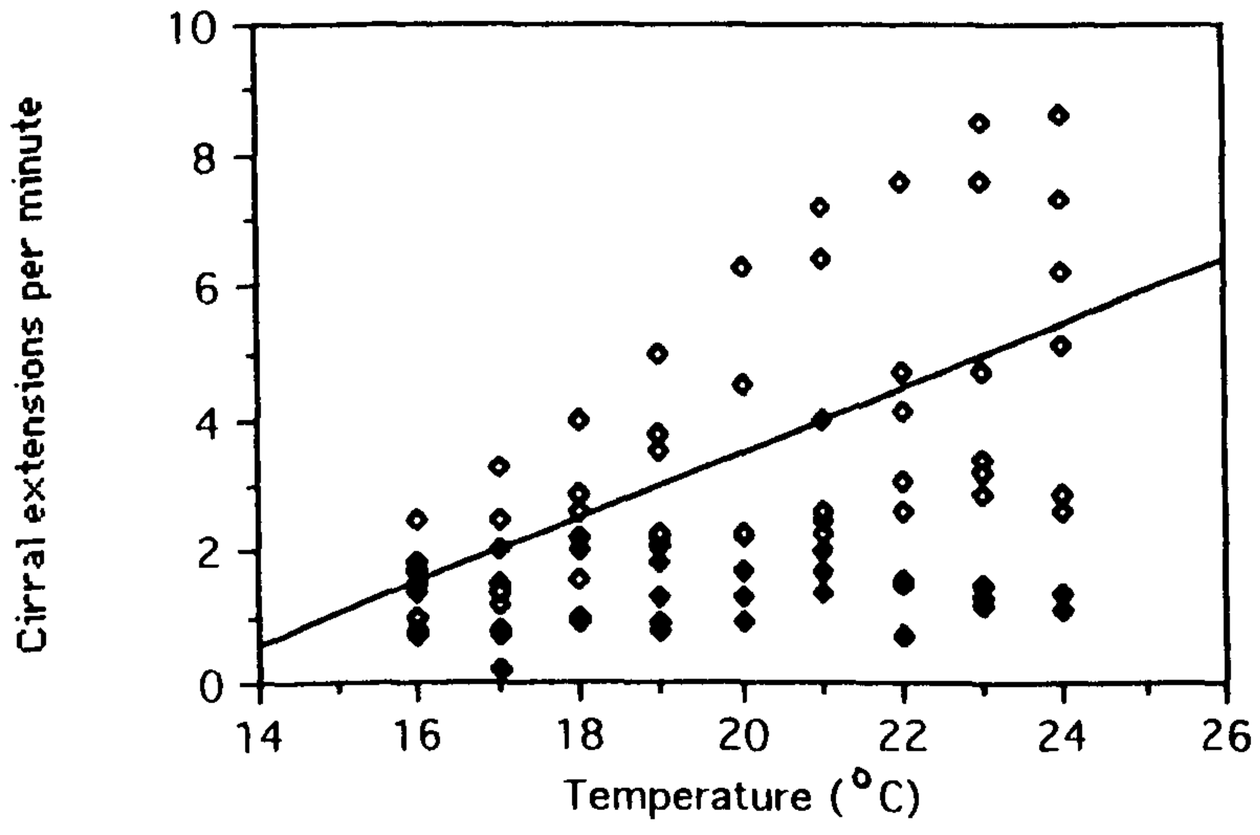


Figure 5.4b. Cirral beat rate of juvenile *P. pollicipes* at different water temperatures. Open diamonds denote temperature dependent activity while closed diamonds denote temperature independent activity.

Temperature dependent animals:

Cirral extension frequency = -6.282 + 0.4897 (temperature).

r = 0.63 (df = 52, P < 0.001).



Temperature independent animals: r = 0.138 (df = 30, P >0.05)

The effect of aeration and the presence and absence of food on the rhythmic cirral activity of juvenile *P. pollicipes*.

Cirral beat frequency drops when air is bubbled into the water and this decrease is most marked in the presence of food (Table 5.2). When the water is aerated, the rate of cirral beat is slightly higher in the presence of *Artemia* than when no food is present, although lower in the presence of *Skeletonema*.

Table 5.2: Mean cirral beat frequency (beats min^{-1}) ± SD (in brackets) of juvenile *Pollicipes pollicipes* (RC ~3mm) under various conditions of food and aeration.

	Food			
	None	*Artemia*	*Skeletonema*	Average ± SE
No aeration	1.61 (0.95)	1.91 (0.93)	1.76 (0.81)	1.76 (0.15)
Aeration	1.07 (0.74)	1.26 (0.72)	0.64 (0.41)	0.99 (0.15)
Average ± SE	1.34 (0.18)	1.58 (0.18)	1.20 (0.19)	

By Tukey's method, 95% CI of difference between means of factor 'Food' = 0.38 b.p.m.

The data exhibited no significant heterogeneity of variance (Cochran's C = 0.2399 for 6 and 8 df, $P > 0.05$). An analysis of variance (blocked by animal) was performed to assess the effect of food and aeration on the cirral beat frequency (Table 5.3). Cirral beat rate varied significantly from individual to individual ($F = 12.58$, 8 df, $P < 0.001$), aeration significantly reduced the rate of rhythmic cirral extension and retraction ($F = 37.83$, df = 1, $P < 0.001$) and the presence of food also significantly affected the rate of cirral activity ($F = 4.69$, 2 df, $P = 0.012$). The interaction term was not significant ($F = 1.63$, 2 df, $P = 0.209$).

Table 5.3. Analysis of variance table for the effect of animal, aeration and food on the frequency of rhythmic cirral activity of juvenile *P. pollicipes*.

Variation	DF	Sum Sq.	Mean Sq.	F-Value	Probability
Animal	8	22.3604	2.7950	12.58	< 0.001
Aeration	1	8.4017	8.4017	37.83	< 0.001
Food	2	2.2026	1.1013	4.96	0.012
Interaction	2	0.7233	0.3617	1.63	0.209
Error	40	8.8841	0.2221		
Total	53	42.5720			

The extension frequency with *Artemia* is significantly greater than that in the presence of *Skeletonema* but the average with no food present is not significantly different from the other two. There may be a general tendency for *Artemia* to increase extension frequency and *Skeletonema* to depress extension frequency but within the variability of the experiment only the largest mean differences can be confidently distinguished.

There were also other behavioural differences in animals in the presence of algae. The drop in beat frequency measured was attributable to longer cirral extensions caused by cleaning of cirri, which was not seen under any of the other experimental conditions. Cleaning involved incurling of cirri III-VI in towards the mouth, while cirri I and II seem to move across them, combing alternately. Because of these behavioural changes further investigations into the effect of algae on beat frequency were carried out.

The effect of *Skeletonema costatum* density on the rate of rhythmic cirral extension and retraction of juvenile *P. pollicipes*.

The frequency of cirral extension decreased slightly with increasing algal suspension density (Table 5.4). The behaviour of the barnacles changed when algae were added to the water and this was most marked when air was bubbled into the water. When the water contained no food only four out of ten barnacles showed full cirral extension while beating. However, eight out of nine did so when algae and aeration were present. With or without food, the animals not showing full cirral extension were beating with partially extended cirri.

Table 5.4: The mean rates of cirral beat (± SD) for juvenile *P. pollicipes* (RC = 3.0 ± 0.13 mm) in *Skeletonema costatum* suspensions of various densities. N = number of animals.

Density (cells/μl)	Beat Frequency (Beats/min)	SD	N
0	0.86	0.58	7
4.2	0.46	0.42	7
11.1	0.54	0.46	8
14.7	0.43	0.29	7
15.8	0.33	0.26	7
28.9	0.39	0.38	9
31.9	0.18	0.12	8
52.5	0.42	0.42	9
53.6	0.35	0.22	8
71.4	0.25	0.23	6
80.3	0.17	0.08	6
131.1	0.28	0.22	8

Fig. 5.5 shows a significant correlation between log average extension rate and log *Skeletonema* concentration plus 1. The rate decreased logarithmically with algal concentration again as longer time is spent cleaning the cirri so fewer extensions are possible per unit time.

The effect of prior starvation on the rate of rhythmical cirral activity

Slightly higher mean rates of cirral beating were observed in the previously starved animals (Table 5.5) although the coefficients of variation were very high (48 - 70%). No feeding was observed when *Artemia* were present, and in the absence of aeration, the *Artemia* tended to congregate in parts of the dish where light was focused.

Table 5.5. The mean rate ± SD of juvenile *P. pollicipes* cirral beat (n = 11) after prior feeding or starvation, under different food conditions. CV = coefficient of variation.

	Treatment					
	Starved			Fed		
	Mean	SD	CV	Mean	SD	CV
Food	1.25	(0.871)	70%	1.19	(0.726)	61%
No food	1.30	(0.720)	55%	1.07	(0.516)	48%

The data showed no significant heterogeneity of variance (Cochran's C = 0.3662, P > 0.05). A two-way analysis of variance was performed (Table 5.6) to assess the effect of previous starvation and of the presence and absence of food on the rate of cirral beat. Neither the main effects nor the interaction term proved significant within the variability of measurement. The rhythmic behaviour measured appears to have little relationship to previous feeding history.

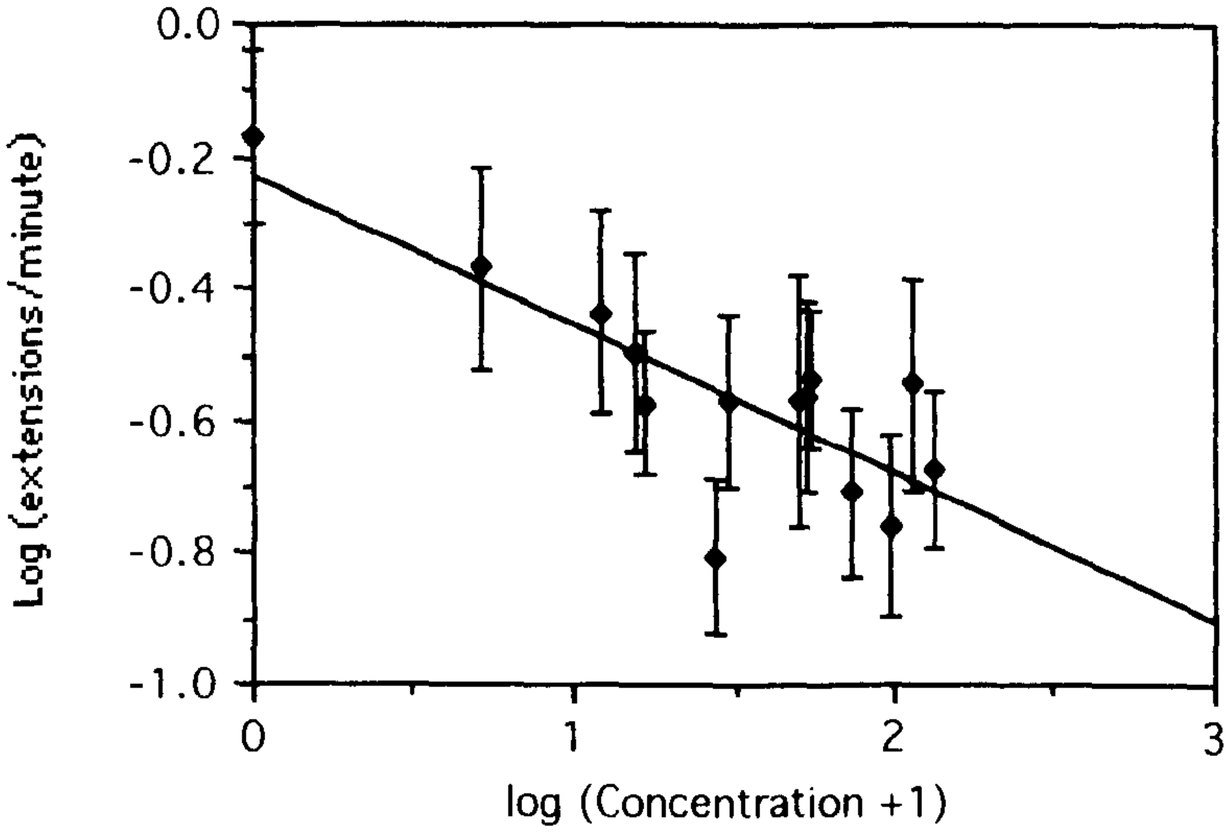
Table 5.6. Analysis of variance table for the effect of prior starvation or feeding (history) on the cirral beat rate of juvenile *P. pollicipes* in the presence and absence of food and for the effect of any interaction between the two.

Source	d.f.	Sum sq.	Mean Sq.	F-value	P
History	1	0.2327	0.2327	0.45	0.507
Food	1	0.0145	0.0145	0.03	0.868
Interaction	1	0.0736	0.0736	0.14	0.708
Error	40	20.7182	0.5180		
Total	43	21.0391			

Figure 5.5. Log mean cirral extension rate (beats /min) of *P. pollicipes* (2.96 ± 0.13 mm RC) against log (*Skeletonema* concentration +1).

log extension = - 0.227 - 0.226 x log (*Skeletonema* concentration + 1) beats per minute.

r = - 0.807, df = 12, P < 0.001



The effect of previous immersion and emersion on the rate of rhythmic cirral activity

Animals that were immersed for the two hours prior to the experiment exhibited marginally higher mean beat rates than did those that were kept out of water for that time (Table 5.7). Beat rates were not consistently higher in the presence of food. Again the relative variability was extremely high (CV range = 72 - 86%).

Table 5.7: The mean cirral extension frequency ± SD of *P. pollicipes* juveniles previously immersed and emersed in the presence and absence of food. N = number of observations. CV = coefficient of variation.

	Treatment							
	Immersion				Emersion			
	N	Mean	SD	CV%	N	Mean	SD	CV%
No food	9	1.22	1.01	83	8	0.93	0.76	82
Food	9	1.32	0.95	72	8	1.10	0.95	86

The data exhibited no significant heterogeneity of variance (Cochran's C = 0.3248, P>0.05). An analysis of variance was performed to ascertain the effect of prior immersion or emersion on cirral beat frequency in the presence and absence of food (Table 5.8). Neither of the main effects nor the interaction term proved significant within the variability of measurement.

Table 5.8. Analysis of variance table for the effect of prior immersion or emersion (history) on the cirral beat rate of juvenile *P. pollicipes* in the presence and absence of food and for the effect of any interaction between the two.

Source	d.f.	Sum sq.	Mean Sq.	F-value	P
History	1	0.3828	0.3828	0.42	0.520
Food	1	0.0703	0.0703	0.08	0.782
Interaction	1	0.0528	0.0528	0.06	0.810
Error	28	25.2288	0.9010		
Total	31	25.7347			

The effect of flow on the cirral activity of adult and juvenile *P.pollicipes*

Cirral extension and retraction frequency decreases in both the adults and juveniles with increased flow (Table 5.9), although observation suggests that while adults are exhibiting full cirral extension, the juveniles still only have their cirri partially uncurled. No adults exhibited beating in high flow, although they had their cirri extended. Perhaps the beat rate was so slow that the ten minute experimental period was insufficiently long to measure it.

Table 5.9. The mean cirral beat rate (± SD) of juvenile and adult *P. pollicipes* under different flow conditions (none, low (6.7 cm s^{-1}), medium (9.74 cm s^{-1}) and high (18.1 cm s^{-1}) and in the presence and absence of food. N = 5 animals.

Flow	No food	Food	Average
Juveniles			
None	0.94 (0.84)	1.42 (1.49)	1.18
Low	1.44 (1.29)	1.34 (1.45)	1.39
Medium	0.32 (0.29)	0.58 (0.69)	0.45
High	0.24 (0.15)	0.28 (0.36)	0.26
Average	0.74	0.91	
Adults			
None	1.10 (0.44)	0.98 (0.42)	1.04
Low	0.92 (0.49)	0.86 (0.54)	0.89
Medium	0.32 (0.19)	0.22 (0.13)	0.27
High	0.26 (0.18)	0	0.13
Average	0.65	0.52	

The variance was stabilised by log(beat+1) transformation giving Cochran's C for juveniles = 0.2556, 8 and 4 df, $P > 0.05$ and for adults C = 0.2806, 8 and 4 df, $P > 0.05$, both no longer exhibited significant heterogeneity of variance so two two-way analyses of variance were performed, for the beat rate of juveniles and adults (see Table 5.10a and b). The presence or absence of food (*Artemia* nauplii) has no significant effect on the cirral beat rate of either juvenile or adult *P. pollicipes* over the range of flow conditions tested (Table 5.10a, F = 0.12, 1 df, P= 0.729 and Table 5.10b, F = 2.87, P = 0.10).

Table 5.10. Two-way analysis of variance for the effect of flow and food on the cirral beat rate of a) juvenile and b) adult *Pollicipes pollicipes.*

a) juvenile *P. pollicipes*.

Conditions	df	Sum sq.	Mean sq.	F	P
Flow	3	0.3497	0.1166	3.02	0.044
Food	1	0.0047	0.0047	0.12	0.729
Interaction	3	0.0151	0.0050	0.13	
Error	32	1.2364	0.0386		
Total	39	1.6059			

b) adult *P. pollicipes*

Conditions	df	Sum sq.	Mean sq.	F	P
Flow	3	0.4553	0.1518	23.35	< 0.001
Food	1	0.0187	0.0187	2.87	0.100
Interaction	3	0.0099	0.0033	0.51	0.680
Error	32	0.2079	0.0065		
Total	39	0.6917			

The level of flow has a significant effect on the cirral activity of juvenile ($F = 3.02$, 3 df, $P = 0.044$) and adult ($F = 23.25$, 3 df, $P < 0.001$) *P. pollicipes* (Table 5.10) with the frequency of cirral extension decreasing with increasing flow.

Tukey's pairwise comparisons were performed to see which flow regimes promoted significantly differing cirral activity (Table 5.11). The beat rates of adults were not significantly different between no and low flow nor between medium and high flow. All other differences were significant. This indicates that there is a threshold flow rate, between 6.7 and 9.7 cm s^{-1} at which there is an appreciable drop in cirral beat rate. Although for juveniles none of the differences were significant at the 5% level, the pattern was very similar.

Table 5.11. Tukey's pairwise comparisons of cirral beat rates of *P. pollicipes* under different flow regimes, averaged over animal size and food availability. The values given are the differences between the mean log(beat+1) for each comparison. Minimum difference for significance in parenthesis, significant differences are denoted by *.

Juveniles (0.274)

	No flow	Low flow	Medium flow
High flow	0.1903	0.2228	0.0482
Medium flow	0.1421	0.1745	
Low flow	0.0325		

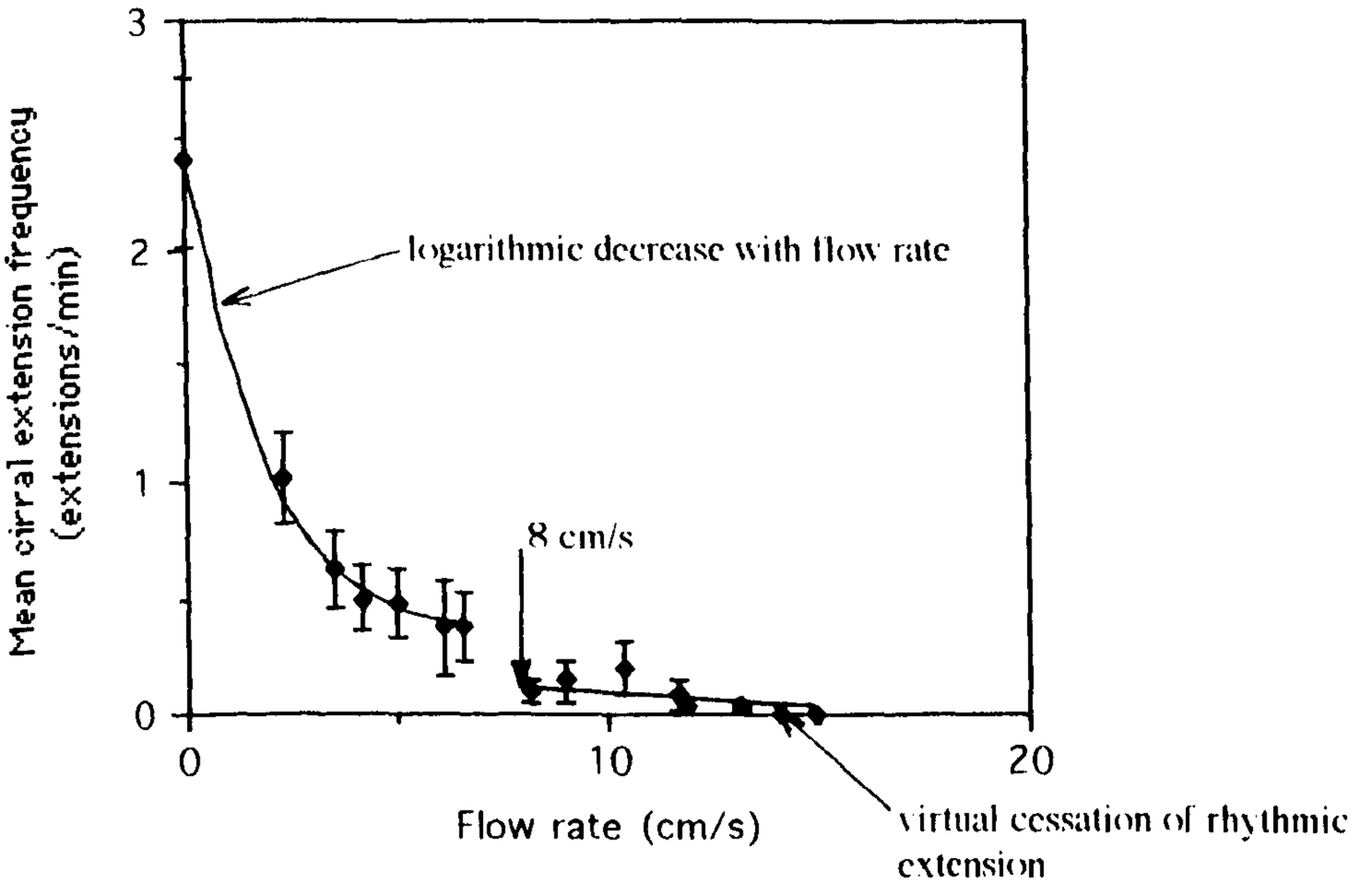
Adults (0.112)

High flow	0.2533*	0.2159*	0.0523
Medium flow	0.2011*	0.1636*	
Low flow	0.0374		

The effect of laminar flow in a flume on rhythmic cirral activity of *Pollicipes pollicipes*.

The rate of cirral beat initially decreases sharply with increasing flow rate (to a flow rate of about 8 cm s^{-1}) after which further increases in flow rate cause beat rate to decrease more gradually, until there is no beating exhibited at all at flow rates above 14 cm s^{-1} (Fig. 5.6). The 8 cm s^{-1} discontinuity is the same as the threshold flow measured in the dish under more turbulent conditions. Animals not exhibiting rhythmic extension activity at the lower flows remained inactive with cirri fully retracted. At the higher flow rates rhythmic activity ceased in favour of prolonged cirral extension.

Figure 5.6. The effect of flow rate on the frequency of rhythmic cirral activity of juvenile *Pollicipes pollicipes*. Lines fitted by eye.



The behaviour of animals of different sizes was observed at higher flows. Those animals exhibiting any activity were extending their cirri, either partially or fully. At the highest flow that could be generated (47.6 cm s^{-1}) all barnacles were still able to exhibit full cirral extension (Table 5.12).

Table 5.12. Cirral behaviour of juvenile and adult *P. pollicipes* at flow rates between 18 and 47.6 cm s^{-1}. Key: ++ cirri fully extended into the feeding position, + = furled cirri protruding from the mantle, - = cirri completely withdrawn.

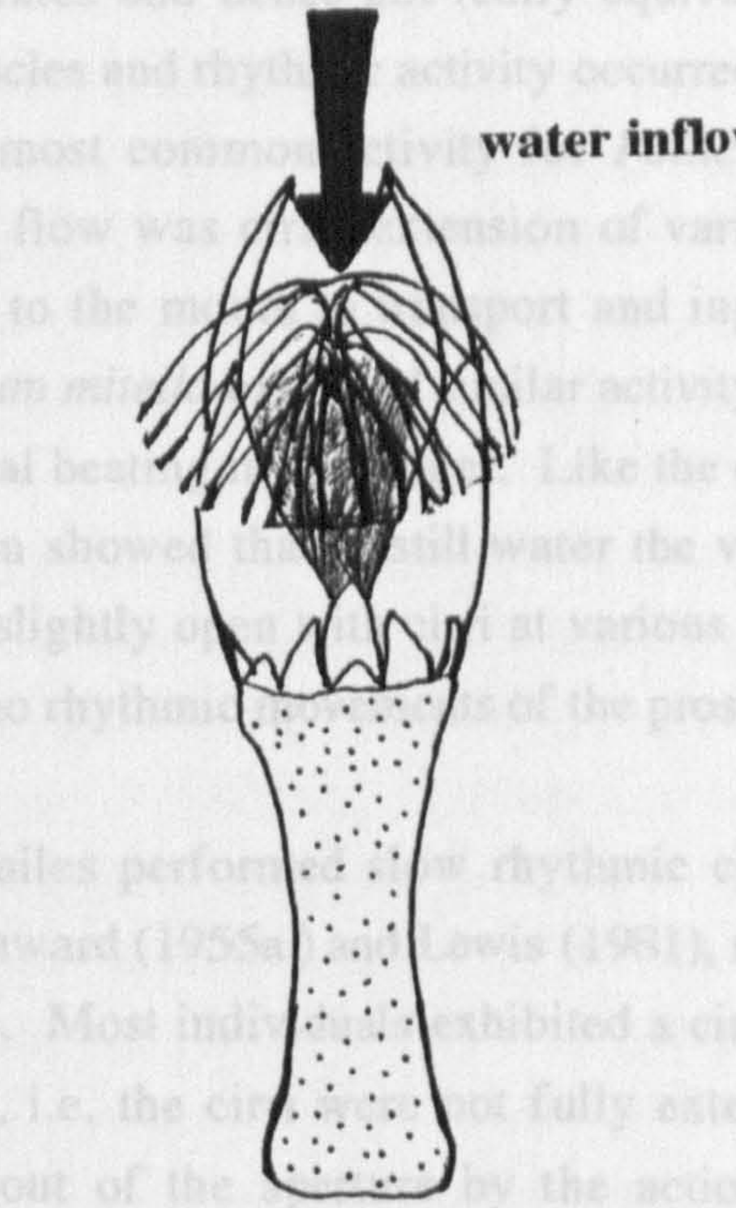
Animal RC (mm)	Flow (cm s^{-1}) 18.5	22.6	30.0	38.2	43.9	47.6
3.3	-	+	+	++	++	++
6.4	+	++	++	++	++	++
6.5	++	++	++	++	++	++
7.45	-	+	+	+	+	+
9.4	++	++	++	++	++	++
11.7	++	++	++	++	++	+
12.55	+	++	++	++	++	++

Water flow through the mantle cavity in association with cirral beating.

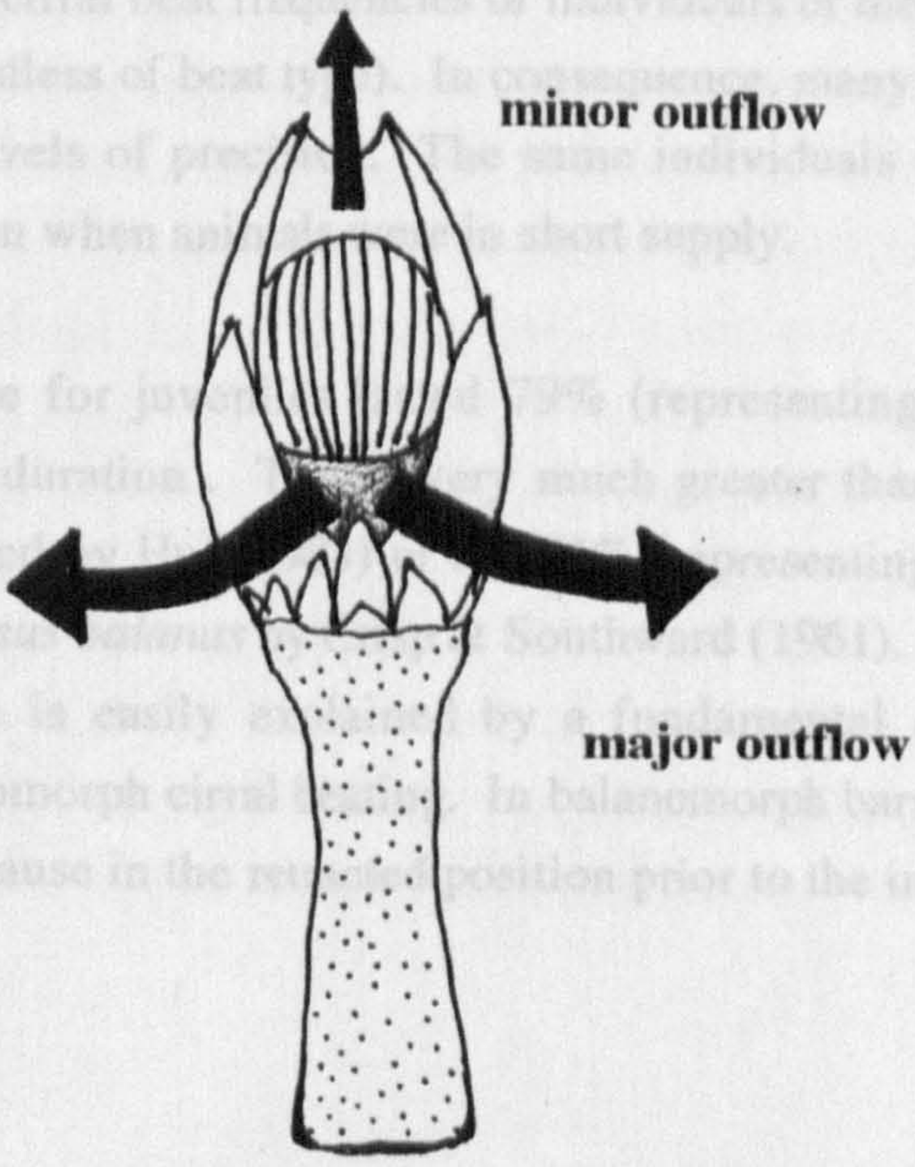
Milk was released above the opercular opening (by the carina) and noticeably drawn into the mantle cavity behind the cirri and prosoma as the cirri were extended (a, Fig 5.7). No milk was seen to issue from the mantle cavity until the cirri were retracted whereupon two plumes of milk streamed from the rostral side of the opercular opening with a residual stream issuing from behind the cirri at the carinal side (b, Fig 5.7). When milk was introduced above the opercular opening on the ventral side none was drawn into the mantle cavity. While the cirri were extended no water movements were discernible around the opercular opening and the milk simply fell, under gravity, down the sides of the peduncle.

Figure 5.7. The patterns of water flow associated with the cirral beating of *Pollicipes pollicipes*.

a) The cirri are extended from the mantle cavity causing an inrushing of water at the top of the capitular opening.



b) The cirri are retracted into the mantle cavity amd there is an expulsion of water. The primary outflow is from the bottom of the capitular opening. However, some water is expelled at the top of the opening.



Discussion

Pollicipes pollicipes showed a restricted range of types of cirral activity in common with most other lepadomorph barnacles: 'testing' - the capitular aperture open but the cirri not extended; 'pumping' - curled up cirri protruded and retracted in a rhythmic manner; very slow 'normal' beat; prolonged cirral extension. All the rhythmic behaviour was at much lower rates and hence not really equivalent to the beating behaviour of balanomorph barnacles and rhythmic activity occurred only in conditions of very low or no flow. The most common activity for *Pollicipes* of all sizes in conditions of moderate to high flow was cirral extension of various types with the incurling of at least one cirrus to the mouth to transport and ingest captured food. Both juvenile and adult *Capitulum mitella* exhibited similar activity when subjected to water flow but exhibited no cirral beating in still water. Like the observations of Hui (1983), the present investigation showed that in still water the valves of *C. mitella* either remained tightly shut or slightly open with cirri at various stages of retraction (generally fully retracted) with no rhythmic movements of the prosoma.

All *Pollicipes pollicipes* juveniles performed slow rhythmic cirral extension and retraction but, as found by Southward (1955a) and Lewis (1981), not all the barnacles 'beat' their cirri at any one time. Most individuals exhibited a cirral activity like the 'beat' described by Hui (1983), i.e. the cirri were not fully extended, but partially uncurled and moving in and out of the aperture by the action of the prosoma. However, some (~20%) did fully extend and retract their cirri in the manner described for *P. polymerus* juveniles by Lewis (1981).

As Hui (1983) studied the behaviour of only three *P. pollicipes* individuals he is likely to have missed the full spectrum of cirral movements. In the present study, there was significant variation between the cirral beat frequencies of individuals of the same size under the same conditions (regardless of beat type). In consequence, many runs were needed to achieve acceptable levels of precision. The same individuals were used under each experimental condition when animals were in short supply.

On average the extension phase for juveniles lasted 79% (representing some 29 seconds) of the total cirral beat duration . This is very much greater than the 51% (representing 9 seconds) measured by Hui (1983) or the 56% (representing 1.1 - 2.8 seconds) measured in adult *Balanus balanus* by Crisp & Southward (1961). The latter percentage and time difference is easily explained by a fundamental difference between balanomorph and lepadomorph cirral beating. In balanomorph barnacles it is a true, rapid beat and there is a pause in the retracted position prior to the initiation of

the next beat but no hesitation in the extended position. In lepadomorph barnacles the beat is characterised by the cirri being held extended and immediately re-extended following retraction (see Anderson & Southward, 1987). However, the extension phase for juvenile *P. polymerus* was found to be 80% (representing 34 seconds on average) of the total beat time (Lewis, 1981) - very similar to the value quoted here for *P. pollicipes* . Why such a low value was measured by Hui (1983) is unclear.

The 'beat' frequencies measured for juvenile *P. pollicipes* by Hui (1983) were 5.5, 2.3 and 2.3 beats per minute, comparable to those measured for *P. polymerus* juveniles (Lewis, 1981), and within the range of ca. 1 - 4.5 beats per minute measured in the current study. Direct comparison with Hui's (1983) results can only be tentative as he used so few animals and gave no indication of the experimental conditions.

Lewis (1981) suggested that the cirral beating behaviour of juvenile *P. polymerus* may be partly respiratory in function having observed average beat rates drop slightly when no food was present and halve from 2.7 to 1.4 b.p.m. (with *Artemia* nauplii) when air was gently bubbled into the water. *P. pollicipes* also showed significant decreases (from 1.6 to 1.1 b.p.m., see Table 5.2) in the presence of aeration. Such gentle aeration would cause no significant increase in the oxygen content of the water over the ten minute experimental period and even if it had, cirral beat rates are not altered greatly by normal fluctuations in oxygen tension of water (Anderson & Southward, 1987) although large increases can depress beat rates slightly (Southward, 1965). It seems more likely that it was the water movement generated by the aeration that was causing the measured decreases in the beat rates. Although aeration may make food more readily available and for this reason depress beat rate, the depression of beat rate in the absence of food tends to suggest that this is not the only reason. There is a clear relationship between the frequency of *P. pollicipes* cirral beat and the rate of flow which can almost certainly be related to gas exchange. In *P. pollicipes* the filamentary appendages (see Fig. 6.1, Chapter 6) are used for respiratory exchange, although Darwin (1851) thought they were not of high functional importance and that most gaseous exchange occurs over the body and mantle surface. In low flow the slow cirral beat causes an exchange of water in the mantle cavity and surrounding the cirri to improve the efficiency of gas exchange. The rates of shear and hence oxygen concentration gradients around the extended cirri increase at higher flow rates, which markedly improves the efficiency of gas exchange at the cirral surface and probably renders cirral beating unnecessary.

In many barnacle species, conditions of water flow dictate the mode of cirral activity. In sufficiently high water flow, prolonged cirral extension is normal, whilst in still

water or low flow the cirri will beat (Crisp & Southward, 1961). Balanomorph barnacles vary their cirral activity depending on the current speed (Southward, 1965). Both *Balanus balanoides* and *Elminius modestus* occasionally use cirral extension when exposed to strong currents (Southward, 1965). In still water most barnacle species stabilise into a consistent pattern of cirral activity consisting of short bursts of beating interspersed with periods of inactivity (Southward, 1965; Anderson & Southward, 1987).

Activity is almost continuous in moving water when the critical flow velocity is exceeded. The critical velocity depends on species, habitat and previous history (Southward, 1965). Southward (1955b) noted that *Chthamalus stellatus* and *B. balanoides* exhibited very different flow-stimulated cirral behaviour even when compared at their optimum temperatures, although both species remained closed in still water. *B. balanoides* and *C. stellatus* initiated rhythmic cirral movement when the flow velocity reached 0.1 cm s^{-1} and 10 cm s^{-1} respectively. At this latter flow rate *B. balanoides* shut the shell valves whereas at even higher flows *C. stellatus* began to favour prolonged cirral extension. In *P. pollicipes*, a critical velocity, when beating ceases and extension takes over, is apparent at flow rates between 8 and 14 cm s^{-1}. *P. pollicipes* were still exhibiting cirral extension at the highest flow rate examined (ca 48 cm s^{-1}).

In balanomorph and lepadomorph barnacles where such a switch in behaviour is seen, the cause may be twofold. Firstly, a high external water flow may be sufficient to drive a respiratory current through the mantle cavity without cirral beating, if indeed it is necessary (the filamentary appendages and mantle surface may only be needed in still water or low flow, with the cirral surface being sufficient for gas exchange at high flows). Secondly, high flow brings food in the water column so there is no need for the barnacle to produce its own feeding current at, presumably, an extra energetic cost.

P. pollicipes and *P. polymerus* are found living on wave battered shores and, although the animals tend to occur in dense stands particularly in crevices and amongst mussels, they will be subjected to high water flow. Thus prolonged cirral extension will predominate. Adult *P. polymerus* maintained in still water will exhibit cirral beating (Barnes & Reese, 1959) although Lewis (1981) did not observe any beating in animals over 14 mm. In still water or low flow in the laboratory 79% of *P. pollicipes* of all sizes, up to 15 mm (the largest available) exhibit rhythmic cirral extensions of some description. However, such hydrodynamic conditions would rarely be experienced by adults in the wild, except, perhaps, by animals in tide pools.

On the shore, it is possible that juvenile *P. pollicipes* and *P. polymerus* are subjected to slightly lower levels of water flow than the adults, living as they do on or around the stalks of the adults, within the boundary layer, in less turbulent (lower Re) flow. If so, it might have been expected that there would be differences between the beating behaviour of adults and juveniles when exposed to varying degrees of water movement. However, both showed cirral beating in still water and low flow conditions and lower cirral extension frequency and prolonged cirral extension at higher rates of flow. If the levels of water movement generated by gentle aeration were high enough to decrease significantly cirral beat rate and a water flow of just 0.14 ms^{-1} was sufficient to eliminate beating of juveniles in the laboratory, it is unlikely that the very high flow rates in surge channels on rocky coastlines do not, when these are usually in excess of 10 ms^{-1} (Jones & Demetropoulos, 1968).

In balanomorph barnacles, cirral beating is for captorial feeding and also generates a respiratory current through the mantle cavity from which small particulate material may be filtered by the maxillipede setae (Southward, 1955a). The presence or absence of food has an important effect on the cirral activity of acorn barnacles. Suitable food stimulates a significant proportion of the animals to beat with a fully extended cirral net. The absence of particles, or presence of inedible particles, reduces the number of animals showing beating activity and increases the number exhibiting pumping beat (Crisp, 1964).

In lepadomorph barnacles slower cirral beating, usually observed only in still or slow moving water, is thought to be primarily respiratory in function (Barnes & Reese, 1959; Anderson & Southward, 1987). However, Lewis (1981) concluded that cirral beating in *P. polymerus* juveniles is a feeding adaptation suitable for the more sheltered microhabitat of the juveniles and that the respiratory function is secondary. Her evidence to support this suggestion was four-fold: She found only juveniles performing cirral beating activity to any extent, none was observed in animals over 14 mm. Beating rate was inversely related to animal size. If juveniles are subject to lower current speeds in the wild than the adults they may be at a competitive disadvantage if they eat the same things and feed in the same manner as the adults. Lewis found that juveniles in the wild had a larger proportion of small particulate material in their guts than the larger animals, suggesting that juveniles utilised a different feeding mechanism. The presence of *Artemia* nauplii (prey) resulted in increased beat rates lending further support to the suggestion that cirral beating is the feeding mechanism the juveniles employ.

The fact that the small juveniles have a larger proportion of microparticulate organic material in their guts than the adults does not necessarily indicate that they are reliant on another feeding mechanism. Small animals are likely to be more efficient than adults at capturing small particles (having a smaller setal mesh size, Chapter 4), but the reduced rate at which they are likely to ingest larger prey may be sufficient to account for the different gut contents without hypothesising different feeding mechanisms.

P. pollicipes adults can detect the physical presence of food organisms touching the cirral net and can respond by curling the cirri or withdrawing the cirral net in response to chemicals (mostly amino acids) likely to be associated with food organisms (Hicks, 1993). However, the current study indicates that the presence of either micro- or macro-food particles had no significant effect on the rate of rhythmic cirral behaviour of either adult or juvenile *P. pollicipes* even when hungry. Most juveniles exhibited a cirral beat of type II (Fig. 5.1) where the cirral net was not fully extended into a feeding position during a beat. The function of such 'beating' is reminiscent of the 'pumping' beat in balanomorphs and is primarily to generate a water current through the mantle cavity probably for respiratory purposes. The flow of water generated by the cirral beat was clearly demonstrated in *P. pollicipes* (see Fig. 5.7) and did not vary in character between the two types of 'beat'.

However, two hours emersion did not seem to have a significant effect on 'beat' rate whether food was present or not. The continuously immersed animals had slightly higher average 'beat' rate (1.3 compared to 1.0 b.p.m.) but the variability was very high. If 'beating' behaviour is respiratory in function and assuming that emersion builds up a respiratory stress, then an increase in beat rate on immersion could have been expected. Lewis (1981) found a much reduced percentage of *P. polymerus* juvenile 'beating' after 24 hours of emersion compared to those continuously immersed. The degree of aerial respiration shown by *Pollicipes* is not known and other energetic stresses may be evident on aerial exposure, hence it is difficult to draw a firm conclusion from these two negative results. Both show a consistent reduction in activity immediately after emersion but insufficient data on the physiological effect of emersion limit any conclusions.

The differences between the beating behaviour of *Pollicipes pollicipes* and *Capitulum mitella* observed by Hui (1983) were supported by current observations and may, as he suggested, be related to the differences in the habitats occupied by the juveniles of the two species. Hui (1983) noted that *Capitulum* juveniles have elongated peduncles so would be subjected to higher levels of flow than *Pollicipes* juveniles (assuming that

the natural flow regimes are similar). They may therefore never need to use cirral beating for respiratory ventilation as, like the adults of *P. pollicipes*, *P. polymerus* and *C. mitella*, they can rely on strong externally generated currents.

Most *P. pollicipes* juveniles exhibited rhythmic cirral extension and retraction that was largely unaffected by temperature, whilst for a few animals, 'beat' rate was strongly and positively correlated with temperature. Temperature dependent cirral beat rates have also been documented in *B. amphitrite*, *B. eburneus*, three species of *Chthamalus*, *B. balanoides* (Newell & Northcroft, 1965; Crisp & Ritz, 1967), *Elminius modestus* (Ritz & Foster, 1968) and *P. polymerus* juveniles (Lewis, 1981). Although there was high variability in the beat rate between individuals of the same size, the rate for an individual barnacle at a given temperature was constant, as it was for *Balanus* (Cole, 1929; 1932). The Q_{10} for *P. pollicipes* juveniles whose beat was affected by temperature was 5.1, very similar to the value of 4.6 calculated for juvenile *P. polymerus* by Lewis (1981). However, as Lewis (1981) plots only mean beat rates it is unclear whether there are two types of behaviour or not. The temperature range used in both studies is similar to that experienced by animals in the wild. Extremes of temperature have been shown to depress beat rates (Southward, 1955a; 1957; Anderson & Southward, 1987).

At a constant temperature, the frequency of cirral beat in *P. pollicipes* was inversely related to animal size, broadly in agreement with the findings of Lewis (1981) for *P. polymerus*. The most notable difference between these two studies is the slope of the relationship; that of *P. polymerus* is about twice that measured for *P. pollicipes*. Lewis (1981) observed no cirral beating in animals >14 mm RC and noted that the beating in *P. polymerus* animals over 10 mm was intermittent. In the present study cirral beating of the larger animals was rhythmic and continuous but at a lower rate than that seen in the smaller animals. Cirral beating was exhibited by all smaller animals but not all adults. Decreasing frequency of beating with increasing size was thought by Anderson & Southward (1987) probably, to represent a hydrodynamic effect, but with younger and therefore smaller specimens probably also having a higher relative metabolic rate (Newell & Northcroft, 1965). The higher relative metabolic rates of small animals are driven by the ease of gas exchange permitted by the larger surface area to volume ratio.

In summary, the slow cirral extension and retraction of juvenile *P. pollicipes* observed by Hui (1983) was observed and also documented in adults. However, this behaviour only occurred in conditions of no or very low flow (<1.4 cm s^{-1}) and at flows >8 cm s^{-1}

'beat' rates were very low. Such conditions are thought to be rarely encountered by this species in the wild. All the evidence suggests that the rhythmic cirral activity has a respiratory function with the clear relationship between cirral 'beat' rate and flow attributable to the rate of gas exchange. In fast flow, there is a high rate of shear around the barnacle cirri with steep oxygen gradients and efficient gas exchange over the cirral surface. In the absence of flow, there will be less of an oxygen gradient around extended cirri and very inefficient gas exchange. Pumping the cirri in and out of the mantle cavity generates a throughflow of water (demonstrated using milk, see Fig. 5.7) which will permit gas exchange to occur over the cirri, prosoma and mantle surface. There was no evidence that the cirral beating shown by *P. pollicipes* was for feeding hence no support for the hypothesis of Lewis (1981) and Hui (1983) that there is a juvenile to adult switch in feeding strategies, from cirral beating to cirral extension, in *Pollicipes*.

The present study has found that *P. pollicipes* juveniles are able to capture and ingest larger food just as the adults are capable of ingesting diatoms (Chapters 6 & 7). The higher proportion of small organic material in the guts of small animals may be explained if they catch fewer large prey per unit time than a large barnacle. Small barnacles would have to use the whole cirral net not one ramus or cirrus and have a smaller cirral net, catching less food per unit time. Large adult *P. polymerus* have microparticulate material in their guts, but show no rhythmic cirral extension, hence this behaviour is not essential for small particle capture.

Chapter 6

The gut contents and faecal production of *Pollicipes pollicipes*

Introduction

The faeces of several barnacle species have been described previously; those of *Balanus balanoides* by Stubbings (1975), *B. tintinnabulum* by Edge (1934), *B. eburneus* and *B. improvisus* by Kraeuter and Haven (1970) and *B. amaryllis* by Arakawa (1971). In all these species the faecal pellets are rod-shaped with smooth sides and no longitudinal grooves. The faeces of *Balanus balanoides* have been described as being frequently curved or twisted with the irregular packing of the gut contents often resulting in constrictions along the length of the pellet. The pellet ends are tapered and the final portion is often long and translucent - a mucilage sheath containing no food but large numbers of enterobacteria (Stubbings, 1975). The faecal pellets of *B. balanoides* were measured by Rainbow (1975) and found to be about 1.2 mm in length (one third of the length of the midgut). *B. balanoides* fed on diatoms were found to produce faecal pellets 2 hours after feeding and then at 20 minute intervals.

The gut contents of *Pollicipes polymerus* were analysed by Barnes (1959) who found that copepods, algae and unidentifiable particulate material were always present, cirripede moults, amphipods, cyprids, small clams and hydroids occurred frequently, while polychaetes and barnacle nauplii occurred only occasionally. Very few sand grains or diatoms were found. The gut contents of *Lepas* were found to be very similar. Generally, the stomach contents of barnacles reflect the material available in the surrounding water, as the capture method is largely non-selective for bigger particles. The size range taken by cirral activity must be largely related to the mesh size of the filtering surface that the cirral net presents to the water.

A relatively unselective filter feeder such as *P. polymerus* living on an exposed shore might have been expected to have more sand in its gut than was found by Barnes (1959). He showed that in the absence of a rhythmic cirral beat the cirri are individually retracted in response to tactile stimuli and that this reaction was not readily provoked by inorganic material, hence there was little grit or sand in the gut.

An analysis of the gut contents was made in order to ascertain what these animals eat in the wild and whether there are any apparent differences in food preference associated with barnacle size. Lewis (1981) studying the gut contents of *P. polymerus* considered the gut contents of adult and juvenile *P. polymerus* to be evidence supporting her theory of a juvenile to adult shift in feeding strategy and Hui (1983) felt that *P. pollicipes* exhibited similar behaviour. Any differences in gut contents at different locations and associated with different shore levels was also investigated.

Materials and Methods

Faecal examination

Groups of *Pollicipes pollicipes* of known size were kept in isolated tanks for ease of faecal collection. Animals were attached to slate panels with Velcro and Superglue, placed in 10 litre tanks of UVFSW (10-20°C), kept in the laboratory and were fed to excess on monospecific diets (*Rhinomonas reticulata, Skeletonema costatum, Brachionus plicatilis* and *Artemia* sp.). The faecal production of each group was collected every day and examined under a binocular microscope. This was repeated for a number of days. The appearance of the faeces was described and the length and width of pellets measured using an eyepiece graticule. Taking the shape of the faecal pellets to be approximately cylindrical, the volume of faecal material produced per animal per day was estimated for animals of a given size feeding on each diet.

Twenty faecal pellets were collected from *P. pollicipes* of different of sizes which had been feeding on *Artemia*. The length and widths of the faecal pellets were measured along with rostro-carinal length (RC) of the animals producing them.

Five large *P. pollicipes* (~10.57 mm RC) and 5 small (~4.27 mm RC) were starved for 24 hours to ensure that their guts were empty. They were then placed in UVFSW in a dish on a magnetic stirrer generating a moderate water flow around the dish. Recently hatched (within 24 hours) *Artemia* nauplii were added to the water in a high density (> 20 ml^{-1}) and the *P. pollicipes* were left to feed at room temperature. Regular observations were made and the time of first and subsequent faecal production noted for animals of both sizes.

Estimation of gut evacuation time.

An attempt was made to film defaecation using time lapse video-recording. A 4.5 mm RC animal was placed in a large glass dish containing 450 ml of UVFSW with a

magnetic stirrer but this caused electrical interference with the camera so its effect was replaced with vigorous aeration. Fifty ml of a *Rhinomonas reticulata* suspension (~1000 cells μl^{-1}) was added and the animal left to feed for 6 hours after which it was transferred to a dish of clean UVFSW. The animal was illuminated with a red light (barnacles are insensitive to red light) and filmed (1:40 time lapse) for 30 hours. Despite the presence of faeces in the water, the expulsion of faeces was not recorded. Presumably it happened too quickly for the time lapse recording.

Five animals (4.65 mm RC ± 0.11 SD) were glued to small pieces of slate and placed in a 1 l. glass dish with a dense suspension of *Artemia* (~20 ml^{-1}) and *Rhinomonas* (~100 cells μl^{-1}). The dish was placed on top of an air-powered magnetic stirrer. The water was also aerated and the dish was covered with a sheet of perspex to reduce evaporation. The animals were orientated with rostrum facing into the current and left to feed for 24 hours. The slates bearing the animals were transferred to a 500 ml glass dish of UVFSW (preheated to room temperature, 21°C). This was placed on a magnetic stirrer and left for 1 hour. The water was checked for faecal pellets and the animals were transferred to clean UVFSW and left for a further hour. The water was then checked for the presence of faeces. The procedure was repeated until 3 consecutive hours yielded no faeces and the gut was assumed to be empty.

Measurement of midgut lengths

The length of mid-gut of dissected barnacles of various sizes was measured to the nearest 0.05 mm, using vernier calipers.

Gut contents

Pollicipes pollicipes were collected in June 1994 from the shore at two sites in south-west Portugal, Site 1, Praia do Castelejo and Site 3 near Sagres (see Fig.3.1). At Castelejo, animals were removed from a deep crevice in rocks lying off the beach (see Fig. 3.5). At Sagres animals were obtained from the large horizontal crevice pictured in Fig. 3.6. At both sites three samples were collected, one from the top, middle and bottom of the animal's shore distribution. A wide range of sizes was collected in each sample. At Castelejo, all samples were taken from a vertical rock face whilst at Sagres the top sample was taken from a vertical rock face above the crevice, the middle one from the horizontal surfaces within the crevice and the bottom one from an open rock surface, gently sloping down to sand.

The animals (360 in all) were eased from the rock surface as gently as possible using the back of a penknife blade. They were placed in labelled bottles containing 50%

alcohol. Examination of the gut contents was carried out back in Wales. Several animals were. however, dissected on the shore and the gut contents examined using a ×20 hand lens.

The rostro-carinal length of every animal was measured using vernier calipers. The capitulum was shucked using a knife. The body was removed from the capitulum, the presence or absence of egg masses was noted, and the percentages of animals of each size that were found to have egg masses are given in Table 6.1. It can be seen that the percentage of animals of a given size containing egg masses depends on the shore position. Samples from higher on the shore were found to have a higher proportion of animals of each size containing egg masses and the animals contained egg masses at a smaller size than lower on the shore. Sixty-seven percent of the animals over 9 mm RC had egg masses and are therefore sexually mature adults. Only 16% of the animals less than 9 mm showed evidence of maturity hence 9 mm was selected as a cut off point between large juveniles and adults. The animals collected were therefore subdivided into 3 size categories; <6 mm RC, 6 - 9 RC, and >9 mm RC, equivalent to small juveniles, larger juveniles and sexually mature adults. Sexual maturity was also found to occur at a size of 9 mm by Cardoso & Yule (1995).

The outside of the body was thoroughly rinsed to remove any adherent material. The gut was sliced open with a scalpel (see Fig. 6.1). The contents of the gut were removed by Pasteur pipette and placed in a labelled 1.5 ml Eppendorf microtest tube with 1 ml of 0.5% formalin solution.

The gut contents were examined on a modified Fuchs-Rosenthal haemocytometer. Wherever possible, the material was identified and the approximate percentage of counted particles falling into each of three categories was estimated. The three categories (after Lewis, 1981) were;

1) Particulate organic material $\leq 10\ \mu m$ in diameter
2) Large organic material $> 10\ \mu m$
3) Particulate inorganic material $< 10\ \mu m$

Figure 6.1 Diagram of the lateral view of *P. pollicipes* indicating the position of the gut and the extent of the incision made to remove the gut contents.

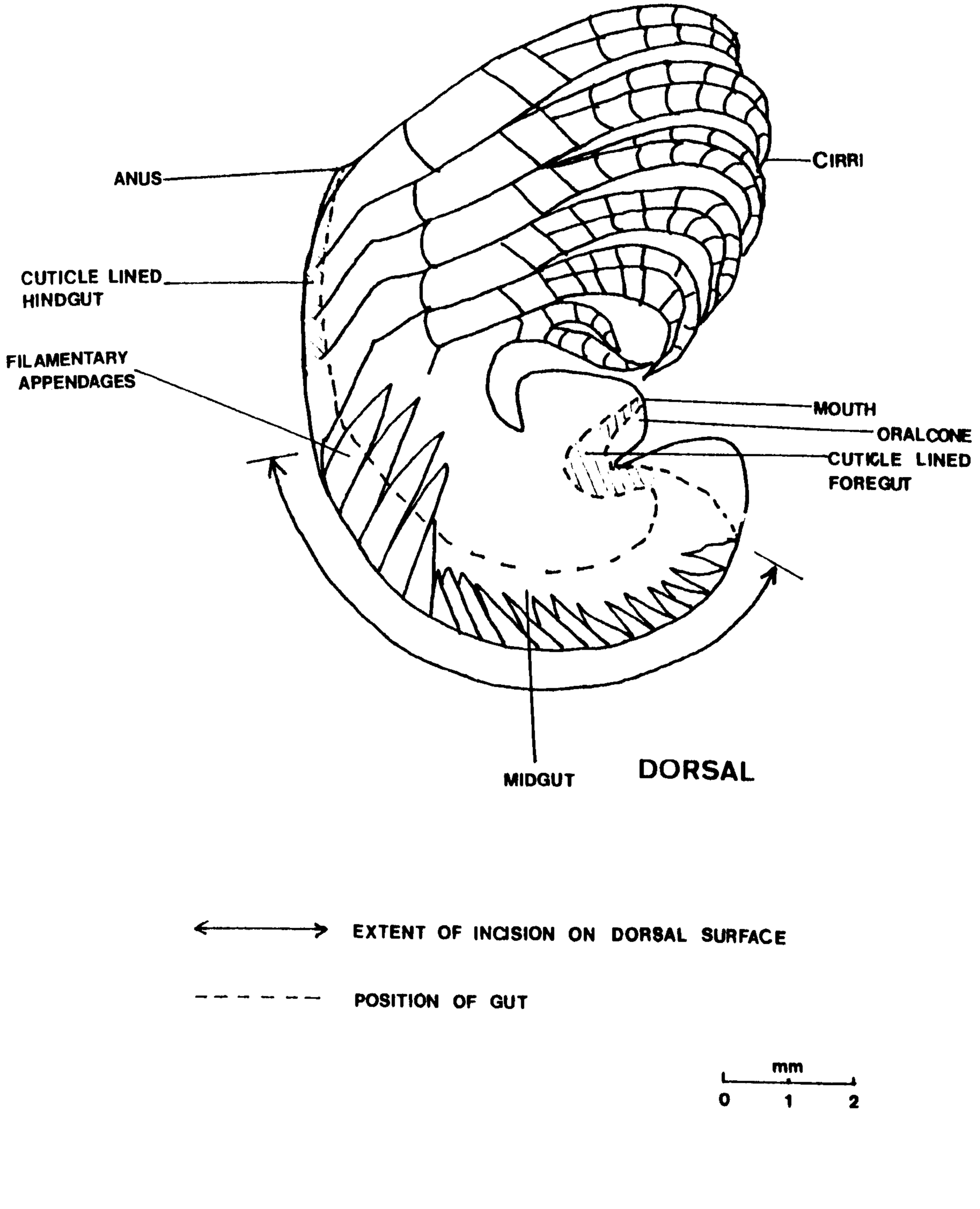


Table 6.1 Percentage of animals from each size category at each site and shore position found to contain egg masses. N= number of animals examined.

Size	Bottom N	Bottom %	Middle N	Middle %	Top N	Top %
Castelejo						
6-6.95	10	0	12	0	4	25
7-7.95	17	0	7	14	10	30
8-8.95	22	9	13	15	12	50
9-9.95	14	50	8	38	13	92
10-10.95	10	50	5	80	8	88
11-11.95	6	67	1	100	1	100
12 & over	3	67	3	100	1	100
Sagres						
7-7.95	7	0	5	0	2	0
8-8.95	7	0	5	20	7	29
9-9.95	8	38	4	100	4	50
10-10.95	7	14	2	100	3	67
11-11.95	0	-	3	33	2	0
12 & over	6	67	6	83	2	50

The gut contents of each barnacle were fully scanned and the presence or absence of identifiable animal and plant food was noted. The results were expressed as the percentage of animals in each size category with each food item in their gut.

The size of the larger particles in the gut (those classified as large organic) were measured for 5 juveniles and 5 adult *Pollicipes* in order to test for differences in macerative capacity between the larger and smaller barnacles.

Results

Description of the faeces of *Pollicipes pollicipes* feeding on zooplankton and micro-algal diets.

Animals fed on *Artemia* sp. produced orange, pink or red faecal pellets. These were rod shaped, roughly cylindrical but with slightly tapering ends (Fig. 6.2a). All the pellets were smooth-sided, occasionally twisted or curved and did not have any obvious constrictions along their length. Most pellets were bound by a membrane which was sometimes so thick as to obscure the contents. Occasionally, pellets were produced that had very long membrane-bound sections devoid of faecal material (Fig. 6.2b). Some pellets were found to be clearly divided into two regions, the majority filled by a dense, apparently homogeneous mass while, at one end, there was a high concentration of *Artemia* cysts (Fig. 6.2d & f).

Pollicipes pollicipes fed on the rotifer *Brachionus plicatilis* produced pale yellow to mid-brown faeces of a similar shape to those produced by animals feeding on *Artemia*. Some faeces were twisted (Fig. 6.2c) but most were straight sided and rod-like. Most pellets had densely compacted contents although some were more loosely packed (Fig. 6.2e). Most pellets, which were not obviously broken, had gently tapering ends.

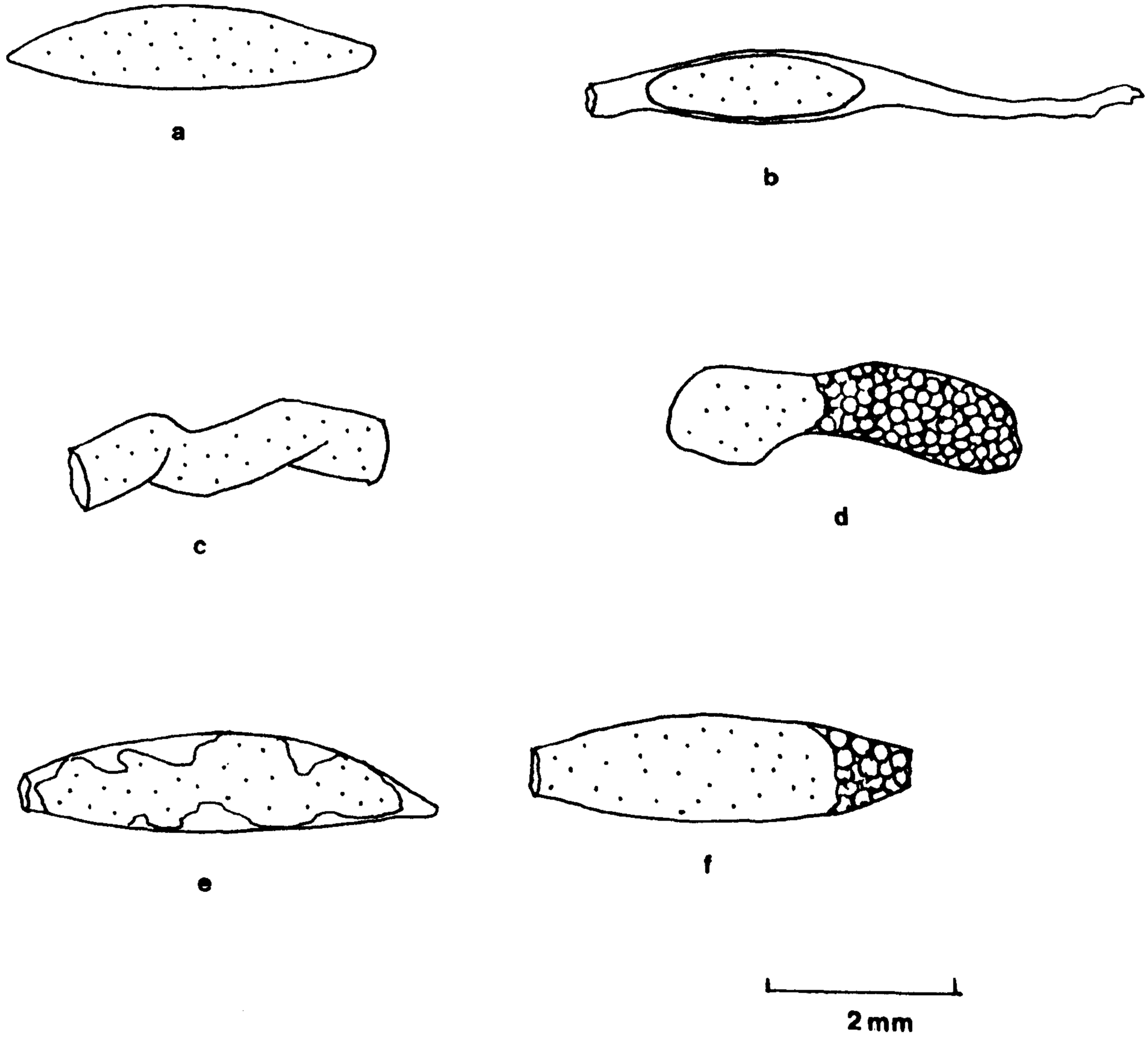
Fewer faecal pellets were produced by animals feeding on micro-algae (*Skeletonema costatum*) - faeces being produced only every six days or so. The faeces were dark chocolate brown in colour. Again they were rod-shaped and smooth sided, rarely curved or twisted and sometimes seen with very loosely packed contents. Irregularities in the contents often formed constrictions along the length of the pellet. The faeces produced by barnacles feeding on *Rhinomonas reticulata* were very pale brown-pink in colour. Table 6.2 gives the dimensions of faeces produced by animals feeding on diets of *Artemia*, *B. plicatilis* and *S. costatum*.

Table 6.2: The dimensions of unbroken faecal pellets of juvenile (5 - 7 mm RC) and adult (> 9 mm) *Pollicipes pollicipes* feeding on different diets. Mean pellet length, range, mean pellet diameter and range are given. All measurements are in mm. N= the number of faecal pellets measured.

Diet		N	Mean length(mm)	Length range	Mean diameter (mm)	Diameter Range
Artemia	Juvenile	12	1.55	1.15-2.60	0.36	0.31-0.46
Artemia	Adult	24	2.46	1.23-3.46	0.61	0.46-0.69
Brachionus	Juvenile	57	1.26	0.62-1.85	0.32	0.15-0.62
Brachionus	Adult	38	1.86	0.77-3.54	0.43	0.23-0.77
Skeletonema	Adult	18	2.00	0.71-3.23	0.47	0.31-0.77

Some of the barnacles examined on the shore in Portugal had formed faecal pellets in their guts, which were awaiting evacuation. These were dark brownish-green in colour.

Figure 6.2. Drawings of the types of faecal pellets produced by *Pollicipes pollicipes* .



a) General pellet form. Pellet is cigar shaped with tapering ends and may or may not have a membranous sheath.
b) Small pellet with length of empty membrane.
c) Twisted pellet
d) & f) Pellets of animals fed on *Artemia*. Digested *Artemia* remains fill most of the pellet but there is a concentration of *Artemia* cysts at one end.
e) Loosely packed contents in pellet of animal fed on algae.
g) Pellet with irregularities in the contents causing constrictions along its length.

There are curvilinear relationships between faecal pellet length and RC length (means shown in Fig. 6.3, simple linear regression on all log-transformed data, r = 0.752, 587 df, P < 0.001) and between faecal width and RC length (means plotted in Fig. 6.4, regression performed on all log-transformed data, r = 0.859, 587 df, P < 0.001). The faecal pellet lengths of *P. pollicipes* individuals are more variable than pellet width or volume. There is also a curvilinear relationship between faecal pellet volume and RC length (means plotted in Fig. 6.5, linear regression performed on all log-transformed data, r = 0.880, 587 df, P < 0.001).

The production of faeces by *Pollicipes pollicipes*.

Animals ~10.6 mm RC feeding on *Artemia* nauplii under conditions of flow conducive to feeding produced their first faeces 4 hours after the commencement of feeding while those of ~4.3mm RC produced faeces after 4 hours and 39 minutes of feeding. The larger animals produced faeces at a mean rate of 0.5 per animal per hour and the smaller animals at a rate of 0.25 per animal per hour (calculated from the total number of faeces produced by all the animals divided by the number of animals and the number of hours over which the faeces were produced).

Table 6.3 shows the estimated faecal volume produced per animal per day. Not surprisingly larger animals produce larger volumes of faecal material. Animals greater than 9 mm RC, feeding on *Brachionus plicatilis* produced five times, and on *Skeletonema costatum* nineteen times, less faecal material (approximate volumes) than those feeding on *Artemia* nauplii. However, juvenile *Pollicipes* feeding on *Brachionus* produce 74% of the volume of faeces produced by those animals feeding on *Artemia* .

Table 6.3 The mean faecal volume of juvenile (5 - 7 mm RC) and adult (> 9 mm) *Pollicipes pollicipes* feeding on *Artemia* nauplii, *Brachionus plicatilis* and *Skeletonema costatum*. The number of animals used, number of pellets collected and measured, the number of days over which collection was undertaken and the volume of faecal material produced are given.

Diet	Barnacle size	N Animals	N Pellets	mean faecal vol. (mm^3)	N Days	Production (mm^3/an./day)
Artemia	Juvenile	4	5	0.160	2	0.100
Artemia	Adult	7	17	0.720	2	0.870
Brachionus	Juvenile	39	57	0.100	2	0.074
Brachionus	Adult	32	38	0.267	2	0.159
Skeletonema	Adult	8	18	0.340	17	0.045

Figure 6.3. Plot of log mean faecal pellet length (means from ~20 measurements ± SE) against log rostro-carinal length for *P. pollicipes*. Line fitted to whole data set by least squares linear regression.

log (pellet length) = -0.339 + 0.638 log (rostro-carinal length)
r = 0.752 587 df P < 0.001

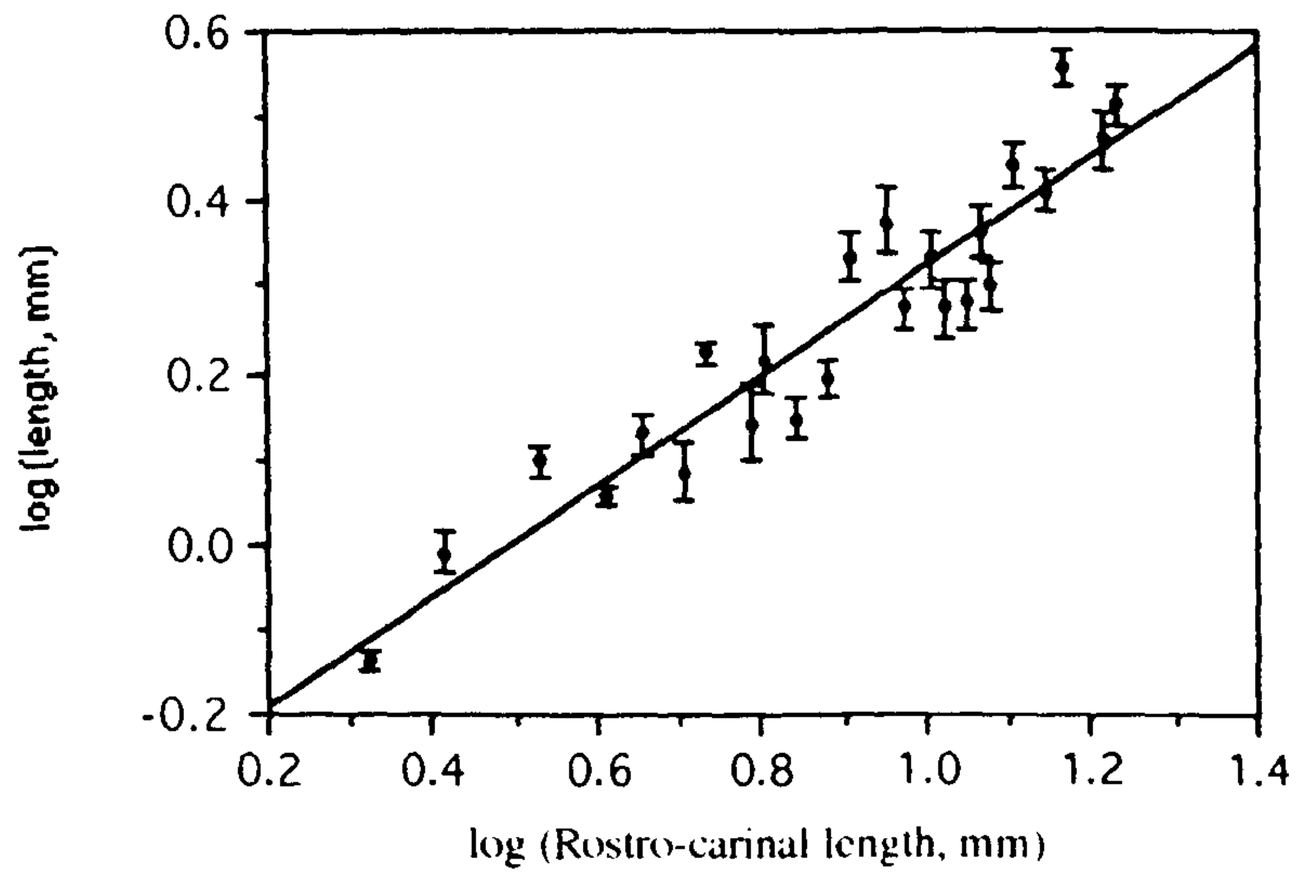


Figure 6.4. Plot of log faecal pellet width (means ~20 measurements ± SE) against log rostro-carinal length for *P. pollicipes*. Line fitted to whole data set by least squares regression.

log (pellet width) = -0.981 + 0.747 log(rostro-carinal length)
r = 0.859 587 df P < 0.001

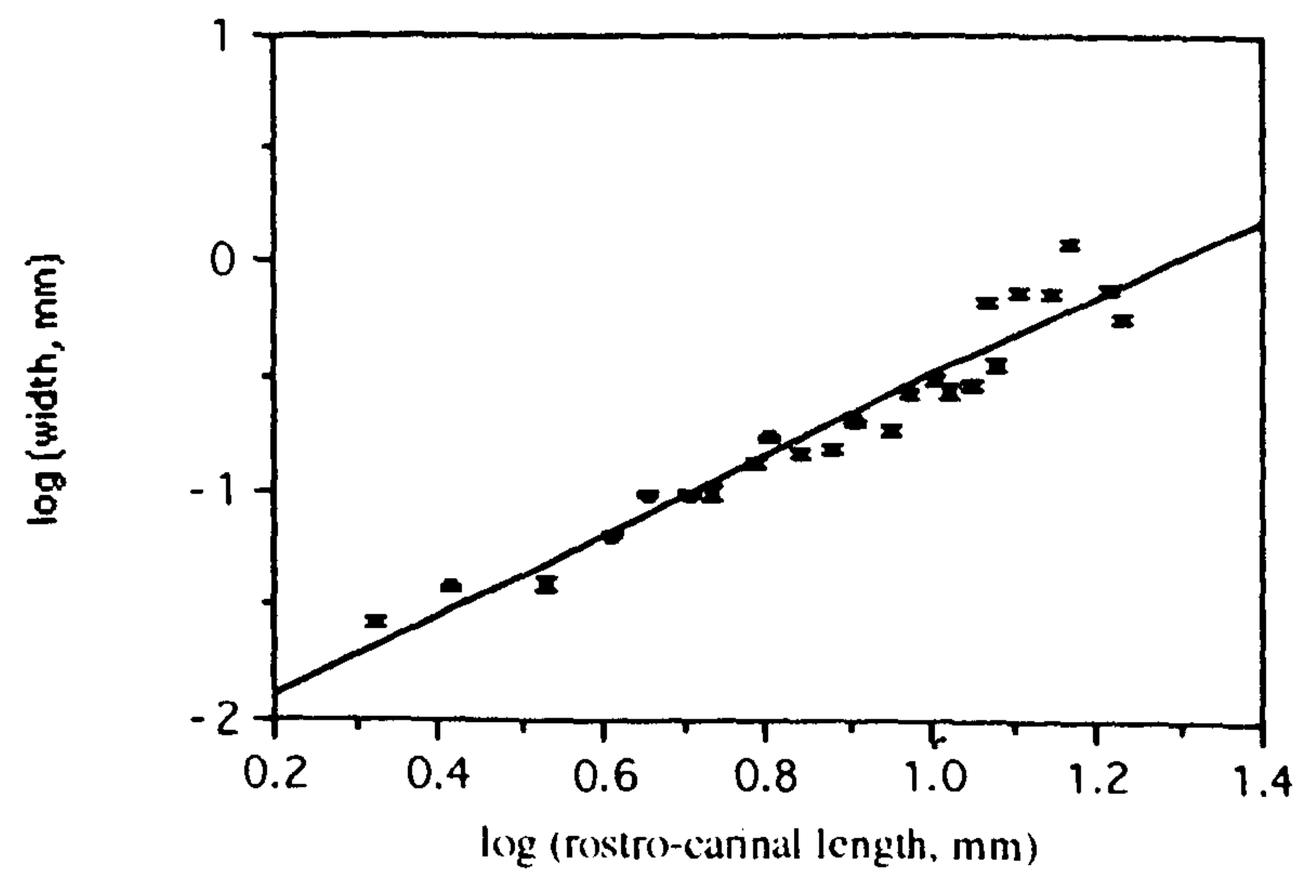


Figure 6.5. Log mean faecal pellet volume (~20 measurements ± SE) against log rostro-carinal length. for *P. pollicipes* Line fitted to full data set by least squares regression.

log (pellet volume) = -2.406 + 2.132 log (Rostro-carinal length)
r = 0.88 587 df P < 0.001

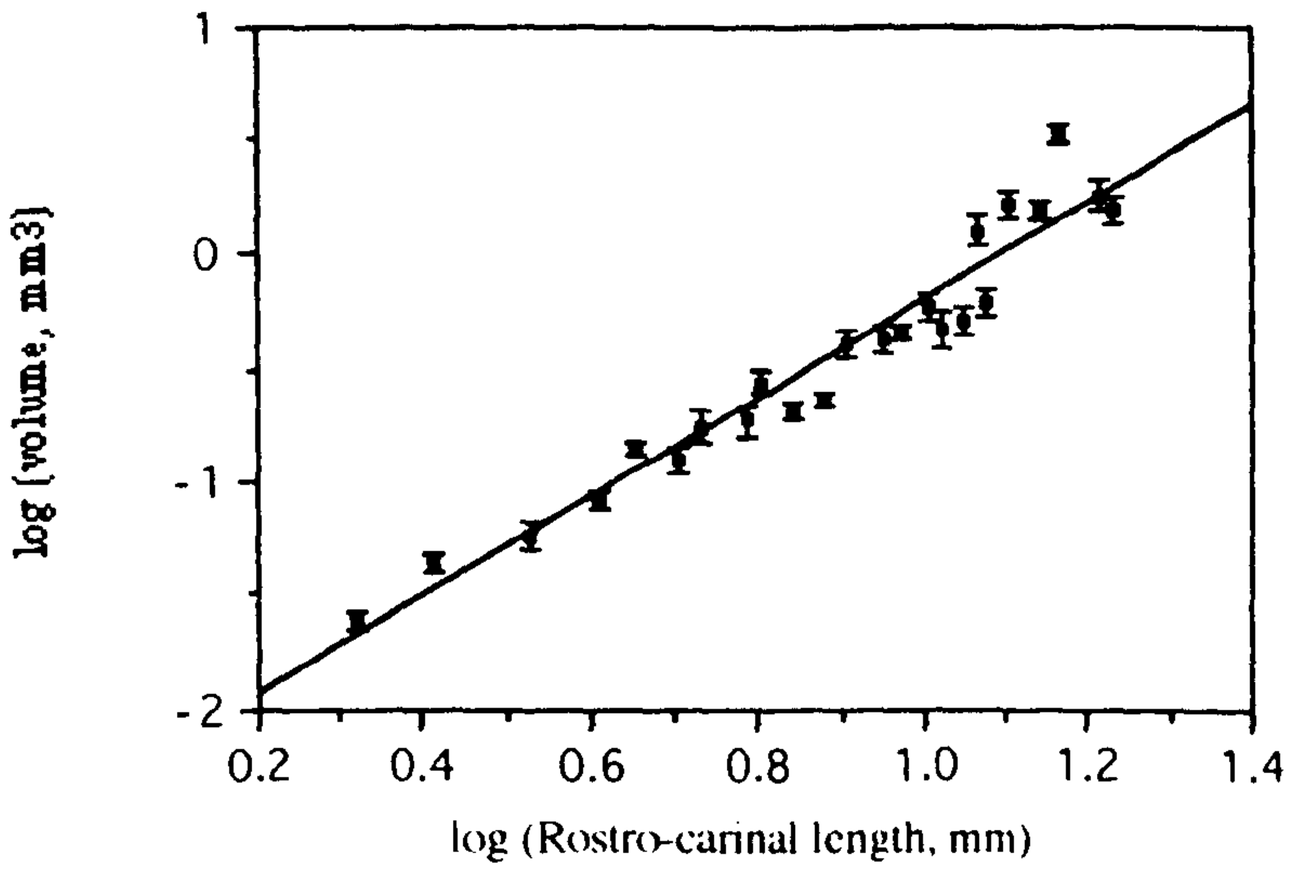
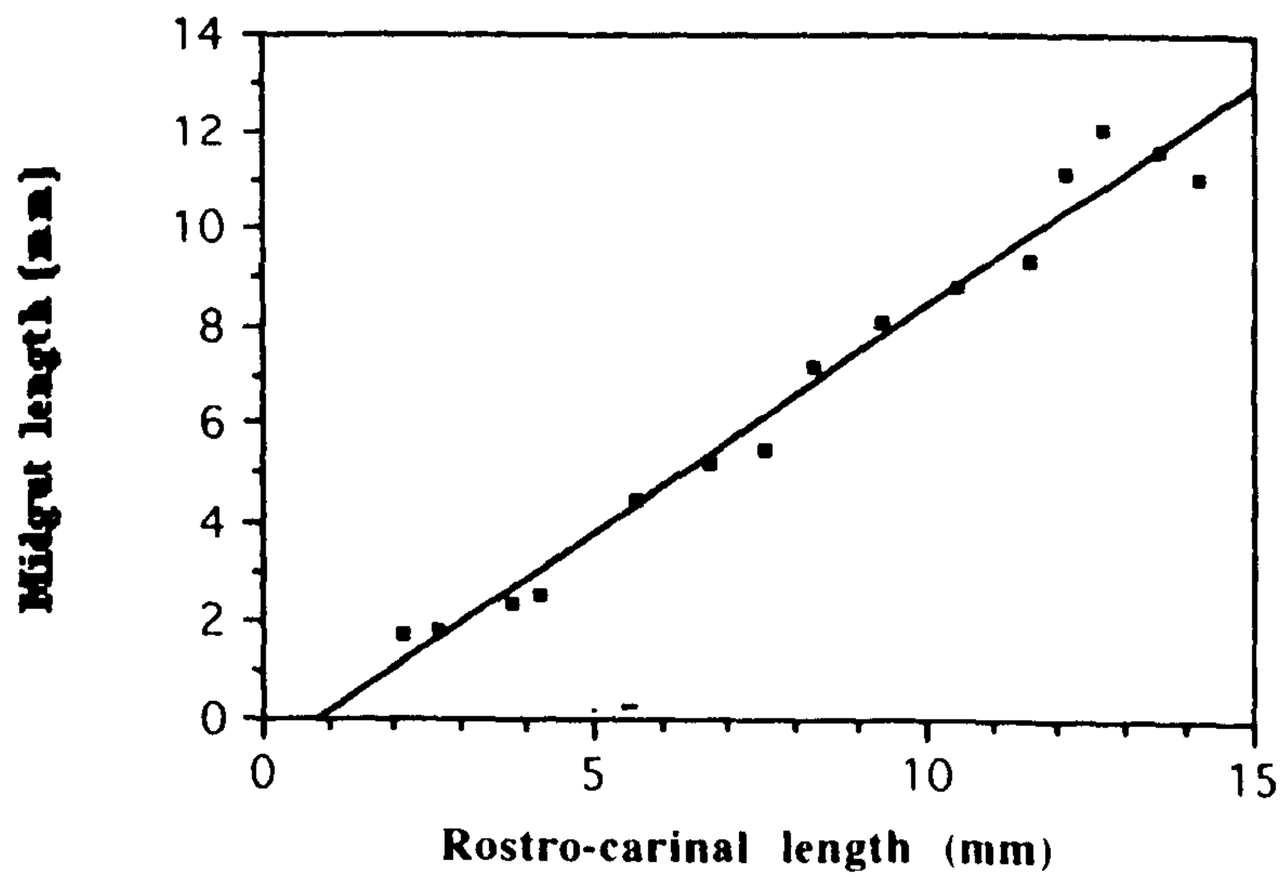


Figure 6.6. Midgut lengths of *Pollicipes pollicipes* of different rostro-carinal lengths. Line fitted by least squares regression

midgut length = -0.7594 + 0.9162 (Rostro-carinal length)
r = 0.986 13 df P < 0.001



There is a strong linear correlation ($r = 0.987$, 13 df, $P<0.001$) between gut length and RC length (Fig 6.6). The gut evacuation time of 11 hours was estimated for 4.5 mm RC *Pollicipes pollicipes*.

The gut contents of *Pollicipes pollicipes* in the wild.

The gut contents of the animals examined on the shore consisted of a thick, dark green-brown fluid. Once back at the lab the fluid in most animals was much paler, less dense but still green-brown. In a few individuals the guts looked pale pink or yellow. However, once they were opened up it appeared that this was caused by pigmentation of the gut wall rather than to the colour of the contents.

Precise identification and counts of food material were difficult to obtain. Diatoms, other unicellular phytoplankton and large algae were common, as were crustaceans and their remains, cirripede larvae and moults. Sand grains and shell fragments (10 - 250 μm) were fairly common. Blue-green algae, eggs and molluscs were less common. Echinoderm larvae and shrimps were only found in a few animals. There were large amounts of unrecognisable organic debris in all the guts examined. Annelids, ostracods, isopods, amphipods and even a fish larva were found. In some animals, cirripede larvae constituted the bulk of the gut contents. The cyprids found were 300 - 500 μm in length and 150 - 250 μm wide. Nauplii were 250 μm wide and 250 μm long (including caudal process).

In general, animals from the high shore had paler, more homogeneous, watery gut contents with less identifiable material than the lower shore animals. Smaller animals appeared to have more thoroughly digested gut contents than the larger animals. The high shore animals at Sagres had more sand and shell particles in the mantle cavity and adhering to the outside of the body than those from the lower shore or Praia do Castelejo.

Table 6.4 shows the gross composition of the gut contents of *P. pollicipes* from two sites in south-west Portugal and the relationship with tidal height and animal size. There are very few apparent differences between the two collection sites and between the shore heights. The most obvious differences in the gut contents are, at both sites, related to animal size. In all but Castelejo low shore animals there is a decrease in the percentage of particulate organic material in the gut with increasing animal size. The mean percentage of small organic material in the gut of all animals, regardless of site or shore position, is: 80.5% in animals less than 6 mm, 64.5% in 6 - 9 mm animals and 55.6% in animals over 9 mm.

At both sites the smallest animals have less inorganic particles in their guts than the larger animals (a mean of 5.3% compared to 7.4% in 6 - 9 mm animals and 8.8% in the largest animals). Similarly, the larger animals have the greatest proportion of large organic particles in their guts (mean 36% compared with 27.7% in 6 - 9 mm and 13.9% in the animals < 6 mm).

Table 6.4 : The gut contents of *Pollicipes pollicipes* of various sizes collected from three tidal heights. Results are given as the mean percentage ± standard deviation, SD (in brackets) of the total particles counted for each animal falling into each of three categories; small organic material ≤10 μm, small inorganic material <10 μm and large organic material >10 μm. The number in bold in brackets indicates the number of animals examined.

% Food	Animal Size RC (mm) <6		6.05-9		>9	
a) At Sagres						
Low shore	**(17)**		**(20)**		**(20)**	
Small organic material	79.4	(14.7)	61.5	(16.2)	47.1	(9.9)
small inorganic material	3.5	(2.6)	9.1	(6.1)	8.5	(6.0)
Large organic material	17.0	(15.0)	27.8	(13.3)	44.5	(8.0)
Mid shore	**(11)**		**(11)**		**(16)**	
Small organic material	79.0	(7.0)	54.1	(7.4)	45.6	(11.1)
small inorganic material	2.1	(2.7)	8.5	(5.9)	9.5	(7.4)
large organic material	18.9	(7.3)	36.7	(10.9)	43.5	(10.3)
High shore	**(15)**		**(10)**		**(10)**	
small organic material	83.3	(12.4)	66.5	(14.9)	55.6	(10.0)
small inorganic material	6.7	(5.4)	5.4	(4.6)	8.6	(5.9)
large organic material	9.7	(11.0)	28.1	(14.2)	35.5	(12.7)
(b) At Castelejo.						
Low shore	**(16)**		**(47)**		**(31)**	
Small organic material	69.2	(9.4)	71.4	(11.1)	63.2	(8.0)
small inorganic material	5.6	(3.8)	5.5	(5.0)	5.1	(4.8)
Large organic material	25.2	(10.8)	23.1	(9.3)	31.7	(8.1)
Mid shore	**(23)**		**(29)**		**(16)**	
Small organic material	86.4	(9.1)	67.4	(16.5)	62.4	(13.0)
small inorganic material	6	(3.8)	8.3	(10.5)	9.1	(4.7)
large organic material	7.6	(7.0)	24.2	(13.8)	28.5	(11.8)
High shore	**(20)**		**(25)**		**(23)**	
small organic material	85.7	(8.8)	66.1	(10.6)	59.8	(13.9)
small inorganic material	7.5	(5.6)	7.8	(7.2)	12.0	(11.8)
large organic material	4.8	(5.8)	26.2	(9.0)	30.5	(10.8)

The data were transformed using an arcsine transformation and a multifactorial analysis of variance was performed for each dietary component. The effect of any interaction between factors was also examined (see Tables 6.5, 6.6, and 6.7).

Table 6.5. Multifactorial analysis of variance for the effect of barnacle size, site and shore position on the arcsine transformed proportion of small organic material (≤10 μm) in the gut contents of *P. pollicipes*.

Source	Degrees Freedom	Seq. Sum Sq.	Adj. Sum Sq.	Adj. Mean Sq.	F-value	P
Site	1	1467.4	1218.5	1218.5	18.84	<0.001
Position	2	485.8	457.8	228.9	3.54	0.030
Size	2	14824.4	13932.2	6966.1	107.73	<0.001
Site*Position	2	743.2	761.8	380.9	5.89	0.003
Site*Size	2	663.3	716.1	358.0	5.54	0.004
Pos*Size	4	1207.9	917.5	229.4	3.55	0.007
Site*Pos*size	4	870.4	870.4	217.6	3.37	0.010
Error	342	22114.2	22114.2	64.7		
Total	359	42376.8				

Site, position on the shore and animal size account for a significant amount of the variability in the amount of small particulate organic material in the barnacle guts at the 5% level (Table 6.5). Plots of the main effects (Fig. 6.7) show that the guts of small animals (Fig. 6.7c), animals at Castelejo (Fig. 6.7a) and those animals higher on the shore (Fig. 6.7b) contain more small organic material than larger animals, those at Sagres and those lower on the shore.

All interaction terms proved significant (Table 6.5) indicating subtle departure from the general trends indicated by the main effects. There is quite a distinct difference between the proportion of small organic matter in animals at different sites except in the high shore animals, this is largely because of the high small organic content in animals on Sagres high shore. Figures 6.10b and 6.10e show that medium and large animals differ between sites but small animals have similar gut contents at both sites. Small animals have a much more variable small organic fraction than medium and large animals (Fig. 6.10d). The significant three way interaction results from variability in small animals with tidal height and the particularly high small organic component of animals on the high shore at Sagres.

The effect of these factors on the proportion of small inorganic material in the guts of *Pollicipes pollicipes* collected is given in Table 6.6. There were no significant main effects on the inorganic fraction relating to either site or the position of the animal on the shore. However, the main effect of size was significant. Main

Figures 6.7 - 6.9. Plots of the main effects of a) site, b) position on shore (shore level) and c) RC length on the proportion of certain types of particles in the gut of *P. pollicipes*. The Y-axes are mean arcsine transformed percentages, expressed in degrees.

Fig. 6.7. Small organic material

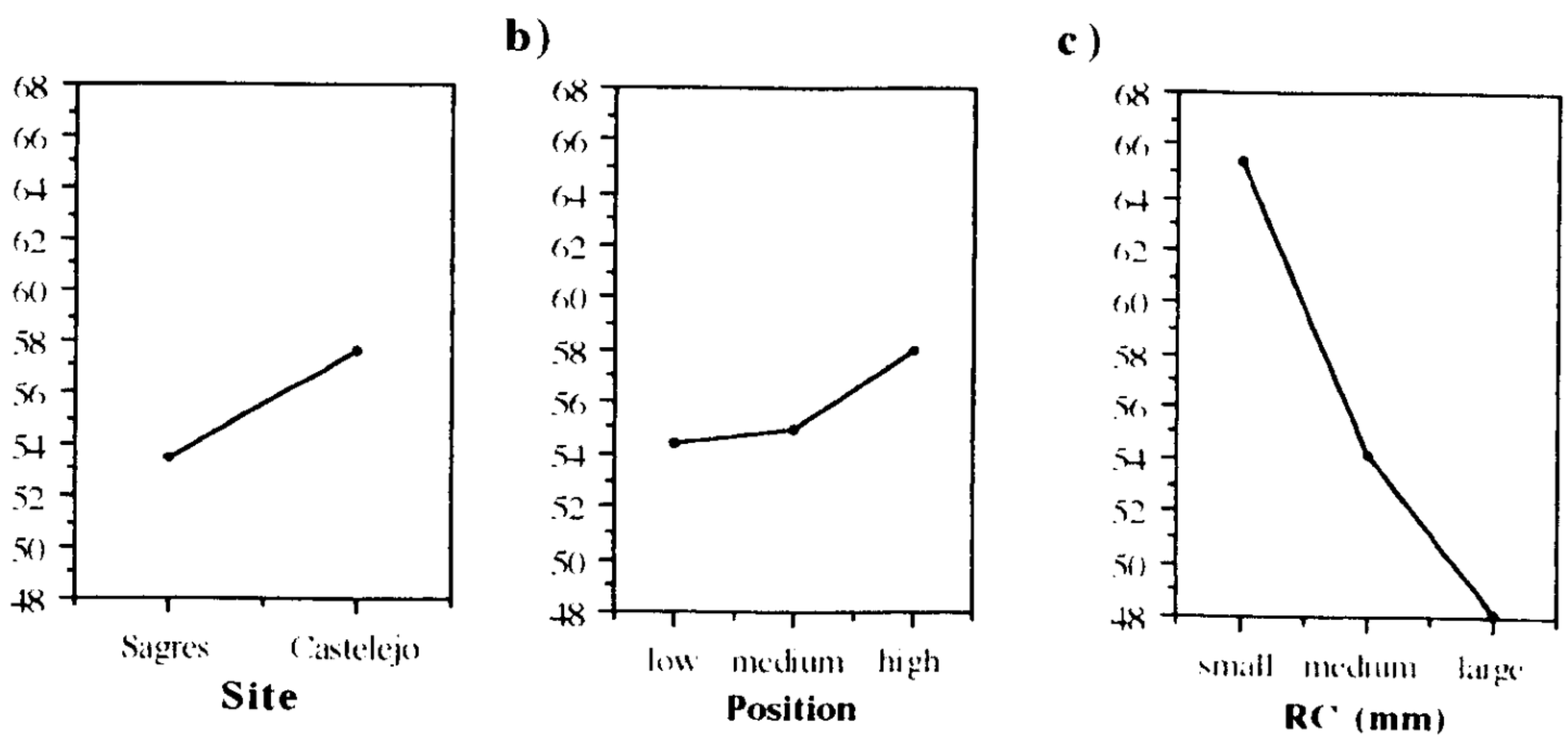


Fig. 6.8. Small inorganic material.

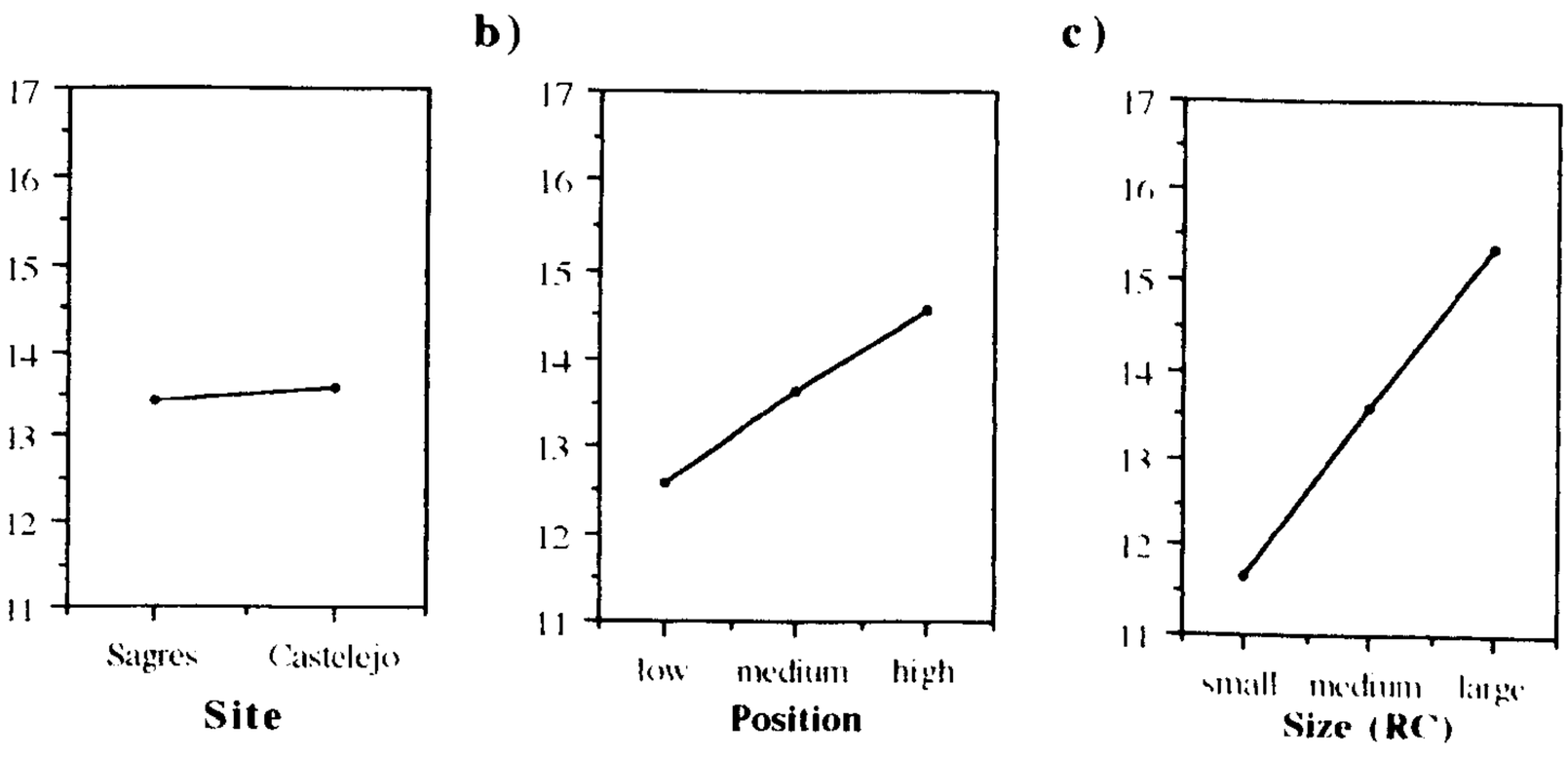


Fig. 6.9. Large organic material

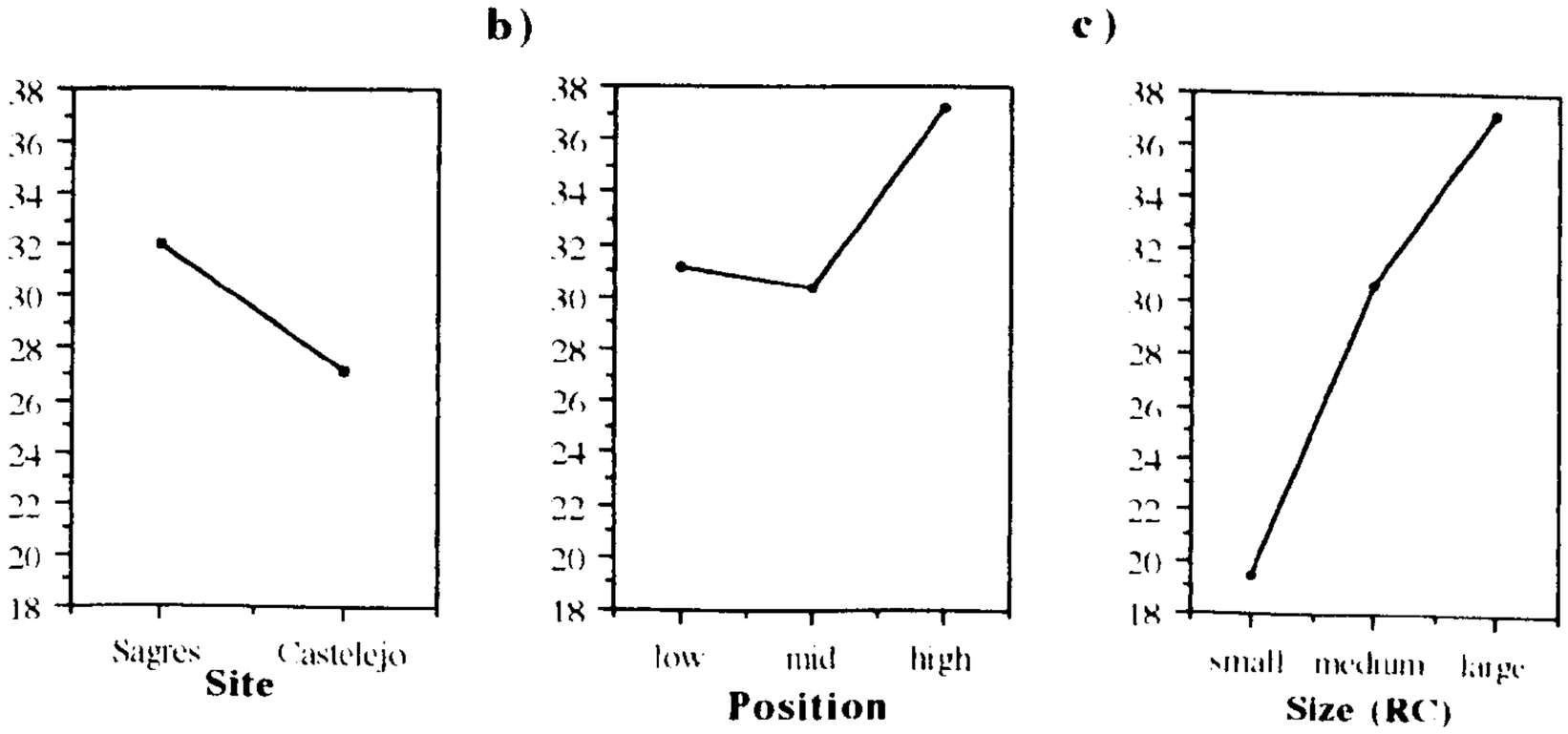
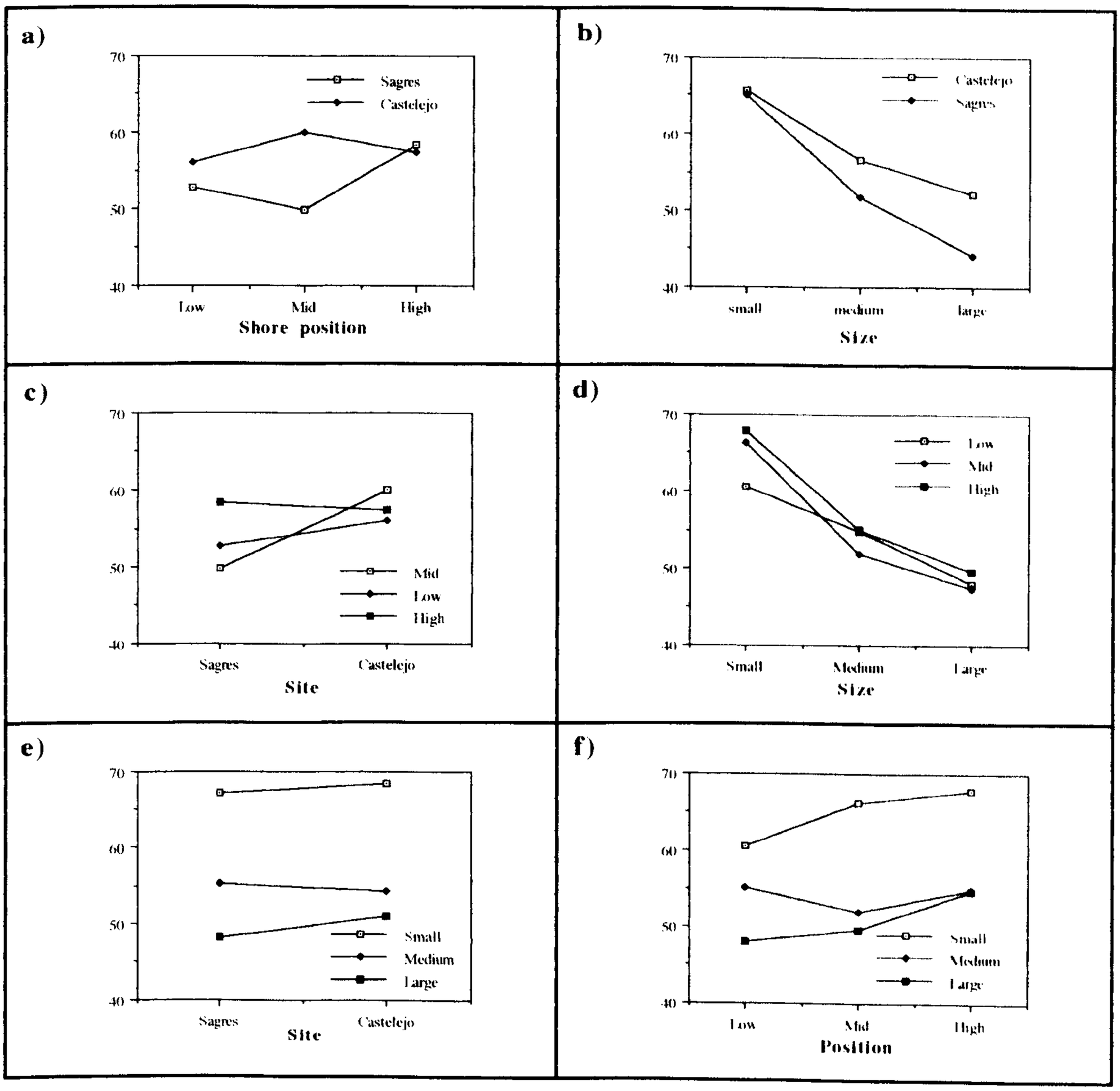


Figure 6.10. The interaction plots for small organic particles (≤1 0 μm) in the gut of *P. pollicipes*. All y-axes are the mean arcsine transformed percentage of the gut contents constituted by particles of this nature and are expressed in degrees. The following plots show the interactions between a) site and position on the shore (shore level), b) site and size, c) shore level and site, d) shore level and size, e) size and site and f) size and position on the shore.



effects plots (Fig. 6.8) show little difference between Sagres and Castelejo. Larger animals have the most small organic material in their guts, small animals the least (Fig. 6.8c). There were also statistically significant interactions between the effect of site and size and between position on shore and size but the interactions between site and shore position and between all three factors were not significant at the 5% level. There was little variability between animals of different sizes at Castelejo but high variability at Sagres, with small animals having particularly low inorganic component to their gut contents (see Fig. 6.11).

Table 6.6. Multifactorial analysis of variance for the effect of barnacle size, site and shore position on the arcsine transformed proportion of small inorganic material (< 10 μm) in the gut contents and the effect of any interaction between factors.

Source	Degrees Freedom	Seq. Sum Sq.	Adjust. Sum Sq.	Adjust Mean Sq.	F-value	P
Site	1	2.51	27.24	27.24	0.45	0.505
Position	2	416.80	233.97	116.99	1.91	0.149
Size	2	681.70	908.50	454.25	7.43	0.001
Site*Pos	2	387.19	333.84	166.92	2.73	0.067
Site*Size	2	358.14	373.56	186.78	3.05	0.048
Pos*Size	4	687.96	698.57	174.64	2.86	0.024
Site*Pos*Size	4	262.5	262.50	65.62	1.07	0.370
Error	342	20917.58	20917.58	61.16		
Total	359	23714.38				

Table 6.7 shows significant main effects on the proportion of large organic material in the gut relating to size, position on the shore and site. Figure 6.9 gives the main effects plots. The proportion of large organic material in the guts of animals at Castelejo is lower than that of animals at Sagres (Fig. 6.9a). High shore animals have a much higher proportion of large organic material compared with the low and mid shore animals (Fig. 6.9b). Small animals have very little large organic material in their guts whilst this component becomes progressively more important with increasing size (Fig. 6.9c). All interaction terms bar site by size proved significant, again indicating subtle departures from the main trends. Plots of the interactions are given in Fig. 6.12.

Figure 6.11. The interaction plots for small inorganic particles in the gut of *P. pollicipes*. All y-axes are the mean arcsine transformed percentage of the gut contents constituted by particles of this nature, expressed in degrees. The following plots show the interactions between a) site and position on the shore (shore level), b) site and size, c) shore level and site, d) shore level and size, e) size and site and f) size and position on the shore.

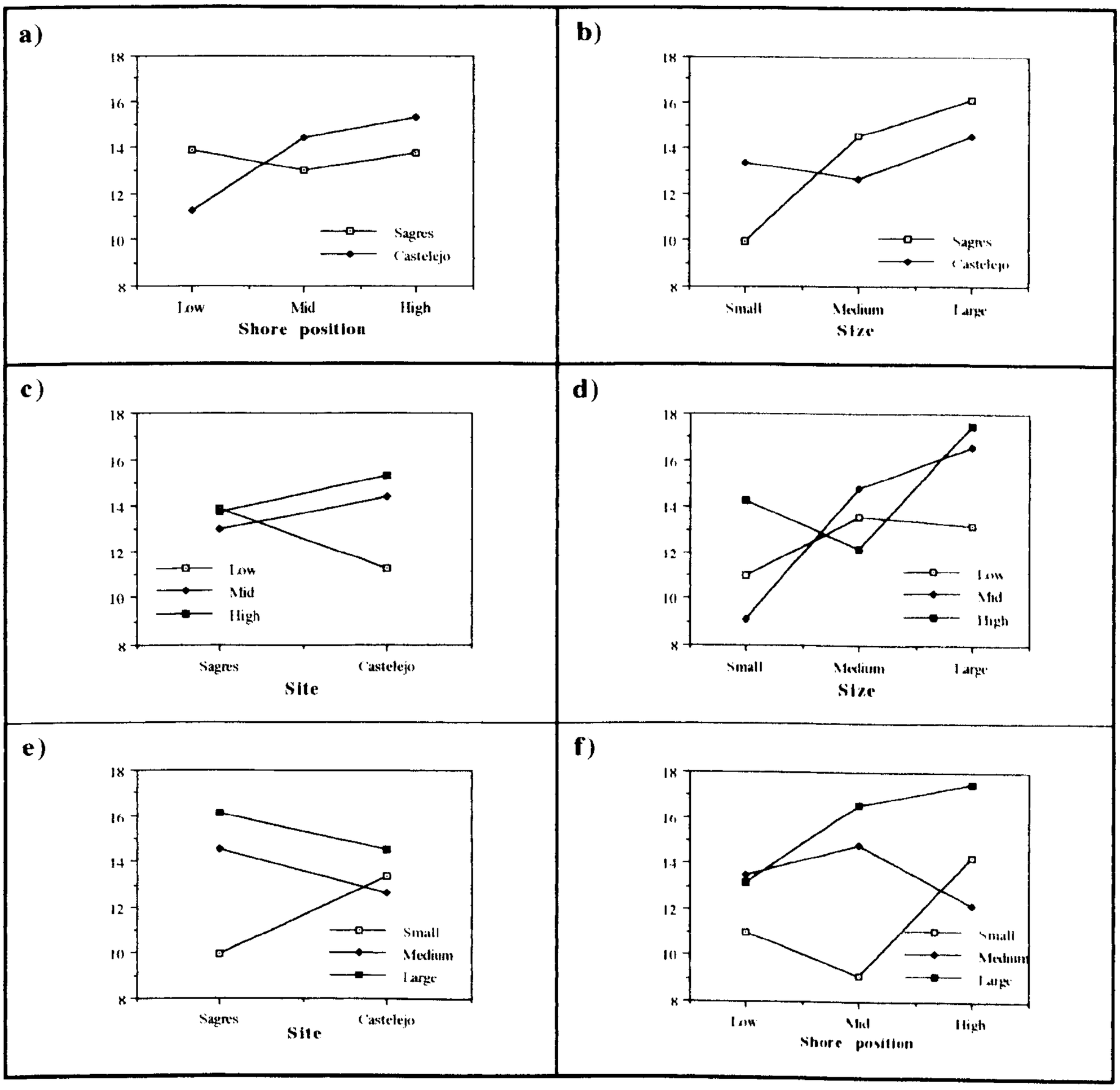


Figure 6.12. The interaction plots for large organic particles in the gut of *P. pollicipes*. All y-axes are the mean arcsine transformed percentage of the gut contents constituted by particles of this nature, expressed in degrees. The following plots show the interactions between a) site and position on the shore (shore level), b) site and size, c) shore level and site, d) shore level and size, e) size and site and f) size and position on the shore.

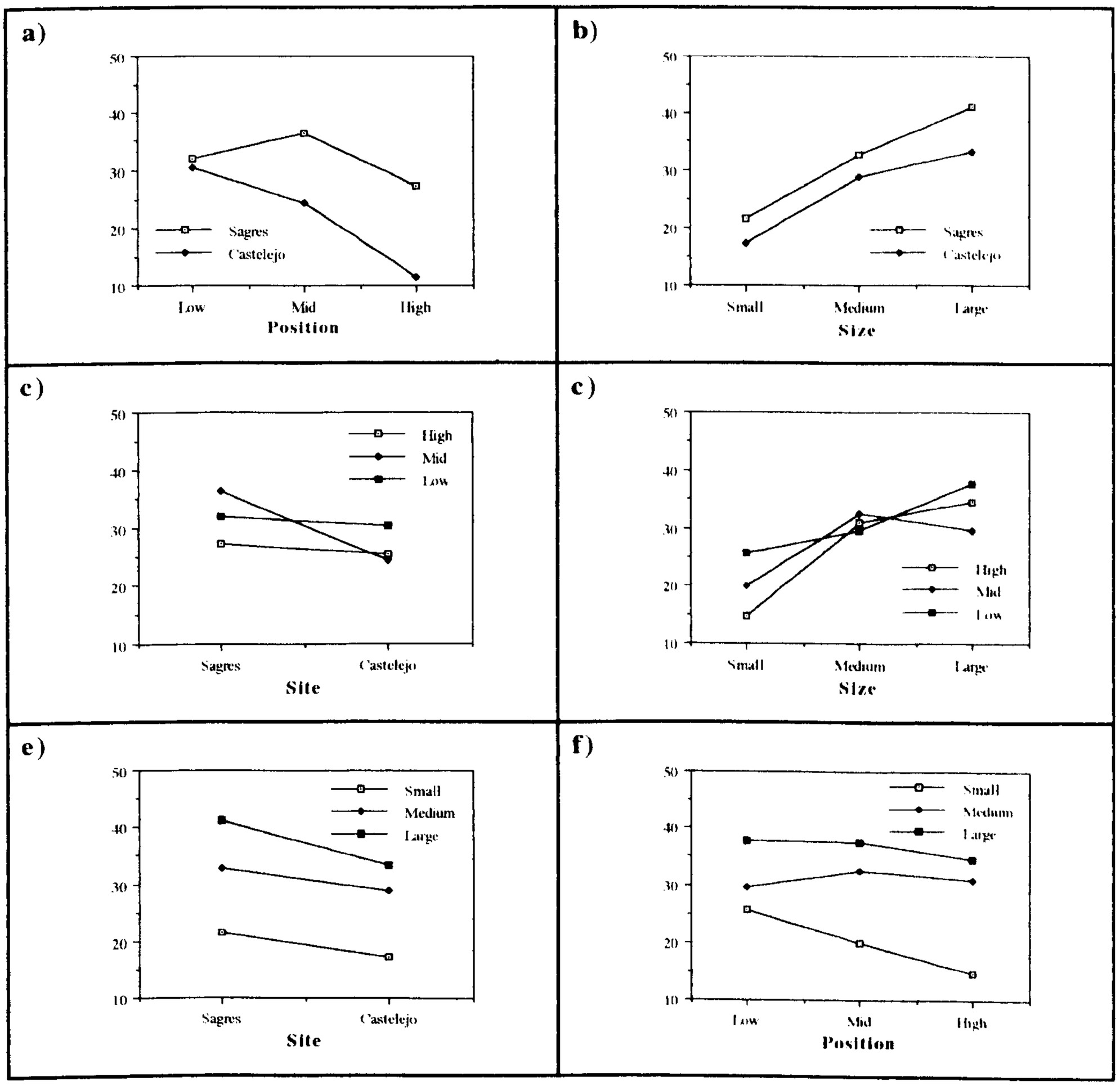


Table 6.7. Multifactorial analysis of variance for the effect of barnacle size, site and shore position on the arcsine transformed proportion of large organic material (>10 μm) in the gut contents and the effect of any interaction between factors.

Source	Degrees Freedom	Seq. Sum Sq.	Adjust. Sum Sq.	Adjust Mean Sq.	F-value	P
Site	1	1939.1	1973.4	1973.4	32.05	<0.001
Position	2	1340.6	1003.7	501.8	8.15	<0.001
Size	2	16027.5	14110.4	7055.2	114.57	<0.001
Site*Position	2	917.7	1299.6	649.8	10.55	<0.001
Site*Size	2	254.1	233.5	116.7	1.90	0.152
Pos*Size	4	1927.9	1473.1	368.3	5.98	<0.001
Site*Pos*Size	4	1223.9	1223.9	306.0	4.97	0.001
Error	342	21060.5	21060.5	61.6		
Total	359	44691.3				

The statistically significant interaction terms make simple interpretation of the results difficult but indicate considerable variability in gut contents which must reflect patchiness of foodstuffs in the water column. However, for all three dietary components, animal size appears to be the most important factor (consistently having the largest F-value) whilst shore position is the least, having the smallest F-values.

The size of larger particles found in the guts of juvenile and adult barnacles are given in Table 6.8. The mean particle size found in the guts of juvenile barnacles is greater than that found in the adults, indicating that large animals macerate their food more thoroughly. However, both the minimum and maximum particle sizes are smaller in juveniles.

Table 6.8. Mean length (μm) ± SD, standard error of the mean (SE) and the range of lengths for the larger gut particles found in 5 adult and 5 juvenile *Pollicipes pollicipes*. All measurements are in μm and N = the number of particles measured.

	N	Mean	SD	SE	Range
Adult	75	246.8	300.3	34.7	28.9 - 1879.5
Juvenile	75	331.0	275.6	31.8	24.1 - 1212.0

The two samples were tested for equality of variance which indicated no significant heterogeneity (F = 1.187, 2 df, 74, P = 0.622). Plots of the two data sets indicated heavily skewed non-normal distributions. Correlations of the particle lengths against the (normal score x mean length) gave coefficients of 0.754 and 0.928 respectively

which were less than the tabulated value of 0.984 . A Mann-Whitney test gave a W value of 5015.5 (P = 0.015). Hence, the medians differ significantly and we can be 95% confident that the median particle diameter in the guts of juvenile *P. pollicipes* is between 10 and 121 μm larger than that of the particles found in the adult guts.

It can be seen from Table 6.9 that there are a few differences between the two locations and shore positions sampled. A higher proportion of Castelejo than Sagres animals, of all sizes, appears to have small particulate material (diatoms, other unicellular phytoplankton and blue-green algae) in their guts. A similar trend is seen regarding echinoid larvae, cirripede cyprids and nauplii. At Sagres a higher proportion of animals have sponge spicules, copepods, molluscs, eggs and polychaetes in their gut contents. There were no apparent differences between the sites regarding occurrence of large algae, detritus, cirripede moults and unidentifiable crustacean remains.

The most obvious differences were seen in the proportion of animals of a given size that had certain food items in their guts. In larger animals cyprids, nauplii, copepods, moults, polychaetes, eggs, echinoid larvae and molluscs were more common.

Table 6.9. A detailed description of the identifiable gut contents of *Pollicipes pollicipes*. The results are given in terms of the percentage of the animals examined that contained the food item in question, *****all, ** >75%, *** 50-75%, ** 25-49.9%, * <25%, - none. The number of animals of each size examined is given in brackets. Un ID = unidentified. Food items are listed within categories in order of occurrence frequency.**

a) Site 3 near Sagres

	Animal Size RC (mm)								
	Low shore			Mid-shore			High shore		
	<6	**6 - 9**	**>9**	**<6**	**6 - 9**	**>9**	**<6**	**6 - 9**	**>9**
	(17)	(21)	(20)	(13)	(13)	(15)	(16)	(11)	(11)
Particulate organic:									
Detritus	*****	*****	*****	*****	*****	*****	*****	*****	*****
Diatoms	****	****	*****	****	****	****	****	****	****
Unicell. phytopl.	****	****	*****	****	****	***	****	***	***
Blue-greens	-	-	*	-	-	-	-	-	-
Particulate inorganic:									
Shell & Sand	***	***	****	***	**	****	***	**	***
Sponge spicules	*	*	*	-	*	-	-	**	**
Large organic:									
Un ID Crustacea	****	****	*****	****	****	*****	***	*****	****
Large algae	****	****	****	****	****	****	****	****	****
Copepods	**	****	****	*	**	***	**	**	***
Adult barnacle moults	*	**	***	*	**	***	**	**	***
Barnacle cyprids	*	**	***	*	*	**	*	***	**
Mollusc larvae	-	*	**	-	*	**	*	**	**
Un ID Eggs	*	**	*	-	-	*	*	**	**
Barnacle nauplii	-	-	-	-	*	**	*	**	*
Shrimp larvae	-	*	*	-	-	-	-	*	**
Polychaete larvae	-	*	*	-	-	-	-	-	*
Echinoid larvae	-	-	-	-	-	*	-	-	-

Table 6.9b. From Site 1, Praia do Castelejo

	Animal Size RC (mm)								
	Low shore			Mid-shore			High shore		
	<6	6 - 9	>9	<6	6 - 9	>9	<6	6- 9	>9
	(17)	(50)	(32)	(28)	(33)	(16)	(16)	(26)	(22)
Particulate organic:									
Detritus	*****	*****	*****	*****	*****	*****	*****	*****	*****
Diatoms	*****	****	****	*****	****	*****	*****	****	*****
Unicell. phytopl.	****	****	****	*****	****	*****	*****	****	*****
Blue-greens	-	-	*	*	*	*	-	*	**
Particulate inorganic:									
Shell & Sand	****	***	***	***	***	***	****	***	****
Sponge spicules	-	-	-	-	-	-	-	-	-
Large organic:									
Un ID Crustacea	****	****	****	****	****	*****	****	****	****
Large algae	****	****	****	****	****	****	****	****	****
Barnacle cyprids	***	***	****	**	***	****	*	**	***
Adult barnacle moults	***	***	***	*	**	***	*	*	**
Copepods	*	**	**	*	***	**	-	**	**
Barnacle nauplii	-	*	*	-	*	***	*	*	*
Un ID Eggs	-	*	*	*	*	*	-	*	*
Echinoid larvae	-	-	*	-	*	*	-	-	*
Shrimp larvae	-	*	*	-	*	*	-	-	-
Mollusc larvae	-	-	-	-	*	-	-	-	-
Polychaete larvae	-	-	-	-	-	-	-	-	*

These data were summarised with a multivariate principal component analysis (see Table 6.10). The first three principal components (Pc) account for 77.6% of the variability in the data. Pc 1 accounts for 43.8%, Pc 2 21.8% and Pc 3 12%. Pc 4 and 5 each account for about 6% and Pc 6 and 7 account for ≤3.5% each. These data may reasonably be summarised by the first three principal components. Plots of Pc 1 scores against Pc 2 and Pc 2 against Pc 3 may be seen in Figs 6.13 and Fig. 6.14 respectively.

It can be seen from Fig 6.13 that Pc 1 is effectively animal size. Larger animals from both sites and all shore positions are generally found at the top of the graph, having high Pc 1 scores irrespective of the Pc 2 score. Smaller animals always have lower Pc 1 scores, regardless of site and shore position.

Table 6.10. Principal component analysis of the frequency of occurrence of various gut contents in *Pollicipes pollicipes* of different sizes, shore positions and sites.

Eigenvalue	1340.6	668.2	367.8	191.9	180.0	107.1	75.2	44.0	
Proportion	0.438	0.218	0.120	0.063	0.059	0.035	0.025	0.014	
Cumulative	0.438	0.656	0.776	0.839	0.898	0.933	0.957	0.972	
Eigenvalue	34.3	20.4	15.7	6.7	4.4	3.9	1.6	0.1	0
Proportion	0.011	0.007	0.005	0.002	0.001	0.001	0.001	0.000	0.0
Cumulative	0.983	0.989	0.995	0.997	0.998	0.999	1	1	

Variable	Pc1	Pc2	Pc3	Pc4	Pc5	Pc6	Pc7	Pc8	Pc9	Pc10
Detritus	0	0	0	0	0	0	0	0	0	0
Diatoms	0.053	0.076	0.032	-0.175	-0.127	0.292	0.055	0.088	0.219	0.15
Unic. alg.	-0.011	0.335	-0.229	0.126	-0.190	0.218	0.113	0.316	0.075	0.39
Bg	0.046	0.114	-0.062	-0.095	-0.262	0.028	-0.420	-0.486	-0.574	0.29
sand shell	0.045	0.332	-0.521	-0.668	0.123	0.119	0.092	0.091	0.021	0.25
sp. spicules	0.060	-0.320	0.199	-0.069	0.002	0.359	-0.159	0.305	0.124	0.04
crustacea	0.142	-0.129	-0.066	-0.034	0.188	0.123	0.191	0.632	0.298	0.30
cyprids	0.645	0.416	0.259	0.216	-0.124	0.312	0.051	0.048	0.176	0.14
nauplii	0.158	-0.067	0.310	-0.397	-0.604	-0.440	0.055	0.024	0.131	0.14
moults	0.484	0.117	0.197	-0.197	0.545	-0.316	0.007	0.024	0.229	0.27
copepods	0.490	-0.373	-0.632	0.303	0.127	-0.184	0.014	0.123	0.075	0.08
polych. l.	0.030	-0.100	-0.066	-0.029	0.111	0.026	0.369	0.158	0.009	0.12
eggs	0.158	-0.227	0.027	-0.154	-0.172	0.361	0.282	-0.098	0.193	-0.28
mollusc l.	0.135	-0.479	0.062	-0.295	0.124	0.338	0.360	0.277	0.083	0.25
large algae	-0.009	-0.021	-0.108	-0.168	0.131	0.063	-0.184	0.094	0.471	0.51
echinoid l.	0.033	0.026	-0.006	-0.056	-0.083	0.123	0.015	0.050	-0.097	0.11
shrimps	0.089	-0.133	0.073	-0.120	0.230	-0.140	-0.594	0.123	0.361	0.05

The above principal components account for 98.9% of the variation so the other seven are not listed.

Looking at Fig. 6.14, Pc 3 is effectively position on the shore. The strength of its influence is less than that of Pc 1 ('size') or Pc 2 ('site') and patterns are less detectable because of the modifying influence of the Pc it is plotted against. Castelejo animals are found towards the top of the graph with high Pc 2 scores whilst Sagres animals are found in the bottom half of the graph, having low Pc 2 scores. Essentially there is strong separation of shore height at Sagres but at Castelejo the differences between the shore heights are not nearly so marked. Again animals with large Pc 3 scores are from the high shore at Sagres but the low shore at Castelejo. This indicates an interaction between site and shore position.

Identification of the barnacle larvae within the guts was attempted by M. Kugele. The carapace length and width of cyprids, carapace texture and shape were noted and the size of nauplii measured. By comparison with laboratory reared larvae, the nauplii and cyprids taken from the gut contents of *Pollicipes pollicipes* adults were most likely those of *P. pollicipes*.

Figure 6.13. Labelled plot of Pc 1 scores against Pc 2 scores.

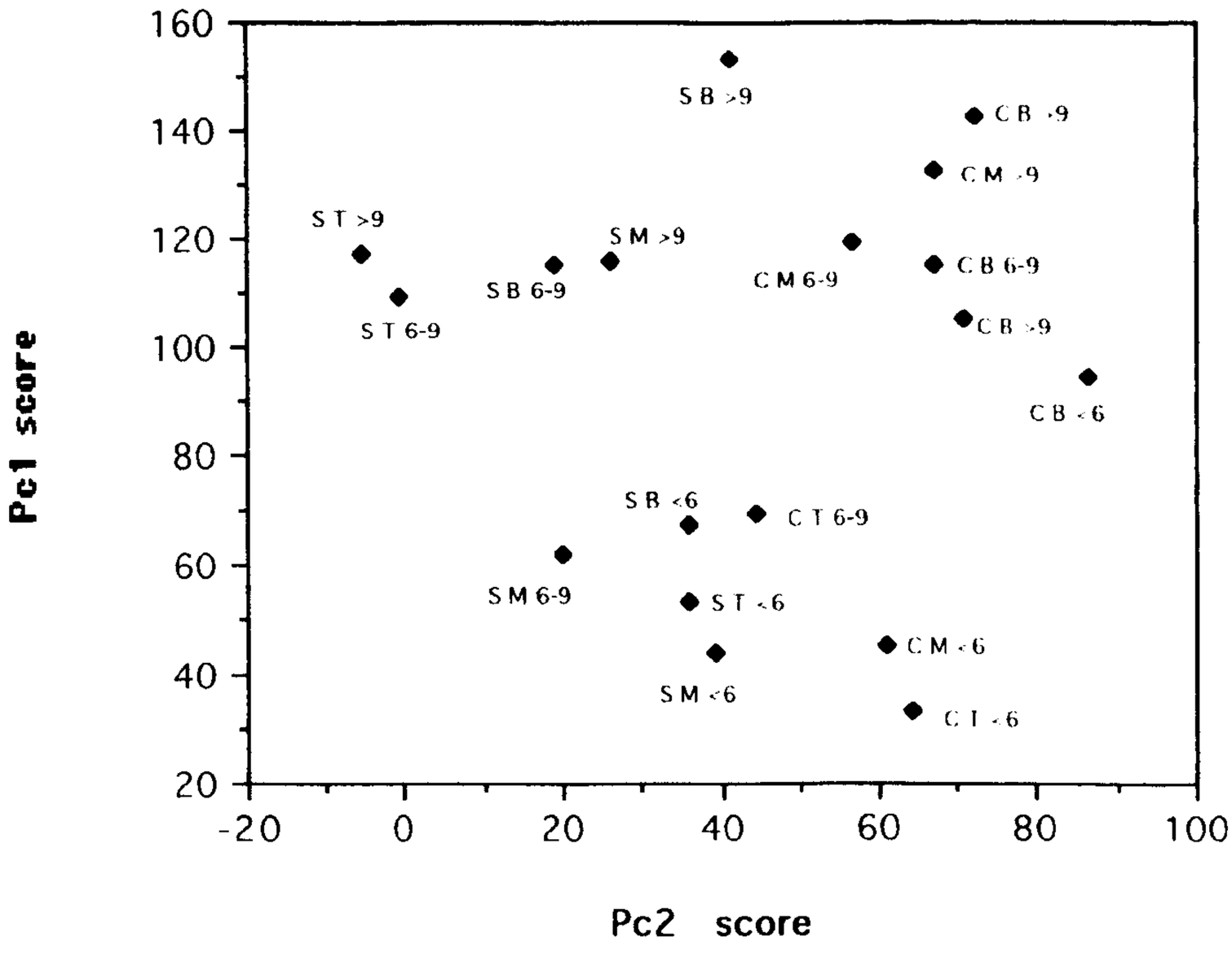
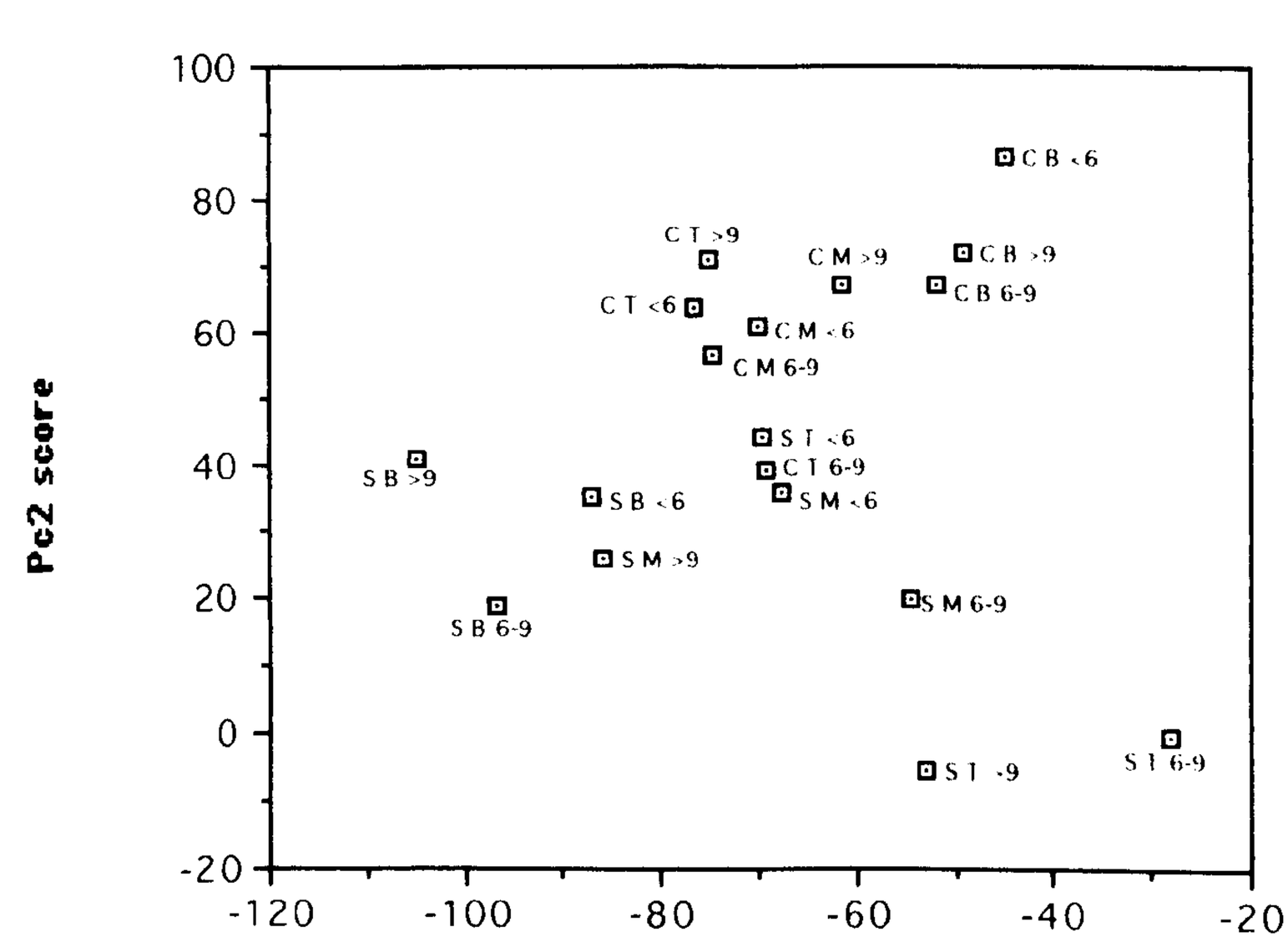


Figure 6.14. Labelled plot of Pc 2 scores against Pc 3 scores.



Key: S = Sagres C = Castelejo;
T = Top M = Mid-shore B = Bottom
<6, 6 - 9, >9 = animal sizes (RC) in mm.

Discussion

The size of *Pollicipes pollicipes* faecal pellets depends on animal size, with larger animals producing larger pellets. This is the case for many invertebrates (Edge, 1934). There is a curvilinear relationship between rostro-carinal length and pellet length, width and volume. A higher variability was evident in the relationship between log pellet length and log animal size ($r^2 = 57\%$) than between log pellet width and animal size ($r^2 = 74\%$). There is also a stronger correlation between log RC length and log faecal pellet width. Pellet width is primarily determined by anus diameter which is probably related to animal size whilst pellet length may be determined by hind gut length or by whether they remain intact or break into shorter lengths. Broken pellets were, where identifiable, excluded from the analysis.

A study of the faeces of *Balanus improvisus* and *Balanus eburneus* (Kraeuter & Haven, 1970) found more variation in the width of faeces, both when looking at the production of a single animal and at the production of several of a known size. The variability was not discussed although it was contrary to their findings for other invertebrates where pellet width was always found to be proportional to animal size, whilst length measurements were more variable (see Kraeuter & Haven, 1970).

Pellet size of *P. pollicipes* also depends on diet. Animals feeding on *Artemia* nauplii produce larger faeces, both in length and girth, as well as in greater numbers than those feeding on *Skeletonema costatum* or *Brachionus plicatilis*. The pellets of *Balanus improvisus* and *B. eburneus* collected and measured by Kraeuter and Haven (1970) were taken from all sizes of barnacles found in Chesapeake Bay including adults and juveniles. It is therefore possible to compare the average size ranges of these and other species (see Table 6.11) with average size ranges of *Pollicipes*.

Table 6.11. Barnacle faecal pellet sizes documented in the literature.

Species	Pellet length (mm)	width (mm)	Source
Balanus eburneus	0.63 - 3.10	0.23 - 0.54	Kraeuter & Haven (1970)
B. improvisus	0.76 - 2.11	0.11 - 0.4	Kraeuter & Haven (1970)
B. tintinnabulum	2.92	0.62	Edge (1934)
B. amaryllis	1.0 - 1.1	-	Arakawa (1971)
B. balanoides	1.2	-	Rainbow (1975)

All values are all very similar to the average range of 0.62 - 3.54 mm long and 0.15 - 0.77 mm wide measured for *P. pollicipes*, although even the largest *Balanus balanoides* are quite small in comparison to even a medium sized *P. pollicipes*. No

faecal sizes for any other species of lepadomorph barnacle appear currently available. The faecal pellets of *P. pollicipes* were around one third of the length of the midgut, as were those of *B. balanoides* (Rainbow, 1975). The midgut runs along the length of the animal body hence correlates strongly with other linear dimensions (e.g. RC length, Fig. 6.6, r = 0.986).

The faecal pellets of *Pollicipes* are rod-shaped, generally straight-sided but occasionally twisted. The pellets of *Balanus amaryllis* (Arakawa, 1971) and *B. improvisus* are similar in shape, but those of *B. eburneus* are generally curved (Kraeuter & Haven, 1970). The pellet ends of *P. pollicipes*, like those of *B. eburneus* (Kraeuter & Haven, 1970) and *B. balanoides* (Stubbings, 1975) are usually tapering or rounded and occasionally broken. Pellets of *B. amaryllis* (Arakawa, 1971) and *B. improvisus* (Kraeuter & Haven, 1970) appear to break more readily. No barnacle faecal pellets yet described have any external sculpturing, all have smooth surfaces, are approximately circular in cross-section and have occasional, irregular constrictions. Edge (1934) noted that not only the amount but also the kind of food present in an invertebrate's gut at the time of faecal pellet formation influences its shape.

Faecal pellets produced by *B. eburneus* are yellowish tan to greenish brown in colour (Kraeuter & Haven, 1970) while those of *B. amaryllis* are dark chocolate brown (Arakawa, 1971), although neither author comments on the diet. The colour of *Pollicipes pollicipes* pellets varies with diet. *Pollicipes* examined in the wild had faecal pellets that were a dark greenish brown in colour. In captivity, a diet of *Artemia* produced red, orange or dark pink pellets, rotifers pale yellow to mid-brown pellets, *Skeletonema*, dark chocolate brown faeces and *Rhinomonas*, pale red-brown pellets. Presumably, the colour of faeces is determined by the pigments present in the food and the degree to which the barnacle is able to digest them.

Some *Pollicipes* feeding on *Artemia* were found to produce pellets divided into two discrete areas. One end containing only a fairly homogeneous mass of digested *Artemia* and the other a concentration, occasionally dozens, of *Artemia* cysts. There are at least two plausible explanations. *Pollicipes* feed selectively on *Artemia* nauplii initially hence the first part of the pellet consists wholly of digested naupliar remains. The animals start ingesting all particles (including cysts) as the density of nauplii is reduced through feeding. Cysts thus ingested appear in high numbers at the rear end of the pellet, that which is formed last. Alternatively, the segregation of cysts in the pellet may be evidence of sorting of ingested particles in the gut. Perhaps indigestible material that has been ingested is rapidly moved through the gut, allowing the slower

passage of more digestible food. The cysts may then become concentrated in the first part of the pellet to be evacuated.

If the former explanation is correct, it would seem more likely that there would be a gradient of increasing cyst abundance along the pellet, with more being ingested as their relative abundance increased. The two portions of the pellet were, however, very markedly discreet, favouring the latter suggestion that there is sorting of the gut contents. Such a mechanism would be beneficial as it effectively increases the digestion efficiency by reducing the volume of refractory material in the part of the gut where digestion occurs.

Pollicipes pollicipes feeding on *Artemia* produced faeces after 4 hours, the larger animals producing pellets at a rate of one every two hours and the smaller animals at a rate of one every 4 hours (6 day^{-1}). The passage through the gut appears slower than was found for *Balanus balanoides* feeding on diatoms by Rainbow (1977); pellets were produced after two hours and at a rate of three per hour. Making comparisons is difficult when the two species are so different, when the size of the *B. balanoides* examined was not given and the diet is different, and as current results indicate that both size and diet are important in determining production. However, although the faeces of small *Pollicipes* are not dissimilar in size to those described by Rainbow (1977) the rate of production is much lower.

Balanus balanoides appears to be better adapted to feeding on diatoms than *P. pollicipes*. Rainbow (1977) found that *B. balanoides* feeding on *Skeletonema costatum* produced faeces at 20 minute intervals while *P. pollicipes* produced faeces at 6 day intervals. However, the gut evacuation time for *P. pollicipes* juveniles feeding on *Artemia* was found to be 11 hours. Perhaps *P. pollicipes* is less able to capture and ingest diatoms than *B balanoides* because its maxillipedes and mouthparts are less highly setose than those of *B. balanoides* (see Chapter 4) and unlike *B. balanoides*, *P. pollicipes* do not exhibit the rapid cirral beating behaviour associated with microphagous filter feeding (Chapter 5). *P. pollicipes*, being larger than *B. balanoides*, also has a larger gut which is likely to take longer to fill or may have a longer passage time than *B. balanoides*.

The less densely packed pellets produced by *Pollicipes* feeding on diatoms does perhaps indicate a problem ingesting sufficient cells to fill the gut, and that *P. pollicipes* is poorly adapted for feeding on algae. Dagg & Walser (1986) looked at the effect of food concentration on the faecal pellet size of three species of marine copepods. It was found that pellet size increases with food concentration up to a value

of 3 μg Chl. l^{-1}; at higher concentrations pellet size was constant. Pellets were less compact and appeared to be more fragile at low food concentrations. The small size of *Skeletonema* cells may result in slow filling of the gut of *P. pollicipes* which in turn produces smaller, partially filled pellets. Even at high algal cell concentrations the barnacle may be effectively in an environment with a low food concentration if it is unable to efficiently exploit this food source. This may lead one to conclude that *P. pollicipes* may not feed efficiently on mono cultures of phytoplankton.

Pollicipes in the wild were, however, found to have plenty of phytoplankton in their guts. In small juveniles, small particulate material accounted for over 80% of the total gut contents, but only 50% in the largest animals. These results are very similar to those obtained by Lewis (1981) working on *Pollicipes polymerus*. Lewis (1981) found that small particulate organic material accounted for 80% in the smallest juvenile animals, 63% in larger juveniles and 52% in adults, very similar to the present study, although a chi-square analysis found there were significant differences, probably because of the large numbers of particles counted. Lewis (1981) concluded from her results that the smaller barnacles were more dependent on microscopic food.

Lewis (1981) assumed that a high proportion of small particles in the guts of smaller animals was synonymous with a dependency on them for food. What is important to a feeding animal is the energetic contribution of the food it ingests. The proportion of particles in the gut that are of a particular food type gives no indication of their relative energy contribution. Nor does the presence of microalgae in the gut prove they are selectively ingested, or even digestible. In energetic terms one *Artemia* is roughly equivalent to sixty eight thousand *Skeletonema* cells (Kurmaly *et al.*, 1989a) and therefore the latter's energetic contribution would be insignificant assuming equivalent digestion efficiencies. Very tiny *P. pollicipes* may be able to get all the energy they require from the small proportion of large organic material they ingest and hence may be no more dependent on small particulate material than the adults.

The smaller proportion of small organic material in the guts of larger animals may not reflect a reduced ability to capture small particulate material, but an enhanced ability to catch, manipulate and ingest larger prey. Adult *P. polymerus* were found by Lewis (1981) to have large organic particles accounting for 40% of their gut contents, large juveniles 31% and small juveniles 8% again equivalent to those obtained for *P. pollicipes* (36%, 28% and 14% respectively).

Inorganic particulate material accounted for 5% in small juvenile *Pollicipes pollicipes*, 7% in larger juveniles and 9% in adults. The proportion of inorganic material is low

considering that the water appeared laden with sand and silt and at Sagres the mantle cavities of the barnacles contained noticeable quantities of sand. Barnes (1959) found only a few sand particles in the gut contents of adult *P. polymerus* and concluded that large, inorganic, inanimate material does not readily elicit a feeding response from the cirri. *P. pollicipes* also have a capacity for chemoreception and inorganic material will not stimulate feeding (Hicks, 1993; M. Cottam, pers. comm.). Finer inorganic material is wiped off the cirri, carried like other small particulate material to the mouth but is then carried forward over the inactive mouthparts and not ingested (Barnes & Reese, 1959). A mechanism for the capture and transfer of all material by *P. polymerus* was proposed by Barnes & Reese (1959). Cirral setae intercept the particles, which are then bound together in glandular secretions and transported to the mouth. Inorganic particles thus bound up with organic food are presumably not rejected and some inorganic material is ingested. The magnitude of the inorganic fraction in the gut contents would thus be lower than expected if the density of organic particles in the water were much lower than the density of inorganic particles.

The gut contents of several species of barnacles have been described and there are notable similarities. Detritus, diatoms and arthropod appendages have been found in varying proportions in the gut contents or faeces of *B. eburneus*, *B. improvisus* (Kraeuter & Haven, 1970), *Verruca stroemia* (Stone & Barnes, 1973), *V. recta* (Anderson, 1980b), *Lepas anatifera* (Barnes, 1959), *P. polymerus* (Barnes, 1959; Lewis, 1981) as well as *Pollicipes pollicipes*. In some species such as *V. recta* (Anderson, 1980b) large particles dominate while in others, for example *V. stroemia* (Stone & Barnes, 1973), small particles do.

Cirripede moults (adult) and cyprids were frequently found in the guts of *P. polymerus* (Barnes, 1959) but nauplii only occasionally. *P. pollicipes* also had cirripede remains in their guts and in many large individuals cirripede larvae dominated the gut contents. These were tentatively identified as *P. pollicipes* (M. Kugele, pers. comm.) although the large *Balanus perforatus* and *Chthamalus sp.* populations present on the shores may also contribute larval prey but none were identified in the current study. Conspecific nauplii were also found in the gut contents of *Verruca stroemia* by Stone & Barnes (1973). Hydroids (unspecified whether adults or larvae) were found in *P. polymerus* by Barnes (1959) and Lewis (1981) but none were present in *P. pollicipes*.

It appears that the gut contents of *P. pollicipes* cover a wide range of food types which reflect the material expected to be present in the surrounding water, both in terms of small particulate material such as phytoplankton and larger particles such as larvae and zooplankton and shreds of thalloid and filamentous algae. It appears that these

animals are relatively unselective feeders, although there is evidence for the active rejection of inorganic material.

The greatest differences in gut contents were associated with animal size. Larger animals generally had a greater proportion of larger particles in the gut contents compared to small animals. This may reflect the inability of smaller animals to capture, to manipulate and macerate large prey. Comparison of the sizes of the larger organic particles in the gut contents of adults and juveniles showed significant differences, but those in the juveniles were larger than those in the adults. This probably reflects an enhanced ability of adults to macerate larger prey rather than juveniles more regularly capturing larger prey than adults.

The difference in gut contents between adult and juvenile *P. pollicipes* is quantitative rather than qualitative. Both are capable of ingesting large and small particulate matter. The juveniles appear less able than the adults to macerate the larger particles and possibly lose a larger proportion of material at that stage. There is no evidence from the gut contents that *P. pollicipes* undergo a distinct shift in feeding habits as they enter adulthood and emerge from the shelter of the adult clumps as has been proposed by Lewis (1981) for *P. polymerus*. The results of the gut content analysis tend to confirm the conclusions of Chapter 4, that morphological changes in cirral structure are progressive with size, and Chapter 5, that *P. pollicipes* do not discernibly alter feeding behaviour with increasing size even if frequencies of cirral extensions in still water decrease with increased barnacle size.

Smaller animals appeared to have more completely digested gut contents than larger animals. Juveniles have a larger proportion of small organic material in their gut which, by its nature, has less substance and looks 'more digested' than larger organic particles. However, measured faecal production rates showed that juveniles produced faeces half as often as adults when feeding on *Artemia* which might suggest that the digestive strategy differed between the two. Juveniles may digest ingested food more thoroughly by retaining it in the gut for longer while adults process larger amounts of food but with a lower digestion efficiency.

Animals higher on the shore also had apparently better digested gut contents than those lower down the shore. There were no obvious fundamental compositional differences between the gut contents in the different positions so one explanation could relate to the time elapsed between emersion and collection. When the samples were taken, the animals lower on the shore would have been immersed and feeding more recently than those higher on the shore. The more recently an animal has fed the

more likely their gut will contain some undigested food although whether the difference in emersion time is sufficient to account for the observed difference is not known.

Gut contents varied significantly with the position of the animals on the shore, but largely due to small animals at the top of the shore (interactive effects, see Figs. 6.10-6.12). The relatively large sample size produces a small error mean square value. When the error mean square is small, F-values become large, so even quite small differences relating to the factors become statistically significant. It is surprising that the gut contents vary when the whole *Pollicipes* zone extends for only 1.5 - 2.0 m in vertical height and the water is so thoroughly mixed by the action of the waves and wind. The differences must reflect the patchiness in the distribution and abundance of particular material in the sea and presumably relate to the propensity of small animals for smaller particulate matter, the relative proportion of large/small particles in the water column and that small animals higher up the shore get less time to feed than their counterparts lower down.

The composition of the gut contents differed at different sites but was not consistent with shore height or animal size. The two sites were about 17 km apart. Both are subjected to cross shore currents, at Castelejo a current moving south down the west coast of Portugal and at Sagres a westward current travelling along the Algarve coast. These currents meet at Cabo do Såo Vicente (which separates the two sites) and move offshore. Differences in the abundance of particular material in the guts of animals at the two sites which is clearly demonstrated by the principal component analysis may reflect differing abundance in the water, relating to the origins of the water masses and large scale patchiness. Differences in the physical nature of the sample sites affecting the way small animals fed also seems likely judging by the interactive effects evident in the gross gut fraction data (Table 6.5). Gut contents may simply reflect differences in the local availability of dietary components. For example, at Sagres mussels are common and *P. pollicipes* contained numerous lamellibranch veliger larvae, whereas at Castelejo, which has few mussels, barnacles contained few such larvae. The large numbers of mussels elsewhere along the coast might have been anticipated to contribute sufficient larvae to negate this effect.

In summary, the faecal production of *P. pollicipes* feeding on algae in captivity is negligible, probably reflecting an inability to fill its gut and generate an appreciable throughput of material. In the wild, both juvenile and adult *P. pollicipes* have a wide variety of animal and plant material in their guts suggesting a truly omnivorous existence. Although the gut contents of *P. pollicipes* do not change qualitatively

with increasing size, there are quantitative changes; small juveniles have a higher proportion of small organic material in their gut than the adults, which generally have a higher proportion of small inorganic and large organic material in their guts. This is not felt to reflect a size-related shift in feeding strategies such as was hypothesised for *P. polymerus* by Lewis (1981).

Chapter 7

Ingestion and growth rates of juvenile *Pollicipes pollicipes* feeding on live diets

Introduction

Early work and observations on the feeding of barnacles suggested that adult barnacles were primarily macrophagous filter feeders. The experiments of Southward (1955a) indicated for the first time the diversity of food organisms that may be exploited by adult barnacles. He demonstrated, by both disappearance from suspension and appearance in the faeces, that an adult *Balanus perforatus* can ingest *Artemia* sp. nauplii (≤ 1 mm), *Peridinium* sp. (30 μm), *Gymnodinium* (10 μm) and rod shaped bacteria (2 μm). The intersetal spaces of the cirral net were ≥33 μm so neither microalgae nor bacteria could have been filtered directly. Southward (1955a) proposed that these were filtered from the water flowing through the mantle cavity by cirri I and II that have intersetal spaces of just 1 μm.

Intertidal barnacles are thought to rely on small crustaceans and diatoms which are readily available in the plankton. Judging by the gut contents, *P. pollicipes* (see Chapter 6) is no exception. Sublittoral barnacles are thought to be dependent on smaller particles as larger ones are less abundant (Barnes, 1959). Howard & Scott (1959) observed that while both *Lepas anatifera* and *Mitella* (= *Pollicipes*) *polymerus* were generally unselective omnivores, both could behave like predatory macrophagous carnivores, more able to capture and manipulate larger prey than was previously suspected. Both *L. anatifera* and *P. polymerus* were able to feed on *Artemia* up to 11 mm in length and were able to catch and retain other prey while compacting and swallowing previously captured ones. Further examples of predaceous feeding include *Lepas anserifera*, feeding on the siphonophores *Velella* and *Physalia* (Bieri, 1966) and *L. anserifera* on *Physalia* and young flying fish (Jones, 1968). *Pollicipes pollicipes* is also able to capture effectively and process large food items (M. Cottam, pers. comm.). Cottam observed entrapment and retention of ghost shrimp *Caprella linearis* with behaviour similar to that seen in *P. polymerus* by Howard & Scott (1959).

Crisp (1964) combined measures of feeding rates of barnacles in the laboratory with the growth rates found in the sea to suggest highly efficient assimilation. Crisp (1964) concluded that, given the high feeding rates measured in the laboratory, both *Balanus balanoides* and *Elminius modestus* require very high concentrations of suspended

organic material to achieve maximal growth rates. Such rich waters are characteristic of the inshore areas in which they are found (Parsons, 1980).

The filtration rates of *Balanus perforatus* were measured by Southward (1955a) and were found to depend on diet. However, as prey densities were not given, ingestion rates cannot be calculated. Feeding rates are likely to depend on many factors; the abundance, size and quality of food particles as well as temperature and animal size. There is marked seasonal variation in the feeding rate of *Balanus balanoides*, when measured as the pigment content of faecal output of barnacles feeding on *Artemia* (Ritz & Crisp, 1970). Peaks in feeding activity were observed between March and May and again in October. Reduced feeding accompanied the onset of breeding in November. When *B. balanoides* feeding rates are high in Spring, increases in temperature result in increases in the feeding rate although rates tend to be independent of temperature at other times (Ritz & Crisp, 1970). Food intake is also related to position on the shore, high shore animals have higher ingestion rates than their low shore counterparts (Ritz & Crisp, 1970). This may counteract the reduced time available to high shore animals for feeding.

Little or no work has been done on the feeding rates of lepadomorph barnacles. The present study attempted to investigate the ingestion rates of juvenile *Pollicipes pollicipes* feeding on animal and plant diets at different suspension densities. The effect of feeding barnacles on a mixed algal-animal diet, as opposed to monospecific diets, was also investigated to see if any selective feeding occurs and what implications this has for the feeding strategy of *P. pollicipes*. An attempt was made to establish the maximum prey size that could be ingested by juvenile and adult *P. pollicipes*. The growth rates of three groups of similarly sized barnacles feeding on different diets were monitored over a six month period to try to establish whether there is a growth advantage to feeding on a mixed diet.

Changes in the rate of ingestion of each diet with increasing barnacle size were explored to further investigate the theory of a switch in diets with increasing barnacle size proposed for *Pollicipes polymerus* by Lewis (1981). From age-related changes in gut contents of *P. polymerus*, Lewis (1981) concluded that small juveniles were most reliant on small particulate organic material such as diatoms whilst adults rely on large organic particles usually of animal origin. Evidence to the contrary is derived from the analyses of the gut contents in *P. pollicipes* (Chapter 6), from the morphology of feeding appendages (Chapter 4) and cirral activity (Chapter 5) which would suggest that juveniles and adults exploit essentially the same food resources.

Materials and Methods

General

Food organisms used

Four species of microalgae were used for the preliminary experiments. The first was the Prymnesiophyte *Pavlova lutheri* (Droop) Green. It is a single-celled, flagellated alga, commonly found in rock pools. According to Green (1975) it is 7 - 9 μm in length and 5 - 7 μm in width and 3 - 4 μm in depth. However, the strain used in this study was smaller (3.5 μm in length). The presence of carotenoids as well as chlorophyll a and c give the alga its yellow-green colour. The second alga was the Prasinophyte *Tetraselmis chui* Butcher. It is a single-celled flagellated green alga about 10 - 19 μm in length. The third was the cryptomonad *Rhinomonas reticulata*. It is a single-celled flagellated alga, commonly found in coastal plankton. It ranges from 9 x 5 μm to 12 x 7 μm (Novarino, 1991). It is reddish brown in colour due to its accessory pigment phycoerythrin. The fourth algal species was *Skeletonema costatum* , a lens-shaped diatom with parallel spines around the cell margin that link with others to form long straight chains. It is a very common species particularly in coastal waters and is frequently associated with the Spring phytoplankton bloom. The individual cells are small, 7 - 15 μm in length according to Newell and Newell (1967). The strain used here ranged from 5 to 18 μm in total length including the spines and 3 - 6 μm in diameter but its chain forming habit increases its effective size to a filter feeding animal.

Both *Tetraselmis* and *Pavlova* were rapidly eliminated from feeding experiments. The former, although of a suitable size, was found to drop out of suspension too readily and did not appear to be ingested to any extent by *P. pollicipes*. *Pavlova*, although remaining in suspension, was also not ingested at a measurable rate. The very small size of this alga may have prevented its capture. Animals presented with monospecific diets of these algae produced no faeces over a period of several weeks.

Two live animal diets were used; the nauplii of the brine shrimp *Artemia* sp. and the rotifer *Brachionus plicatilis* Muller Strain GS74. *Artemia* is a branchiopod crustacean which, although it occurs only in salt lakes and marshes in the wild, is frequently used as a live diet for marine filter feeders and fish larvae. It is easily cultured and is tolerant of a wide range of environmental conditions. The newly hatched nauplii are 511 $\pm$ 33 μm (SD) long and 184 $\pm$ 22 μm across the body excluding limbs so are of a similar size to many of the smaller zooplankters that might be encountered by a barnacle in the wild. They are readily ingested by *Pollicipes pollicipes*. *B. plicatilis* is a marine representative of the mainly freshwater Phylum Rotifera. They are smaller than *Artemia*, measuring 265 $\pm$ 18 μm in length (including the foot) and 213 $\pm$ 43 μm

in diameter. Again they are commonly used as a live diet in aquaculture and are readily captured and ingested by *P. pollicipes*.

Algal culture

All algal species used were routinely cultured in 20 l jars of UVFSW (see Chapter 2) under constant conditions of light provided by fluorescent Grolux tubes and at a temperature of 25°C. Cultures were aerated with an air/CO_2 mixture at a pH of 8.

Algal Counts

Counts made of *Pavlova lutheri*, *Tetraselmis chui* and *Rhinomonas reticulata* to prepare the algal suspensions were carried out on a Coulter Counter model ZM with modal size/volume and size distributions given by an attached channelizer. However, for all post-experimental counts and all counts of *Skeletonema costatum*, a modified Fuchs-Rosenthal (B.S. 748 depth 0.2 mm) haemocytometer was used. Post-experimental counts were made with the haemocytometer because of the inability of the Coulter Counter to discern between algal cells and small particulate material shed by the barnacles (e.g. broken faecal pellets). Use of the Coulter Counter for post experimental counts always resulted in overestimations of cell densities and therefore underestimates of ingestion rates. A haemocytometer was also used for counting the chain-forming *S. costatum* since its shape produces unreliable Coulter Counter counts.

Artemia Culture

For all experiments newly hatched, non-feeding *Artemia* nauplii were used. A suspension of *Artemia* cysts (Sanders) was made up in a 7 l flask in UVFSW and was incubated for 24 hours at around 25°C with vigorous aeration. The hatched nauplii were separated from cysts by removal of the aeration. Within five minutes the hatched cysts floated to the surface, whilst nauplii sank to the bottom. The nauplii were removed via a tap at the base of the jar and were transferred to an 80 μm mesh sieve where they were thoroughly rinsed with UVFSW before being resuspended in UVFSW.

Rotifer Culture

The rotifers *Brachionus plicatilis* were maintained in a 2 l glass conical flask with gentle aeration. They were fed daily with 150 ml (ca 5000 cells μl^{-1}) *Pavlova lutheri* and 150 ml (ca 600 cells μl^{-1}) *Tetraselmis chui*. Three hundred millilitres of the culture were removed to an 80 μm mesh sieve, for experimental purposes, and the

rotifers rinsed with UVFSW to remove algal material before being resuspended in UVFSW.

Artemia and Rotifer counts

All counts were done using a Bogorov tray under a binocular microscope at x12 magnification. All the animals remaining in the vials after the experiments were counted.

Experimental Conditions

All feeding experiments were performed under the same environmental conditions. The barnacles were attached, by means of Velcro and Superglue, to the inside of the lids of 20 ml glass vials. The vials were fixed to a rotating wheel which revolved at 6 r.p.m., and kept in the dark at 16°C. The darkness reduced algal growth and the rotation of the wheel kept food organisms in suspension and therefore accessible to the barnacle. The water movement within the vials also stimulated the barnacles to extend their cirral nets to feed. A similar system has been used to measure ingestion rates in copepods (see Kiørboe *et al.*, 1982; 1985; and Abu-Rezeq, 1992).

For every experimental vial containing a barnacle an identical vial with no barnacle acted as a control. Control and experimental vials were filled from the same stock culture of food organism.

Starving animals prior to feeding experiments can lead to the measurement of unnaturally high feeding rates as the organism rapidly fills an empty gut. However, *P. pollicipes* were found to vary widely in the time taken to begin feeding when placed in a food suspension (see Chapter 5). In experiments of short duration such delays lead to high variability in the measured feeding rates. Consequently, the animals were starved prior to experiments to overcome any rhythms in feeding activity and to stimulate feeding, thereby reducing the variability. Starving the animals increased the likelihood of measuring maximal feeding rates, comparable between the diets even if not representative of those occurring in the wild. Animals were starved for 12 hours prior to experiments, this being the measured gut evacuation time (see Chapter 6).

The effect of suspension density of food organisms on the ingestion rate of *P. pollicipes*

Preliminary suspension density experiments

For every food organism the optimal experiment duration was determined to ensure that the ingestion rate was never limited by food availability. With guidance from literature values, an appropriate suspension was prepared and 17 ml was put into each of ten vials. One animal was fixed to the inside of the lid of five vials and the remaining five vials paired to the first as controls. The vials were fitted to the rotating wheel and every hour an experimental and control vial was removed and for the algal food density was assessed while for animal food, the total numbers remaining in the vial were counted. The plot of time vs. the difference in density between the vials indicated the duration of linear decrease to be used in future experiments.

Four sets of experiments were carried out to investigate the feeding rates of *P. pollicipes* on different diets. In all experiments individual juvenile *P. pollicipes* (all ca 5.8 mm RC) were used. There was no deliberate replication at each cell density; instead, a wide range of densities was used.

The experiments investigating the effect of algal abundance were run over a 24 hour period. *Rhinomonas* is a motile flagellated alga which was fixed, for counting, by the addition of 0.1 ml of 2% formaldehyde solution to the 17 ml sample. The number of haemocytometer fields that were counted depended on the cell abundance (the average count per field) and was calculated to obtain a counting precision of a 95% confidence interval equalling ± 15% of the mean count per field (see Cassell, 1965). When very low cell densities were counted, lower precision was inevitable since the counting of hundreds of fields was necessary to retain a precision even as low as 15%. Ingestion rates were calculated assuming a linear decrease in cell concentration.

$$I_R = |(C \times V)_{con} - (C \times V)_{expt}| / T$$

where I_R = the ingestion rate in cells per hour

C = cell density (cells μl^{-1})

V = volume of suspension in experimental vial (μl)

T = experimental duration (hr)

Experiments using *Artemia* and rotifers lasted 1 - 2 hours which was short enough for there to be a linear decrease in concentration over time, with no density-dependent decreases in ingestion rate (see Paffenhöfer, 1971; Betouhim-El & Kahan, 1972) but long enough to provide a measurable decrease in prey numbers. Ingestion rates were thus calculated as the difference in total prey numbers between control and experimental vials divided by the time course of the experiment.

For all the experiments to provide comparable results, the energetic content of each food organism was estimated and the ingestion rates then expressed in terms of energy intake as well as in numbers.

Preparation of food samples for determination of energy content.

Whatman glass fibre filters (47 mm GF/C 1.2 μm) were freed from oxidisable material by placing them in large aluminium foil boats in a muffle furnace at a temperature of 450°C for 4 hours. The temperature was kept below 500°C to prevent sintering of the glass fibres (see Strickland & Parsons, 1968). The filter papers were weighed on a Cahn C31 microbalance and immediately wrapped in the precombusted aluminium foil to protect them from dust and were handled only with clean metal forceps. The foil packages were kept in a desiccator until required. When samples were ready for filtering, the papers were removed using forceps, and fitted into a Gallenkamp filter holder. Organisms were gently filtered onto the papers, at a suction not exceeding 25 bars, with a hand pump.

(i) Algae

1000 ml each of *R. reticulata* (ca 1000 cells μl^{-1}) and *S. costatum* (ca 2000 cells μl^{-1}) were spun down in a centrifuge at 3000 rpm, 20°C, for 15 minutes. The supernatant was poured off and the cells were resuspended in 3.9% ammonium formate solution which was used to wash salt from the sample. Although previous studies had utilised a 0.9% solution of ammonium formate (see Holland & Walker, 1975), a 3.9% solution was suggested by Collis (1991) to be isotonic with sea water and 100% volatile therefore evaporating to leave no trace. However, the cells were observed to lyse rapidly on addition of this solution, *Rhinomonas* turning from dark red to a bright orange suspension that produced a fluorescent pink filtrate on filtration and *Skeletonema* turned dark green.

An experiment was performed to see whether any other concentrations of ammonium formate solution could be used that would not lyse the cells yet adequately remove the salt. Several dilutions of ammonium formate were prepared and 20 drops of the following were placed individually in twelve 9 ml glass vials; UVFSW, distilled water, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% ammonium formate solution. One litre of *Rhinomonas* (ca 1000 cells μl^{-1}) and one of *Skeletonema* (ca 2000 cells μl^{-1}) were spun down as before and the cells resuspended in 20 ml of UVFSW. Two drops of the *Rhinomonas* suspension were added to each of the twelve tubes and any colour change, indicating lysis, noted. The experiment was repeated using *Skeletonema*. Lysis occurred in both species irrespective of concentration and in distilled water but not in seawater.

The procedure adopted: One litre samples of algal suspension were taken by spinning down as before and resuspending in 20 ml UVFSW, filtering onto a precombusted filter paper and rinsing with 3.9% ammonium formate. When filtration was completed rapidly, lysis of cells was avoided. The cells retained on the filter were gently removed using the back of a scalpel blade and transferred to a precombusted foil weighing boat.

Dry weights

Counts were made of the cell densities of each algal suspension centrifuged and one litre of each was spun down, resuspended in 20 ml UVFSW, filtered, the cells rinsed with ammonium formate and suction reapplied. The filters were dried and reweighed and the weight of cells on the paper was estimated by difference.

(ii) *Artemia* and rotifers

100 ml samples of a dense (100 animals ml^{-1}) day 1 *Artemia* suspension and 500 ml samples of a dense rotifer suspension (100 animals ml^{-1}) were poured through an 80 μm mesh sieve and the retained animals rinsed with several changes of 3.9% ammonium formate solution to remove any seawater. There was no sign of lysis. Each sample was resuspended in 50 ml of ammonium formate solution and then filtered. The animals were gently removed from the filter using the back of a scalpel blade and put into a precombusted foil boat. The sample was dried at 60°C to constant weight. Triplicate 100 ml samples of each suspension of known animal density were filtered onto precombusted and weighed filters. The dry weights of the samples were measured and the dry weight of each animal was estimated.

Measurement of energy content

The energy content of food and faeces was estimated by means of a wet oxidation technique. The method measures the amount of dichromate remaining unreduced after the oxidation of a sample by a mixture containing a known amount of potassium dichromate (see Strickland & Parsons, 1968; Russell-Hunter *et al.*, 1968 and Crisp, 1984). The method is outlined in Appendix 3. Protein content must be measured separately to make a correction for incomplete digestion of protein (Forster, 1970).

Analysis for protein content

A micro-Kjeldahl technique similar to that used by Forster & Gabbott (1971), was used (see Appendix 3) which measures total nitrogen from which an estimate of protein content can be made.

Feeding of *P. pollicipes* juveniles on mixed diets.

Nine animals of similar size (4.16 ± 0.08 mm RC) were starved for twelve hours prior to the start of an experiment. Over the course of three days each animal was presented with each experimental suspension:

1: A mixed suspension of ca 650 cells μl^{-1} *Skeletonema costatum* and 30 *Artemia* nauplii ml^{-1}.
2: A monospecific suspension of ca 650 cells μl^{-1} *S. costatum*.
3: A monospecific suspension of ca 30 *Artemia* ml^{-1}.

Three controls were set up as above but containing no barnacle. The mixed suspension control was intended to account for any ingestion of the algal cells by the *Artemia* rather than the barnacles although the use of newly hatched (non-feeding) *Artemia* should have ensured minimal ingestion.

Previous algal experiments utilised 20 ml vials and were run over 24 hours, in order to provide time for a measurable decrease in cells. Previous *Artemia* experiments were run over 1-2 hours. This was sufficient to give measurable decreases but did not require the initial prey concentration to be so high as to inhibit feeding. The volume of the experimental containers was increased to 100 ml and a larger number of *Artemia* used to allow for differences in the ingestion rates of algal and animal diets. The compromise was adequate but it did make ingestion rates on algae more difficult to measure.

The experimental and control suspensions were all prepared in 100 ml PTFE bottles which had Velcro attached to the inside of the lids. One barnacle was fixed inside the lid of each experimental bottle and all the bottles were fixed onto a rotating wheel (6 rpm.) at 16°C for 24 hours. The barnacles were then removed, algal counts taken on sub-samples using a modified Fuchs Rosenthal haemocytometer and the total number of *Artemia* remaining counted using a Bogorov tray.

The effect of prey size on the ingestion rate of juvenile *Pollicipes pollicipes*

Artemia were ongrown from hatch to provide a range of prey sizes. The same batch of cysts was used and the nauplii were subsequently fed the same diet. In this way compositional variation could be minimised.

Preparation of the diet

11.25 g of *Artemia* cysts were placed in a 10 litre glass flask with 4 litres of UVFSW. These were incubated overnight with vigorous aeration and the nauplii separated from the cysts as before. The animals were poured into an 80 μm mesh sieve and rinsed with UVFSW. They were then transferred to a clean 5 litre glass flask with 4 litres of UVFSW. 500 ml of *Rhinomonas* (ca 1000 cells μl^{-1}) were added and the culture aerated. The water was changed and the animals fed daily.

When required, animals were removed, rinsed in the 80 μm mesh sieve and resuspended in clean UVFSW. Twenty to thirty animals were removed from the stock culture and the total length measured using a microscope fitted with a micrometer eyepiece.

The method used by juvenile *Pollicipes pollicipes* to ingest larger prey items

Two barnacles ca 3.4 mm R-C were attached to a slate panel, by means of Velcro and Superglue, and placed in a 250 ml glass crystallising dish of UVFSW. Many *Artemia* (5 mm length) were introduced and the behaviour of the barnacles noted. The method employed by the barnacle to capture the prey, transfer it to the mouth region and to ingest it was observed.

The maximum prey size that can be ingested by juvenile *Pollicipes pollicipes*

Due to huge variability quantitative estimates of feeding rates on different sizes of *Artemia* were impossible, so faecal production was used instead. Two *Pollicipes pollicipes* of each of two sizes (6.0 and 11.9 mm RC) were starved for 12 hours prior to the start of observations. The length of 20 - 30 *Artemia* was measured. The barnacles were each placed in 500 ml of aerated *Artemia* suspension (ca 15 ml^{-1}). After 48 hours the beakers were examined for the presence of faeces.

Ingestion rates of *Pollicipes pollicipes* of different sizes.

The prey density shown previously to promote maximum ingestion in a 5.8 mm RC animal was used for each diet (*Rhinomonas* 100 cells μl^{-1}, *Skeletonema* 600 cells μl^{-1}, *Artemia* and *Brachionus* 10 ml^{-1}). A large number of barnacles (ca 60) each of different sizes between 1.25 and 10.7 mm RC length provided replication and ingestion rates were measured as detailed above.

The barnacles were set up in glass vials (2.5, 9 or 20 ml depending on the size of the animal) with a known volume of food suspension. Each experimental vial had a

corresponding control vial. The vials were fixed to the rotating wheel. Algal vials were left for 24 hours, rotifer and *Artemia* vials for one hour. Counts were then made and the ingestion rates calculated as previously described.

The growth of *P. pollicipes* juveniles on natural diets.

The growth of 9 *Pollicipes pollicipes* being fed only 100 ml of *Artemia* (20 ml^{-1}), 11 being fed only 300 ml *Skeletonema costatum* (ca 2000 cells μl^{-1}) and 11 being fed a mixed diet of 300 ml *S . costatum* and 100 ml *Artemia* (same densities) per day was measured. The food density in each 8 litre tank was sufficient to ensure that ingestion rates were never limited by food availability. The RC length was measured as described in Chapter 2 as was the wet weight (animals were dried on absorbent paper, this included holding their opercular flaps against the paper to withdraw any water contained within the mantle cavity) measurements were repeated at monthly intervals for six months.

Results

The estimated energy content of the four natural diets is given in Table 7.1.

Table 7.1. The energy content (J mg^{-1} dry weight and J $organism^{-1}$) of each of the four live diets, calculated from mean energy content, corrected for mean protein content. Each mean was calculated from five samples. The dry weight of each food organism is given.

Diet	Dry weight (mg)	Energy (J/mg d.wt.)	Energy (J/org.)
Rhinomonas	9.62×10^{-8}	23.7	2.28×10^{-6}
Skeletonema	3.47×10^{-8}	16.8	5.83×10^{-7}
Brachionus	3.10×10^{-4}	21.14	6.55×10^{-3}
Artemia	1.50×10^{-3}	23.76	3.56×10^{-2}

The effect of organism density on the ingestion rate of juvenile *Pollicipes pollicipes*.

Figure.7.1a)-d) shows the ingestion rate of *P. pollicipes* juveniles at increasing concentrations of *Rhinomonas*, *Skeletonema*, *Artemia* and *Brachionus*. There is a steady increase in the ingestion rate of *P. pollicipes* juveniles at increasing densities of *Skeletonema* (Fig.7.1a). There was a measurable slowing of the rate of increase at higher cell concentrations and a plateau would be anticipated. The maximum

ingestion rate of 5.8 mm RC *P. pollicipes* was probably not measured but from the function can be estimated to be 1.16 x 10^6 cells animal^{-1} hr^{-1} (0.679 J animal^{-1} hr^{-1}), occurring at ca. 5 J ml^{-1} (8500 cells μl^{-1}), an unrealistically high concentration experimentally let alone in nature. The ingestion rate must saturate at lower concentration, suggesting that the predicted maximum ingestion rate is also unrealistically high.

When feeding on *Rhinomonas* (Fig 7.1b) there is a steep, approximately linear increase in the measured energy ingestion rates with increasing suspension density to around 100 cells μl^{-1} (0.23 J ml^{-1}) after which there is a slowing of ingestion rate to a maximum ingestion rate of 0.232 J animal^{-1} hr^{-1} or ca. 100, 000 cells animal^{-1} hr^{-1} at ca. 3 J ml^{-1} (ca. 1300, cells μl^{-1}). Further increases in the cell concentration caused no further increase in the ingestion rate. Fig 7.1e shows that *Skeletonema* is captured more readily and efficiently than *Rhinomonas*, with both the rate of increase in ingestion rate with increasing cell concentration being more rapid and the overall energy ingested greater (ca. 3 times).

The effect *Brachionus plicatilis* abundance on the ingestion rate of juvenile *P. pollicipes* feeding can be seen in Fig.7.1c. The ingestion rate showed a steep and linear increase with increasing prey abundance, slowing until a sustained maximum ingestion rate of 81 rotifers animal^{-1} hr^{-1} (equivalent to 0.53 J animal^{-1} hr^{-1}) at a suspension density of about 35 rotifers ml^{-1} (0.23 J ml^{-1}) was reached. Increases in prey concentration only have a very marked effect between 0 - 0.1J ml^{-1} (0 - 15 animals ml^{-1}). There is a much more rapid increase in the rate of rotifer ingestion than algal ingestion (Fig. 7.1e). This rapid levelling out of ingestion rate suggests that there is a limit as to how many rotifers may be ingested per hour and once a sufficient number of prey items are accessible in suspension, the presence of more animals does not increase the rate at which they are ingested. The estimated maximum energy intake of *P. pollicipes* feeding on rotifers is less than that estimated when feeding on *Skeletonema*.

Fig.7.1d. shows the effect of varying the suspension density of *Artemia* nauplii on the ingestion rate of juvenile *Pollicipes pollicipes*. This relationship is somewhat different from those seen before. There is very high variability in measured ingestion rates and no significant relationship between prey abundance and barnacle feeding rate ($r = 0.267$ for 43 df, $P = 0.076$). The ingestion rate was independent of prey density over the range of densities measured and the mean was 57.6 ± 4.2 (SE) *Artemia* an^{-1} hr^{-1} (2.14 J animal^{-1} hr^{-1}), assuming that prey abundance is not limiting. This suggests that capture and ingestion efficiency are high. Fig 7.1e shows how much higher the

energy intake of *P. pollicipes* is when feeding on *Artemia* compared to all the other diets.

The ingestion of mixed diets by juvenile *Pollicipes pollicipes* .

Table 7.2 shows that mean ingestion rates for each diet in the presence or absence of the other. The ingestion rates appear similar, regardless of the presence or absence of the other diet, although marginally higher in the presence.

Table 7.2. The mean ingestion rate of P. pollicipes juveniles feeding on *Artemia* nauplii in the presence and absence of *Skeletonema costatum* and the mean rate of *S. costatum* ingestion in the presence and absence of *Artemia*. SD = standard deviations and n = number of observations = 9.

Species	Species 2	Ingestion rate (organisms/hr)	SD.
Artemia	Present	14.00	5.87
Artemia	Absent	12.36	8.27
Skeletonema	Present	1098894.00	173750.00
Skeletonema	Absent	944060.00	444988.00

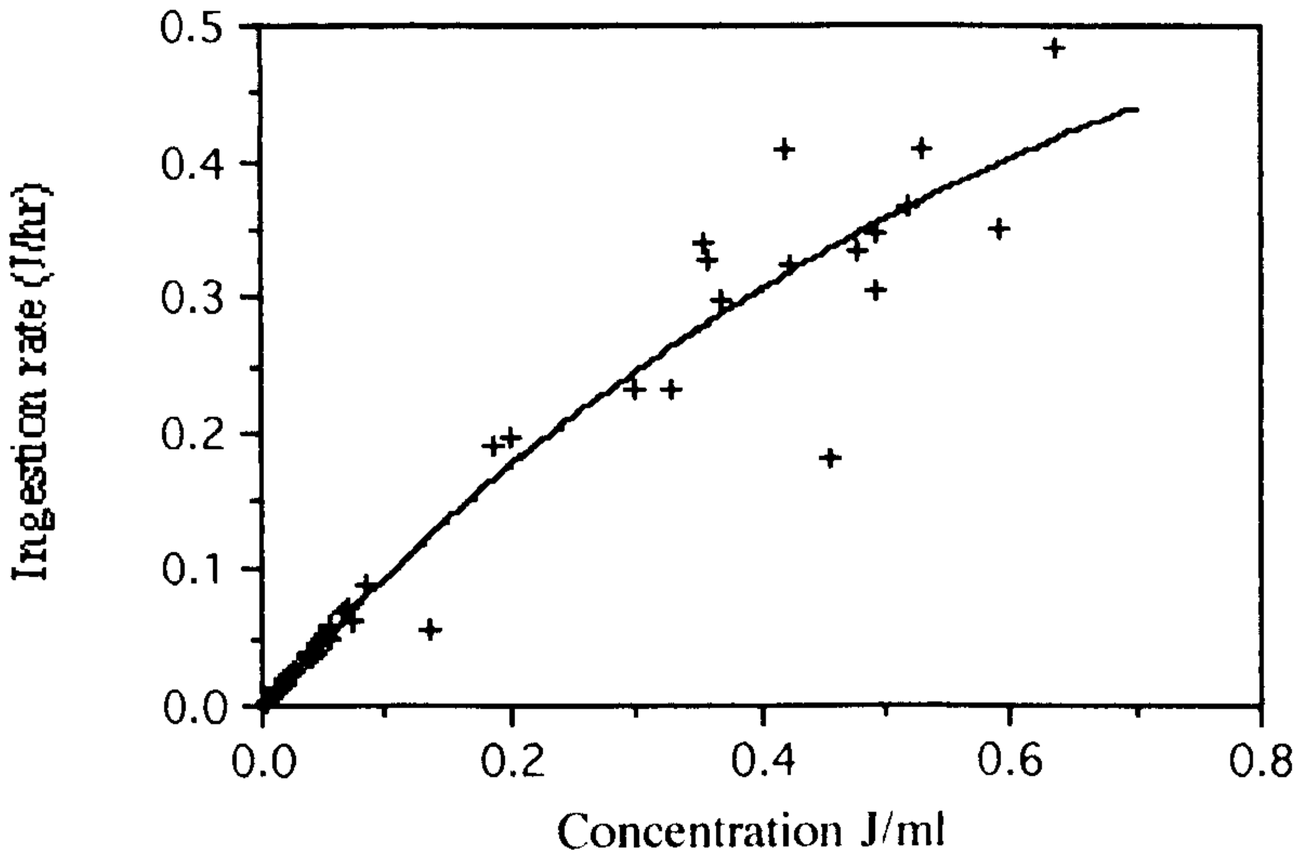
The *Artemia* data showed no significant heterogeneity of variance (Cochran's C = 0.6650, 2 and 8 df) while that of the algae showed significant heterogeneity (C = 0.8676). An analysis of variance on the effect of presenting the barnacles with *Artemia* alone and together with algae on the rate at which they ingest *Artemia* showed no significant difference between the mean ingestion rates (Table 7.3, F = 0.24; P = 0.633).

Table 7.3. Analysis of variance for the effect of *Skeletonema costatum* presence or absence on the rate of *Artemia* ingestion by juvenile *Pollicipes pollicipes*.

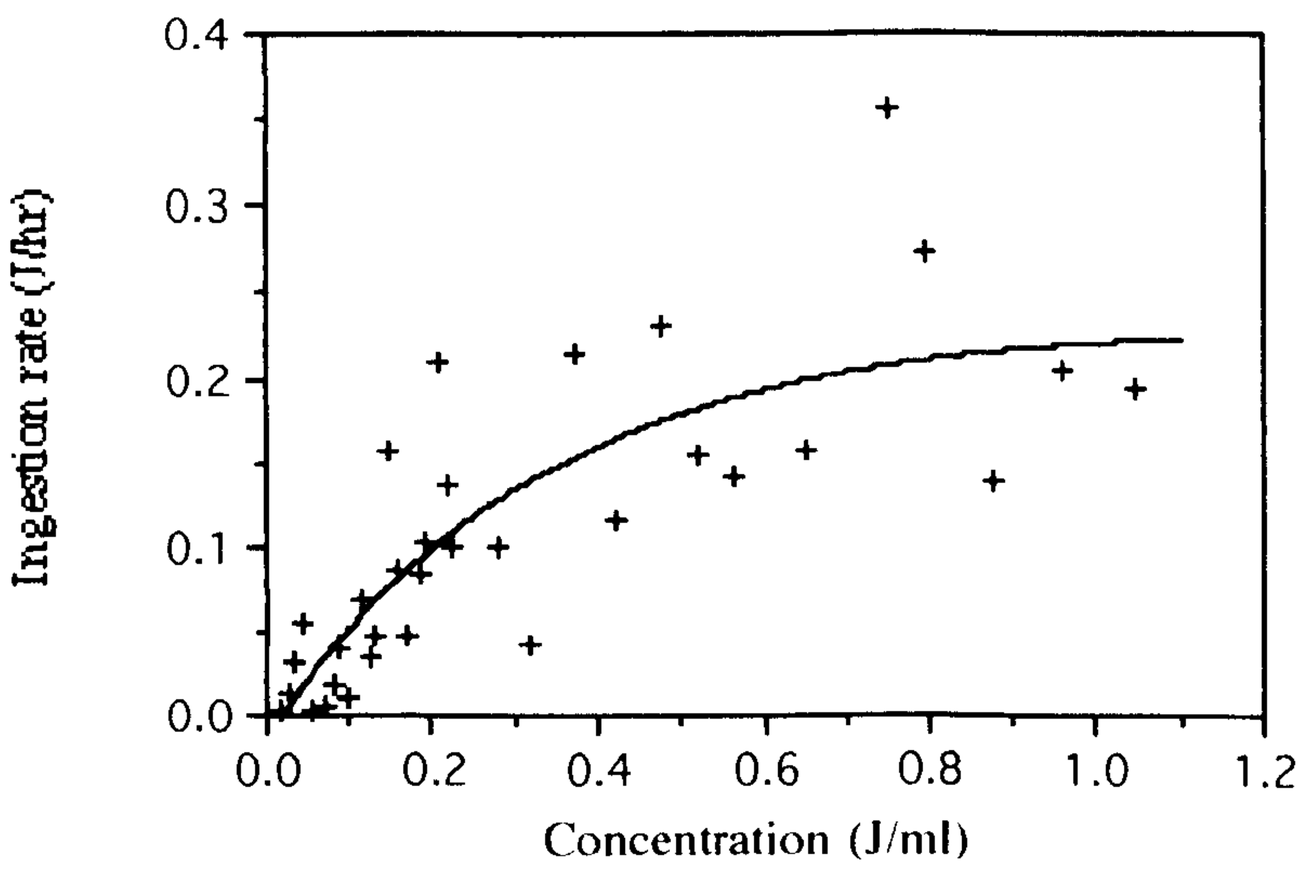
Variation	d.f.	Sum sq.	Mean Sq.	F-value	P
Present/absent	1	12.2	12.2	0.24	0.633
Error	16	823.4	51.5		
Total	17	835.6			

Figure 7.1a) and b). The effect of a) *Skeletonema costatum* and b) *Rhinomonas reticulata* concentration on the ingestion rate of *P. pollicipes* of 5.8 mm RC.

a)



b)

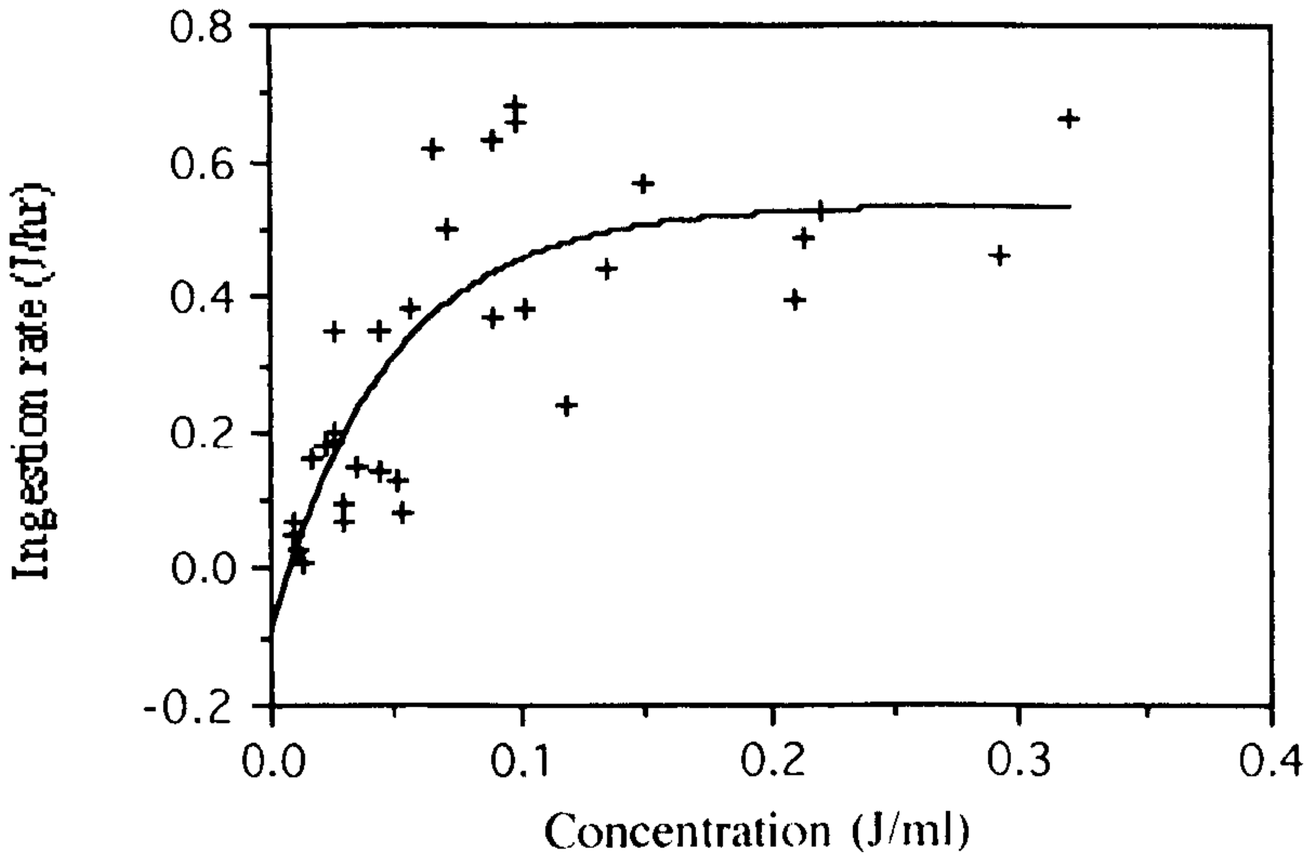


Fitted curves $I_r = I_{max} \{1\text{-}e^{(-k|C-C_0|)}\}$, where I_r = ingestion rate, I_{max} = maximum ingestion rate, K = initial rate of increase and C_0 = Concentration below which no feeding occurs.

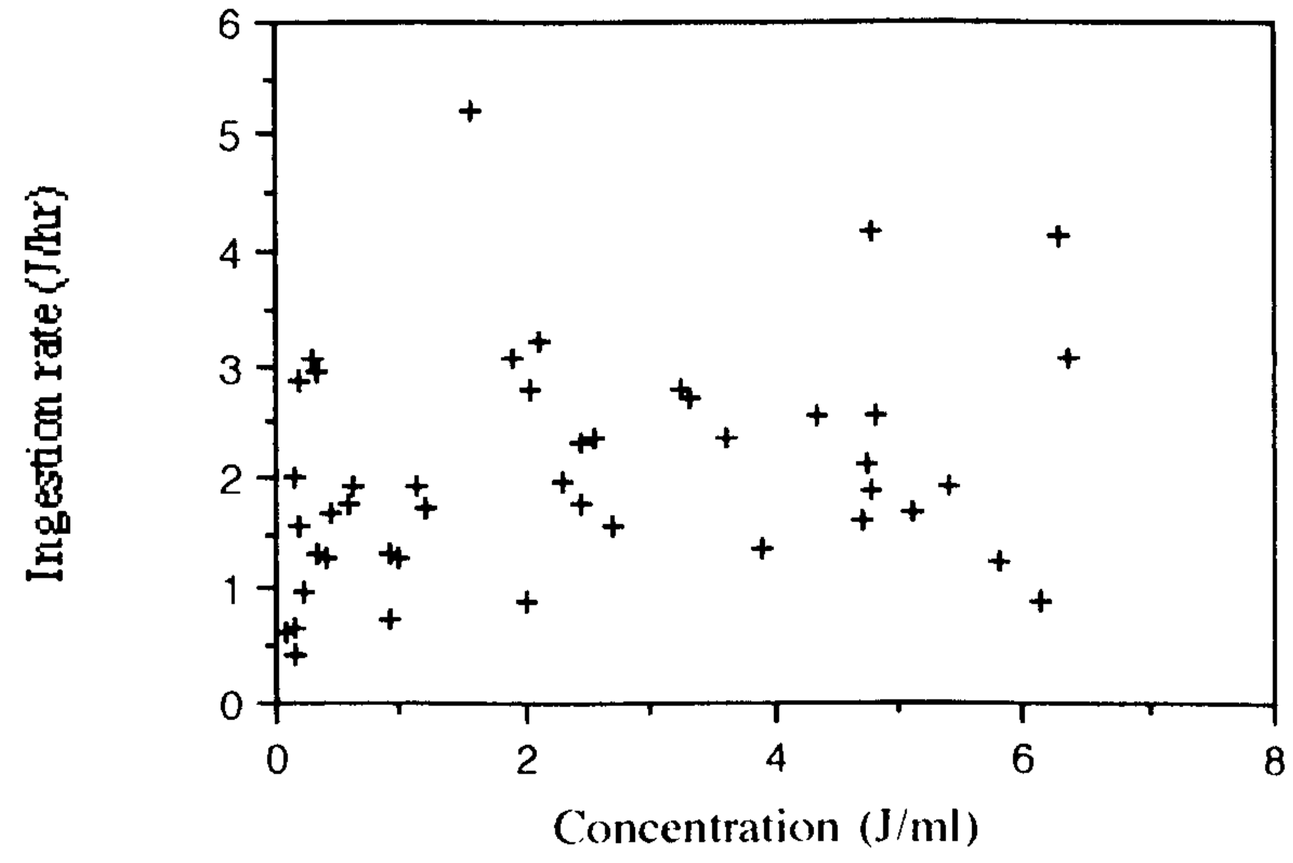
	Parameter	Estimate	Asymptotic SE	CV
a)	I_{max}	6.796×10^{-1}	2.380×10^{-1}	0.3503
	K	1.490	7.729×10^{-1}	0.5187
	C_0	1.070×10^{-3}	1.208×10^{-2}	11.2938
b)	I_{max}	2.32×10^{-1}	3.901×10^{-2}	0.1682
	K	3.037	1.356	0.4463
	C_0	1.757×10^{-2}	3.283×10^{-2}	1.8684

Figure 7.1c) and d). The effect of c) *Brachionus plicatilis* and d) *Artemia salina* concentration on the ingestion rate of *P. pollicipes* of 5.8mm RC.

c)



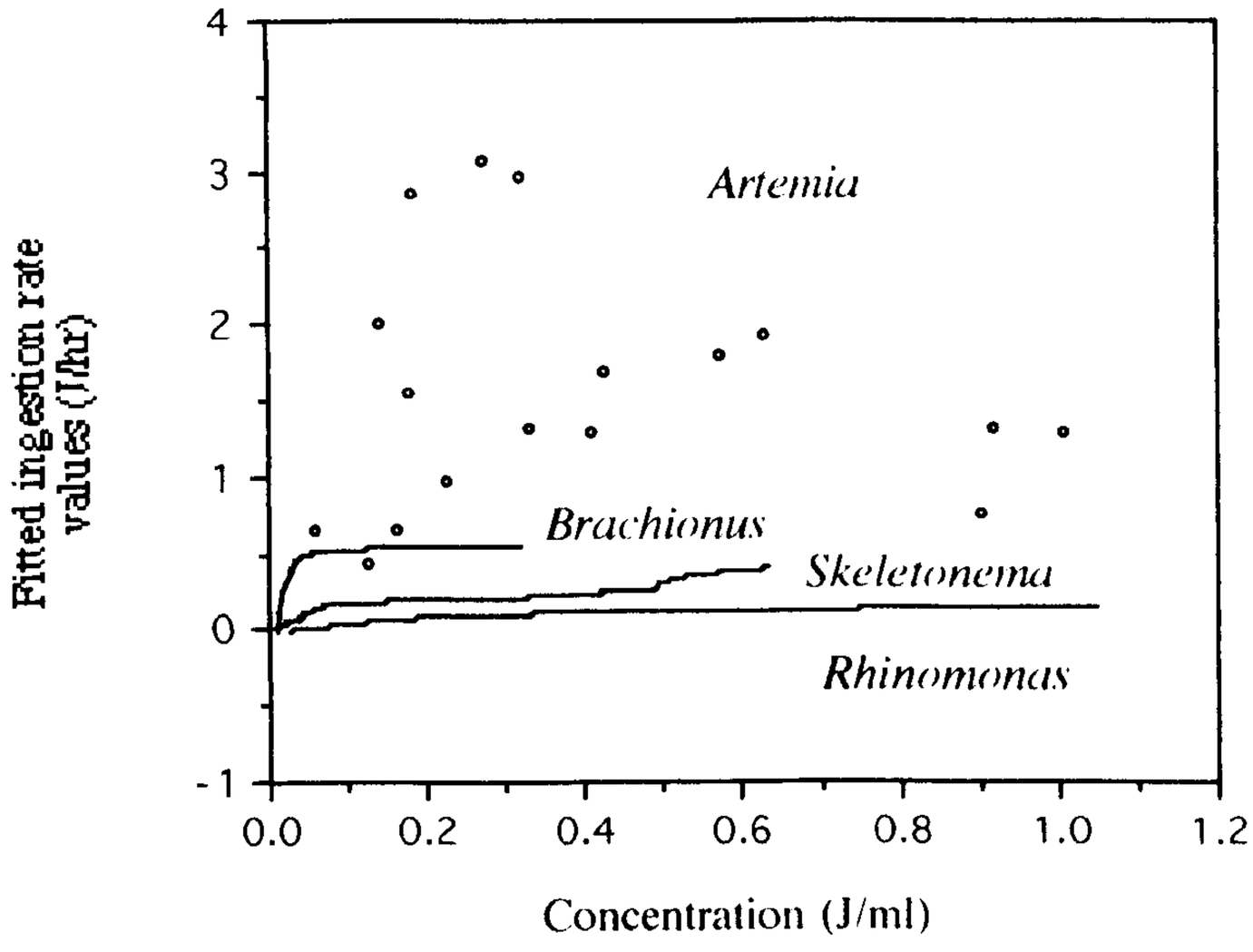
d)



Parameter		Estimate	Asymptotic SE	CV
c)	I_{max}	5.336×10^{-1}	5.833×10^{-2}	0.1093
	K	20.58	7.587	0..3686
	C_0	7.168×10^{-3}	6.024×10^{-3}	0.8405

Figure 7.1e) Combined plot showing the fitted ingestion rate/concentration curves for *Brachionus, Skeletonema* and *Rhinomonas*. and the measured rate of ingestion of *Artemia* for comparison.

e)



The difference between the median ingestion rate of *P. pollicipes* feeding on *Skeletonema* when presented as a monospecific diet and then in conjunction with *Artemia* was tested for significance using the Kruskal-Wallis analysis (Table 7.4). An H-value of 0.24 (1 df, P = 0.627) was obtained. Hence the presence of one food type had no influence on the ingestion rate of another.

Table 7.4. Kruskal-Wallis test for the difference between the rate at which juvenile *Pollicipes pollicipes* ingest *Skeletonema costatum* in the presence of, and in the absence of *Artemia* nauplii.

Level	N	Median	Ave. Rank	Z-Value
Presence	9	1078704	10.1	0.49
Absence	9	1049768	8.9	-0.49
Overall	18		9.5	
H = 0.24	df = 1		P = 0.627	

The growth rates of *Pollicipes pollicipes* feeding on natural diets.

Figs 7.2 and 7.3 show the changes in rostro-carinal length and wet weight observed in animals feeding on the three diets over six months. Animals feeding on a monospecific diet of *Skeletonema* do not grow at all, either in RC length (Fig. 7.2, r = 0.109, 65 df, P = 0.5) or wet weight (Fig. 7.3, r <0.001, 65 df, P = 0.937). Analyses of covariance showed no significant difference between the rate of growth, measured as increased rostro-carinal length (Table 7.5) or wet weight (Table 7.6), of animals feeding on the monospecific diet of *Artemia* or on the mixed diet of *Artemia* and *Skeletonema*.

Figure 7.2. The mean rostro-carinal length of *P. pollicipes* over a 6 month period feeding on monospecific diets of *Artemia* or *Skeletonema costatum* or a mixed diet of the two. Regression performed on all data (only means plotted).

Artemia only	rostro-carinal length = 8.130 + 0.445 (time in months)	
	r = 0.55	P < 0.001
Mixed diet	rostro-carinal length = 0.909 + 0.104 (time in months)	
	r = 0.359	P = 0.005
Skeletonema	r = 0.109	P = 0.5

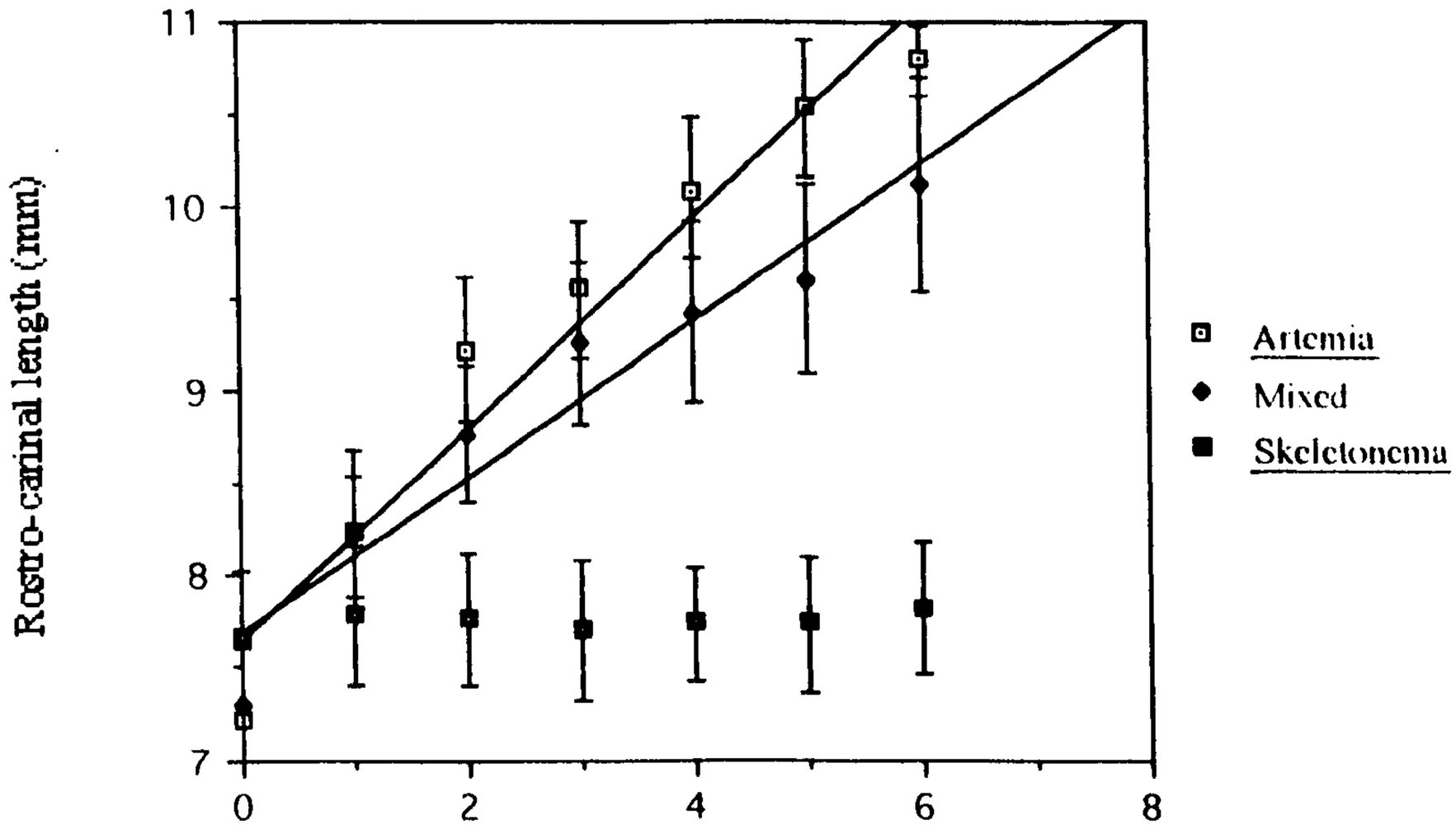


Table 7.5. Analysis of covariance for the effect of diet on RC length over time and the growth rates of *P. pollicipes* feeding on the diets.

Variation	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Month	1	53.998	54.768	54.768	30.49	<0.001
Diet	1	6.184	0.084	0.084	0.05	0.829
Interaction	1	0.793	0.793	0.793	0.44	0.508
Error	116	208.355	208.355	1.796		
Total	119	269.330				

Growth rates of *P. pollicipes*

Diet	Growth rate (mm month^{-1})	SE
Artemia	0.45	0.072
Mixed diet	0.35	0.072

Figure 7.3. The wet weight (g) of *Pollicipes pollicipes* over a 6 month period when feeding on monospecific diets of *Artemia* or *Skeletonema costatum* or a mixed diet of the two. Mean values (± SE) are plotted. Regression and analysis of covariance performed on all data.

Artemia diet	wet weight (g) = 0.293 + 0.928 (time in months)	
	r = 0.770	P < 0.001
Mixed diet	wet weight (g) = 0.349 +0.108 (time in months)	
	r = 0.446	P < 0.001
Skeletonema diet	r = <0.001	P = 0.937

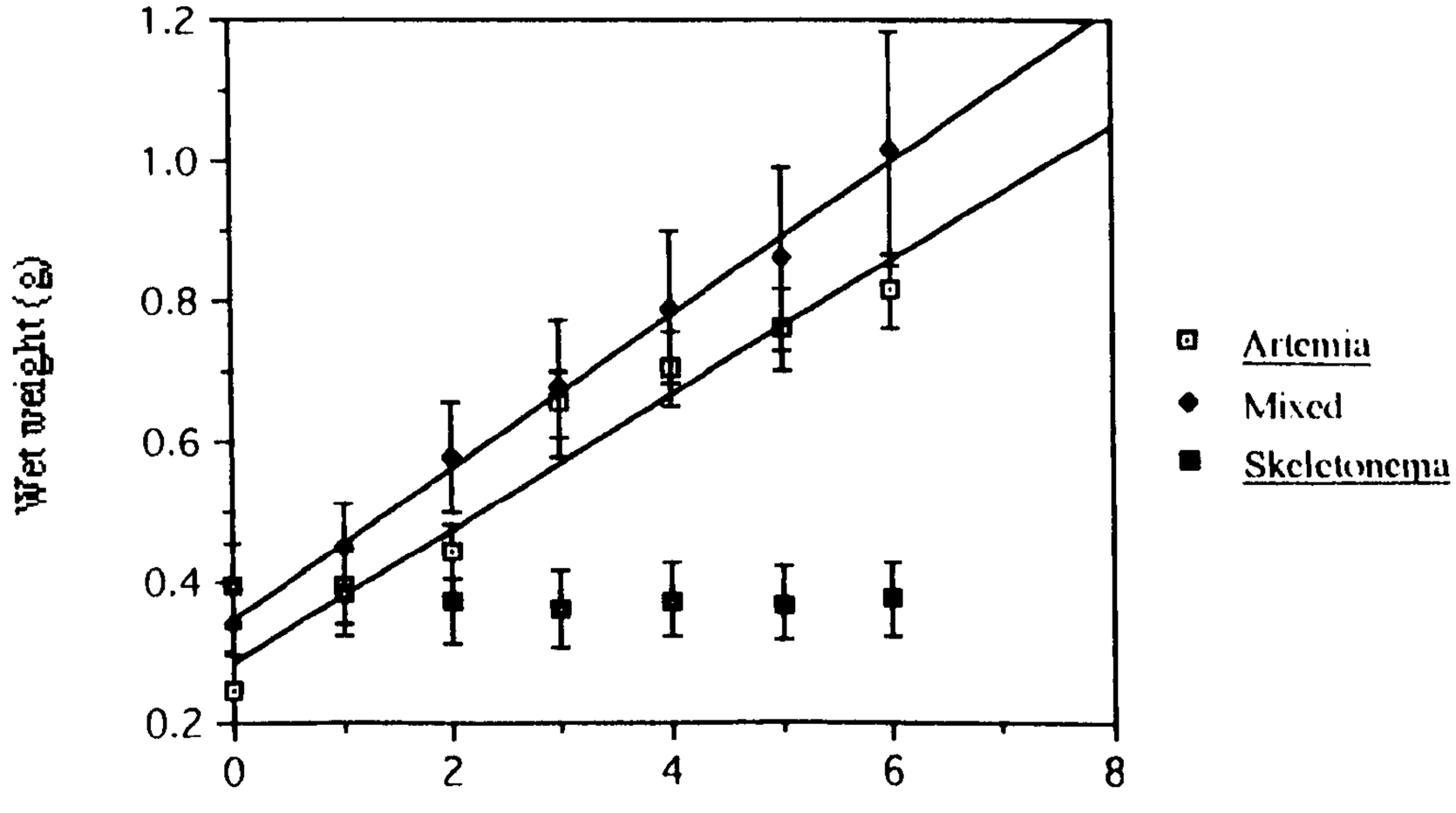


Table 7.6. Analysis of covariance for the effect of diet on the wet weight of *P. pollicipes* over time and growth rates on the diets.

Variation	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Month	1	3.292	3.025	3.025	34.60	<0.001
Diet	1	0.312	0.016	0.016	0.18	0.674
Interaction	1	0.018	0.018	0.018	0.20	0.652
Error	104	9.091	9.091		0.087	
Total	107	12.712				

Growth rates of *P. pollicipes*

Diet	Growth rate(g month^{-1})	SE
Artemia	0.0928	0.0171
Mixed diet	0.1082	0.0171

The moulting frequency of animals feeding on the different diets was also assessed by collecting moults from each tank when the water was changed (Fig. 7.4). The moult frequency of animals feeding on *Artemia* alone and on the mixed diet is similar whereas that of the animals feeding on *Skeletonema* alone is much lower. All moulting rates were very low during month 4 (December) when the water temperatures were particularly low. When moult frequencies were averaged over the seven months, the rates were as follows (± SD); algae only diet 0.442 (± 0.32), mixed diet 1.187 (± 0.26) and *Artemia* only diet 1.051 (± 0.38). A Friedman test of moult frequency by diet and by month indicated that diet had a significant effect on moult frequency ($S = 6.33$, 2 df, $P = 0.043$). Only the moult frequency of barnacles feeding on the mixed diet and algae only were significantly different from one another (although only at the 10% level).

The effects of prey size on the feeding behaviour of juvenile *Pollicipes pollicipes*.

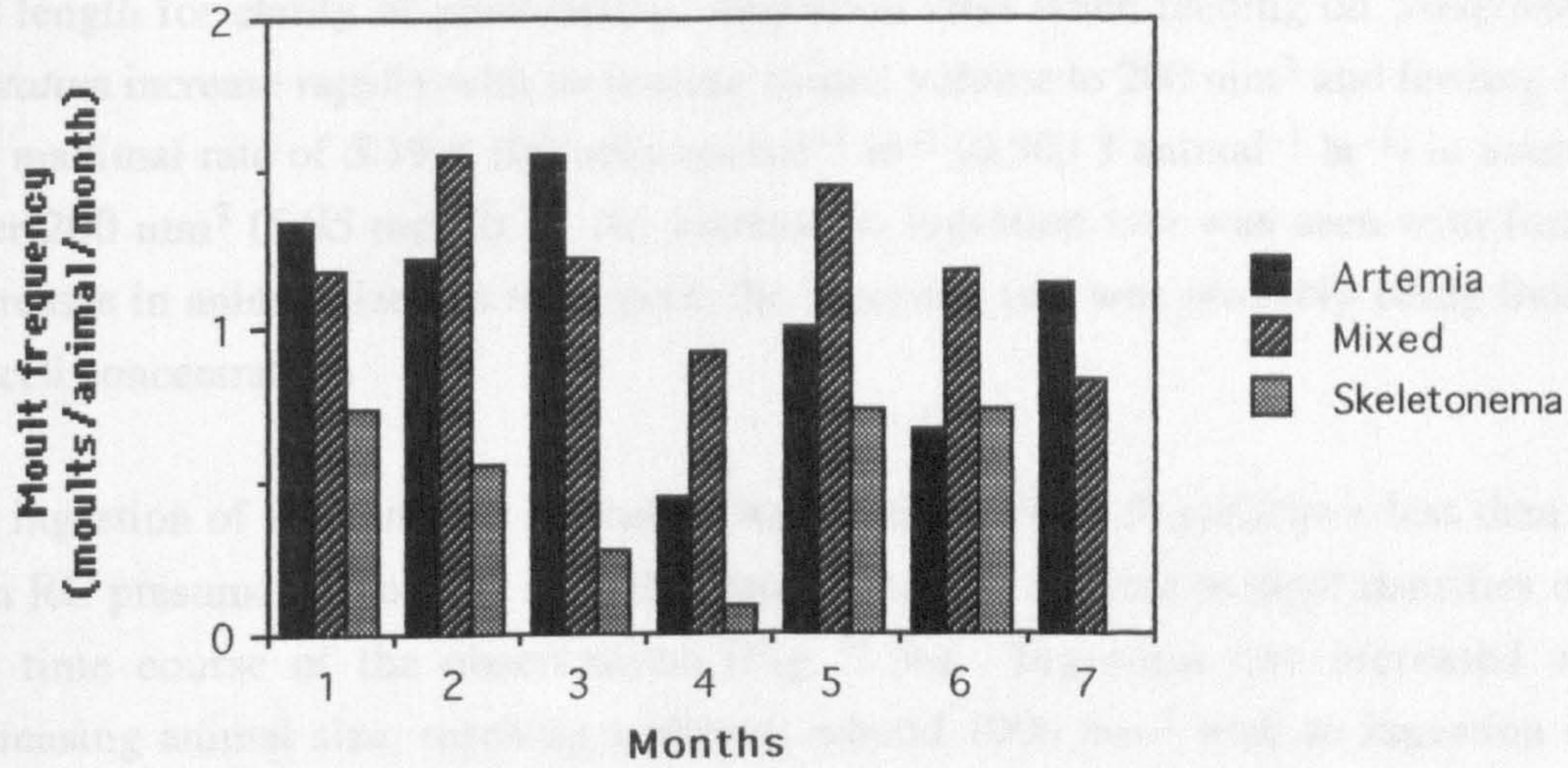
i. The method of capture of larger prey

The cirral net is extended and contact with an *Artemia* on the captorial cirri may result in the curling of the cirrus or whole net in towards the mouth. Some contacts with *Artemia* elicit no response. To see what then happens is very difficult as it is masked by the cirri, but the captorial cirri are wiped across the maxillipedes thereby transferring the captured prey. The alternating action of the maxillipedes (cirri I-III) appears to push the prey item towards the mouthparts and also aids the mouthparts in breaking up the prey prior to ingestion.

ii. The maximum prey size that can be successfully ingested

Adult *P. pollicipes* (RC 11.9 mm) were able to ingest *Artemia* of 6.64 mm in length, the largest size used. Juvenile *P. pollicipes* (5.5 - 6.6 mm RC) were able to successfully capture and ingest *Artemia* up to and including 3.9 mm in length. Larger *Artemia* were captured but they were not successfully ingested. Many *Artemia* were damaged by the barnacles; the heads and limbs were broken off but were not ingested in any great numbers.

Fig. 7.4. Moult frequency of animals feeding on monospecific diets of *Artemia* and *Skeletonema costatum* and on a mixed diet of the two. Moult frequency is expressed in terms of moults an^{-1}month^{-1}.



The effect of *Pollicipes pollicipes* size on the rate at which they ingest live prey.

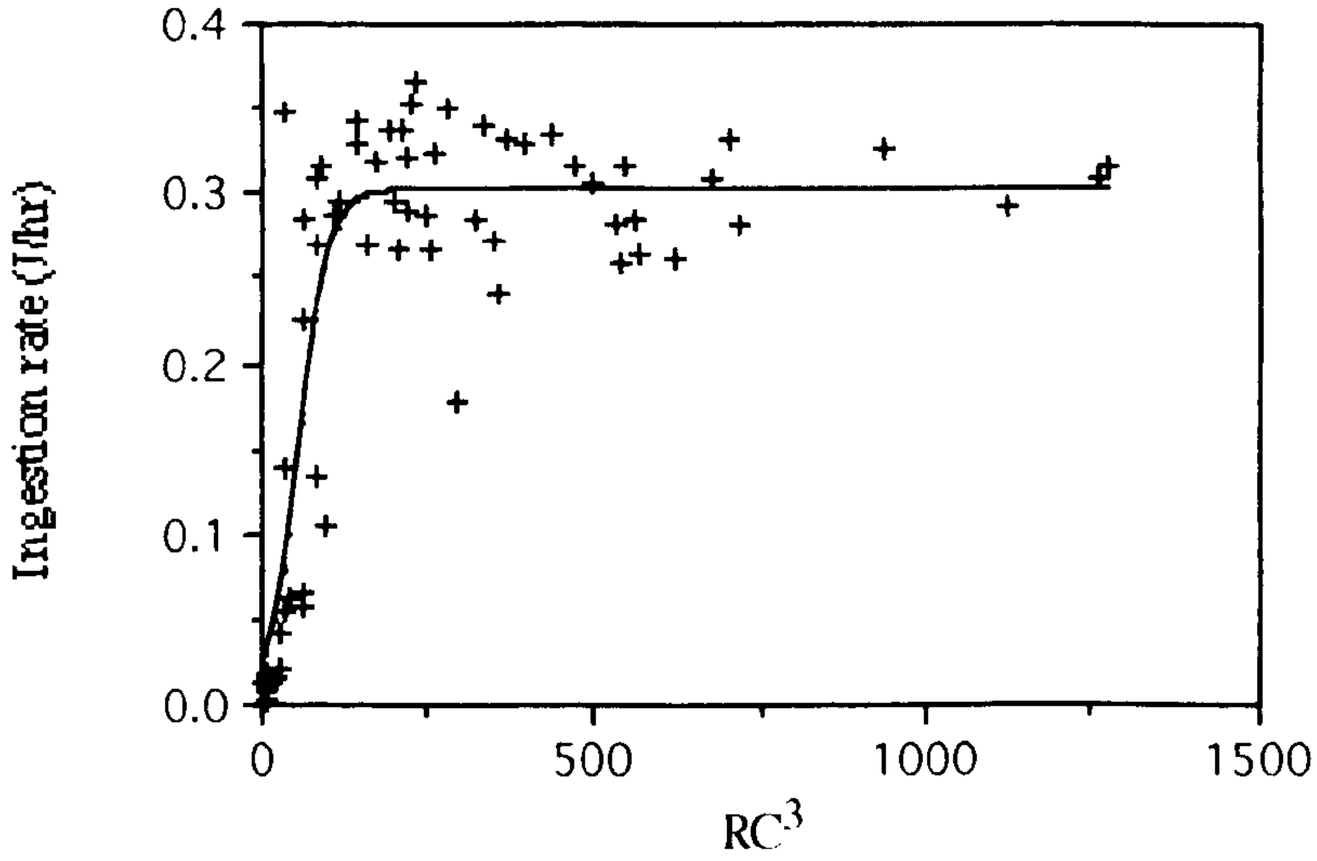
Fig. 7.5a shows the ingestion rates of *P. pollicipes* feeding on *Skeletonema* plotted against increasing animal volume (the cube of RC length assumed proportional to animal volume). Volume is used as the scale of measurement of size in preference to RC length for clarity of presentation. Ingestion rates when feeding on *Skeletonema costatum* increase rapidly with increasing animal volume to 200 mm^3 and feeding is at the maximal rate of 5.19 x 10^5 cells $animal^{-1}$ hr^{-1} (0.303 J $animal^{-1}$ hr^{-1}) in animals over 200 mm^3 (5.85 mm RC). No increase in ingestion rate was seen with further increases in animal size. In retrospect, the ingestion rate was probably being limited by cell concentration.

No ingestion of *Rhinomonas reticulata* was measurable in *P. pollicipes* less than 3.0 mm RC presumably because ingestion rates were low relative to algal densities over the time course of the observations (Fig. 7.5b). Ingestion rate increased with increasing animal size, reaching a plateau around 1000 mm^3 with an ingestion rate around 1.18 x 10^5 cells $animal^{-1}$ hr^{-1} (0.268 J $animal^{-1}$ hr^{-1}). The ingestion of *Rhinomonas* and *Skeletonema* by *P. pollicipes* differs. Maximum ingestion of *Skeletonema* occurs at a much smaller body size than of *Rhinomonas*,; chainforming diatoms are almost equally well handled by all sizes whilst single cell flagellates are only efficiently captured (relative to prey density) by large juveniles (RC ca 8 mm). Although, the measured maximum rates were not dissimilar and clearly related to the limitation imposed by the density of algae offered.

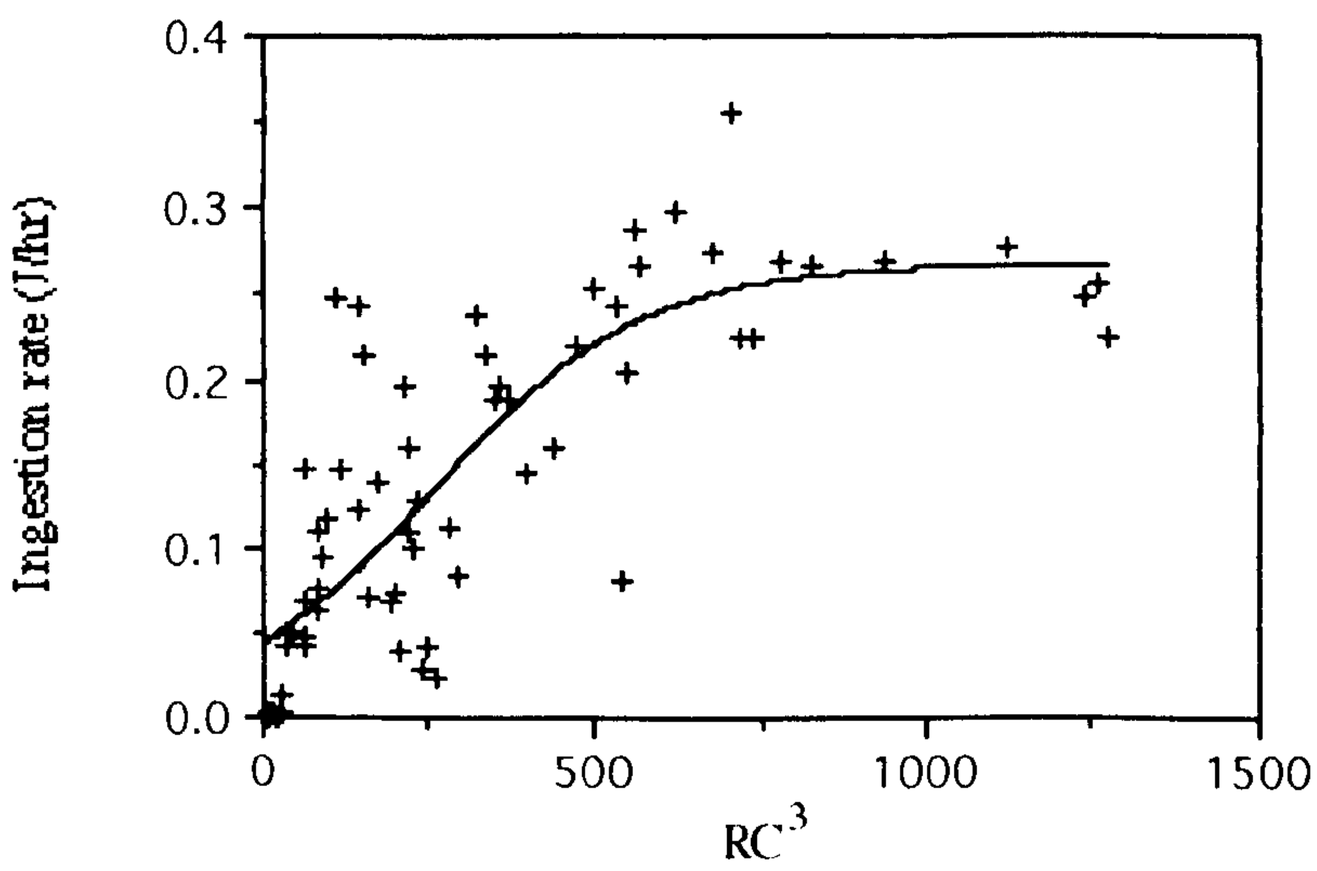
Fig. 7.5c and d show the ingestion rates of *P. pollicipes* feeding on *Brachiomus plicatilis* and *Artemia* respectively. Apart from magnitude the pattern showed is similar for both. Only the very small juveniles are unable to capture rotifers or *Artemia* at the maximal rate possible given the prey concentration. Indeed no juvenile barnacle < 1.5 mm RC length (3.4 mm^3 relative volume) provided any measurable ingestion of *Artemia*. As is inevitable with indirect estimates of ingestion, variability was high on both diets with around 80 rotifers (0.5245 J) per animal per hour and 60 *Artemia* (2.14 J) per animal per hour ingested by *P. pollicipes* > 5 mm RC length (125mm^3).

Figure 7.5 a) and b). The ingestion of a) *Skeletonema costatum* and b) *Rhinomonas reticulata* by *Pollicipes pollicipes* of different sizes. Size is expressed as RC^3 which is proportional to weight. Ingestion rates are expressed in terms of energy ingested (Joules animal^{-1} hour^{-1}) . Prey concentration was 6 x 10^5 cells ml^{-1} (0.35 J ml^{-1}) and 1 x 10^5 cells ml^{-1} (0.23 J ml^{-1}) respectively.

a)



b)

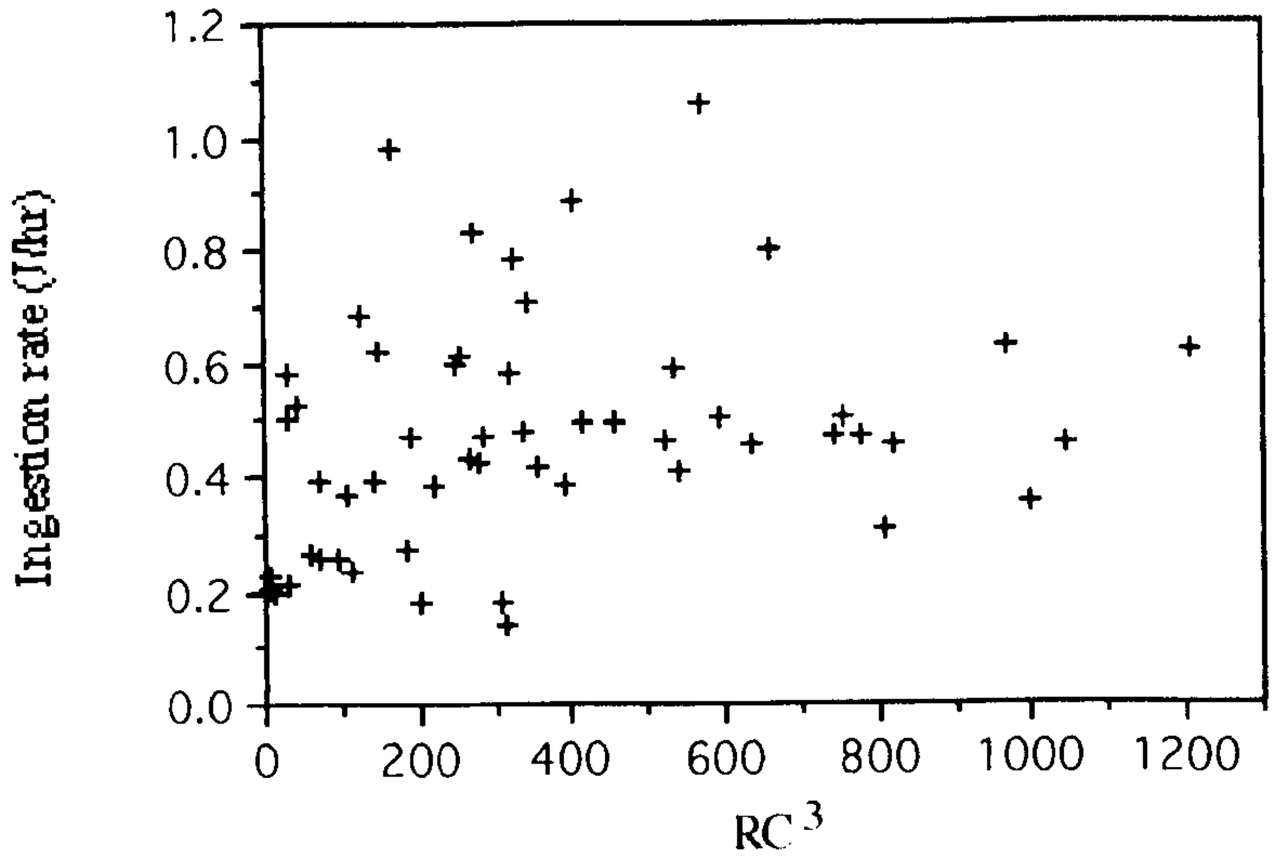


Logistic curves fitted $N(t) = \{ K/|(K-N_0)/N_0| \, |e^{(-rt)}|\}$, where K = maximum ingestion rate, N_0 = size below which ingestion does not occur and r = intrinsic rate of increase in ingestion.

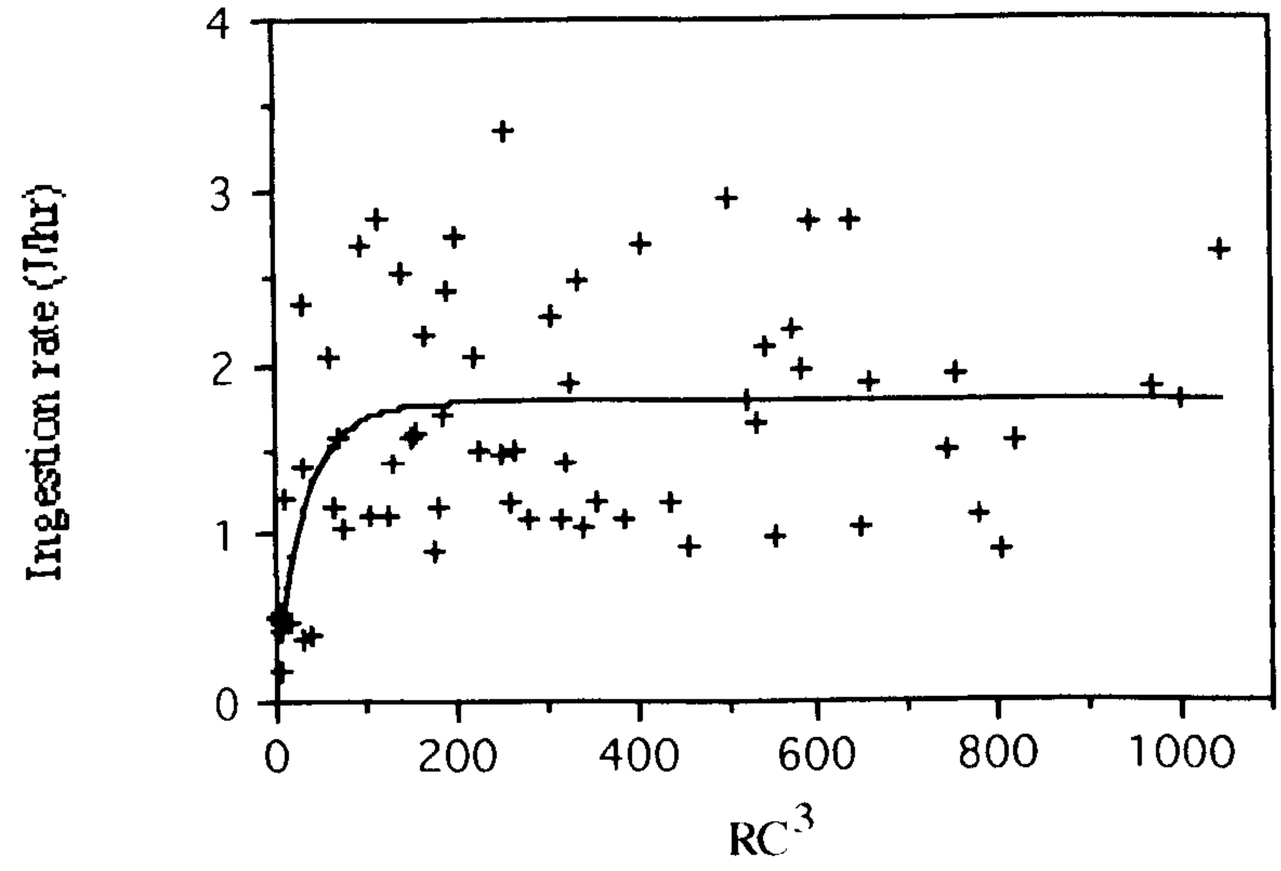
	Parameter	Estimate	Asymptotic SE	CV
a)				
	r	4.356×10^{-2}	9.519×10^{-3}	0.2185
	K	3.028×10^{-1}	9.635×10^{-3}	0.0318
	N_0	2.470×10^{-2}	1.204×10^{-2}	0.4876
b)	r	6.317×10^{-3}	1.386×10^{-3}	0.2194
	K	2.680×10^{-1}	1.976×10^{-2}	0.0737
	N_0	4.408×10^{-2}	1.053×10^{-2}	0.2389

Figure 7.5 c) and d). The ingestion of c) *Brachionus plicatilis* and d) *Artemia* by *Pollicipes pollicipes* of different sizes. Size is expressed as RC^3 which is proportional to weight. Ingestion rates are expressed in terms of energy ingested (Joules animal^{-1} hour^{-1}) . Prey concentration was 10 animals ml^{-1} (0.066 and 0.356 J ml^{-1} respectively).

c)



d)



Von Bertalanffy curve fitted: $I_t = I_{max}\{1-e^{(-K|t-t_0|)}\}$ see Fig 7.1a for explanation of terms.

Parameter	Estimate	Asymptotic SE	CV
d)			
I_{max}	1.772	9.633×10^{-2}	0.0544
K	2.827×10^{-2}	1.654×10^{-2}	0.5850
t_0	-5.854	1.189×10^{-1}	-2.0319

Discussion

Pollicipes pollicipes are able to feed on both an animal and algal diet, as indicated by the gut contents of animals in the wild (Chapter 6). Ingestion rates when feeding on algae are high in terms of numbers (>100,000 cells hr^{-1}) but the energy intake per hour is only a fraction of that when feeding on animal food.

The ingestion rate of *P. pollicipes* juveniles when feeding on *Skeletonema costatum*, *Rhinomonas reticulata* and *Brachionus plicatilis* increased with food abundance until a certain food density was reached, thereafter the ingestion rate was almost constant. These trends are similar to those reported for many planktonic filter feeders. Frost (1972), who tried to model the process in copepods, discovered that the ingestion rate of *Calanus* showed a directly proportional increase with increasing concentration up to a saturation point, the optimal concentration. Above this, the ingestion rate was limited by the rate at which material passes through the gut or by the mechanical speed of the filtering process.

It was estimated that *Pollicipes pollicipes* (5.85 mm RC) can ingest a maximum of 100,000 cells hr^{-1} (0.23 J $animal^{-1}$ hr^{-1}) at food concentrations of >1300 cells μl^{-1} when feeding on *Rhinomonas* and over a million cells per hour (0.68 J) when feeding on *Skeletonema*. Ingestion rates in the tens of thousands have been measured for much smaller copepods (Betouhim-El & Kahan, 1972; Frost, 1972; Abu-Rezeq, 1992).

The ability of an animal to capture algal cells (and therefore ingestion rate) will depend on the relationship between cell size and the mesh size of the filtering apparatus. *Skeletonema* and *Rhinomonas* cells are both smaller than the measured intersetal distances of *P. pollicipes* cirri (see Chapter 4) so it is likely that capture efficiency is low. The measurements were made assuming that the maxillipede setae were arranged in a comb-like manner which was not the case on the more proximal segments, where the setae have a dense brush-like arrangement. Although the distance of each seta base from its neighbour is measurable, there is significant overlapping of the setae that cannot be quantified and as a result there is a tendency to overestimate the functional mesh size. However, a chain-forming or clumping alga is effectively a much larger food item for a filter feeder than a single cell and, for this reason, would be captured more frequently. When a chain or clump of *Skeletonema* cells is captured, this single event can account for the loss of several cells from the suspension compared to the loss of only one cell when ingesting *Rhinomonas*.

There was a 6.5 fold difference between the number of *Rhinomonas* and *Skeletonema* cells required to allow the maximum ingestion rate. Abu-Rezeq (1992) looking at *Tisbe furcata* found a 5.3 fold difference - a close similarity for two very different filter feeders. The differences between the numbers of *Rhinomonas* and *Skeletonema* required to promote maximum ingestion rate reflects the relative sizes of the two cells. When comparing the rates of *Rhinomonas* and *Skeletonema* ingestion by *P. pollicipes*, the overall energy ration taken by the animals is more relevant than the numbers of cells ingested. Yule (1982) found that *Elminius modestus* larvae feeding on *Rhinomonas* ingest far fewer cells than those feeding on *Skeletonema* although the intake was very similar in terms of organic carbon. The difference in the energy content of the two food species at least partially explains the need of the barnacle to ingest much higher numbers of *Skeletonema* cells.

Algal concentrations of thousands of cells μl^{-1} needed to produce maximal ingestion by *P. pollicipes* in the laboratory are extremely rare in the sea where levels are more commonly ≤10 cells μl^{-1} (see Waugh, 1957; Murphy & Haugen, 1975; Throndsen, 1978). The stimulation of maximal ingestion rates by unnaturally high algal densities has been observed by other workers. Yule (1986) found that barnacle nauplii required algal concentrations in excess of 100 cells μl^{-1} in order to achieve a maximal increase in the volume of water handled (volume swept). The harpacticoid copepod *Tisbe furcata* ingested *Skeletonema* cells maximally at a concentration of 80 cells μl^{-1} whilst there was no measurable ingestion of *Pavlova lutheri* by *Tisbe* at concentrations less than 25 cells μl^{-1} (Abu-Rezeq, 1992). Urry (1965) found that *Pseudocalanus elongatus* needed concentrations of *Isochrysis* of 30 - 100 cells μl^{-1} for survival.

Such discrepancies between cell densities in the sea and those shown necessary for physiological processes have been addressed by Dagg (1977) and Yule (1986). They suggest that the algal concentrations reported in the sea are incorrect and underestimate the higher algal abundance in some areas resulting from patchiness. Oyster larvae require at least 50 cells μl^{-1} to survive so there must be patches of high cell density and areas of few or no cells which average out to low density when 10 litres are sampled at a time (Crisp *et al.*, 1985).

The difference in the cell size of *Skeletonema* and *Rhinomonas* is reflected in their rates of ingestion by *P. pollicipes* of various sizes feeding on the two species. The ingestion rates by very small barnacles could not be measured within the variability associated with counting and a more precise technique is required. The intersetal distances of the maxillipedes of small animals (Chapter 4) would enable more efficient algal capture than exhibited by larger animals although their cirral net area is so small

that the number of cells being ingested is likely to be low. The ingestion rates increase with barnacle size until the intersetal distances of the cirri become so large that further increases in cirral net area are offset by loss between the setae, and thereafter, algal ingestion rates remain constant. Hence, when feeding on the smaller cells of *Skeletonema*, the maximum ingestion rate is reached by *P. pollicipes* of a smaller size.

What limits the ingestion rate is unknown, but factors may include those described by Frost (1972): the maximum rate at which algal cells can be successfully captured by the cirral setae, transferred to the mouth and ingested or, once the gut is filled, the rate of passage through the gut. The last possibility is the least likely considering the low defecation rates of *P. pollicipes* feeding on monospecific algal diets. These must be attributable to the inability of these barnacles to ingest sufficient material to fill the gut and sustain a significant rate of throughput. Rough calculations based on estimates of midgut volume (Chapter 6), estimates of algal cell volumes and measured ingestion rates indicate that an adult barnacle feeding on *Rhinomonas* may take as long as 47 days to fill its gut whilst a 6 mm RC juvenile may take 17 days. More refractory food may reside in the gut for longer to enable more thorough digestion.

Eating algae is not necessary for a barnacle to obtain sufficient energy or nutrients essential for health and growth. Algae may simply be captured and ingested incidentally during macrophagous feeding. In the laboratory, the animals fed on algae alone barely maintain their body weight let alone grow, although they survived for up to 12 months. *P. pollicipes* fed on a mixed diet of *Artemia* and *Skeletonema* did not show a significantly higher rate of growth than those fed on *Artemia*, suggesting there is no advantage to feeding on a mixed diet. Although *P. pollicipes* are omnivorous, zooplankton are necessary if body weight is to increase and perhaps also for reproduction, for no egg masses were found over a six month period in the tank of animals receiving the algal diet whereas eggs were frequently found in the other tanks. Lack of reproductive success confirms the lack of energy supplied by the algal diet which was just about sufficient to maintain the barnacles but not enough to allow either somatic or reproductive growth.

Rotifers and *Artemia* were captured individually by the captorial cirri. In general, the rate at which rotifers were ingested depended on their abundance until a concentration of 10 rotifers (1.07 J) ml^{-1} when the ingestion rate became limited, probably by the rate at which a captured animal was transferred to the mouth and successfully ingested. *Artemia* nauplii were larger than the intersetal distances of the captorial cirri so it was likely that the capture efficiency was approaching 100%. The ingestion rate

was constant, regardless of suspension density. This agrees with the findings of Crisp (1961) who noted that the filtration rate of actively feeding barnacles was, for large particles, independent of the particle concentration in the water, provided the minimum requirement was exceeded (the same as for algae). *Artemia* were ingested at a slower rate (numerically) than the rotifer, *Brachionus* (at around 60 *Artemia*, 2.14 J hr^{-1} compared to 80 rotifers, 0.53 J hr^{-1}). *Artemia* contain ca 5.4 times the energy of rotifers yet the maximum energy ingestion when feeding on *Artemia* is only four times that on rotifers so the energy content of the two diets does not, alone, account for the difference in ingestion rates. It seems likely that, because of their larger size, *Artemia* filled the barnacles' guts more quickly than rotifers and the subsequent rate at which they were ingested was determined by the rate of passage of material through the gut and not just limited by the handling time.

Low numerical densities of *Artemia* which allow maximal ingestion rates by *P. pollicipes* represent higher energy and more realistic numerical densities than can possibly be provided by rotifers or algae. Even juvenile *P. pollicipes* are clearly better able to capture sufficient energy from large particulate material than from smaller animals or algae.

P. pollicipes <1.5 mm RC length do not appear able to ingest *Artemia* nauplii or even rotifers with any great efficiency. No intermediate sized food (between rotifers and algae) were used hence optimal particle size for these post-metamorphic barnacles was not obtained.

The fact that *P. pollicipes* >5 mm RC length appear to ingest *Artemia* (and *Brachionus*) at the same rate regardless of size would seem to suggest that either the smaller animals have a much lower digestion efficiency and require a quicker throughput of material or that the limiting step was mechanical but not related to the size of the cirral net. The smaller barnacles clearly ingest more *Artemia* per unit weight than larger animals although their total energetic costs are likely to be of the same order, allowing for higher metabolic costs (Castellani, pers. comm.) and growth rate. There is no published literature on the metabolic costs for *P. pollicipes* and none available regarding digestion efficiency.

Large adult *P. pollicipes* were, however, able to feed on *Artemia* of at least 6.64 mm in length whereas juveniles (6.0 mm RC) could not ingest *Artemia* over 3.9 mm in length. Many *Artemia* were damaged (stripped of all limbs) but not ingested, particularly by the small barnacles. *Lepas anserifera* (Bieri, 1966; Jones, 1968) has been documented as feeding on material 5 - 40 mm long. Howard

& Scott (1959) described the macrophagous predatory behaviour of *L. anatifera* and *Mitella* (= *Pollicipes*) *polymerus*. Both species were allowed to feed on *Artemia* 5 - 11 mm in length and *Tigriopus* 1 mm long for 2 hours and the gut contents examined. The larger barnacles of both species ingested copepods and *Artemia*, the smaller ones took only copepods. Prey animals that were originally longer than the barnacle digestive tract were first bitten into pieces, folded and compacted to fit into the gut. If adult *P. polymerus* are able to feed on prey of up to 11 mm in length, so too might *P. pollicipes*.

Juvenile *P. pollicipes* showed no preference between *Artemia* and *Skeletonema* but can apparently capture both simultaneously, the presence of one in no way affecting the ingestion rate of the other. Captorial feeding involves the alternating combing action of the maxillipedes to transport material to the mouth hence algal cells captured on the maxillipedes would also be ingested. This may indicate that the barnacles are fairly indiscriminate feeders, eating all particles that are retained by the cirral filter. However, the low proportion of inorganic material in the guts of animals in the wild (see Chapter 6) and in the guts of the closely related *P. polymerus* (Barnes, 1959; Lewis, 1981) suggests this is not the case or that a rejection mechanism is operating. Based on observations with *Balanus perforatus*, two independently acting feeding mechanisms were suggested by Southward (1955a); the capture of algae by cirri I and II, and larger organic particles such as zooplankters, by cirri III-VI. The simultaneous use of these two mechanisms allows the full utilisation of all available food resources and maximum energy intake. Lewis (1981) hypothesised that in juvenile *P. polymerus*, the filtration of algae from the water occurs because of the current generated by rhythmic cirral beating. Neither juvenile nor adult *P. pollicipes* exhibited cirral beating in conditions of water flow (Chapter 5) yet algal ingestion still occurs, and the captorial action of cirri IV-VI would be unlikely to generate the necessary water movement to enable algal capture to occur. *P. pollicipes* appear to feed like *Balanus perforatus* (Southward, 1955a) except that the dense setation of cirrus III suggests its involvement in microparticle filtration in addition to cirri I and II.

In summary, *Pollicipes pollicipes*, like other species of barnacle, is omnivorous - ingesting appreciable numbers of micro-algae and zooplankton. However, the maximum energy intake from an algal diet alone is far less than may be obtained from a zooplankton diet and insufficient to allow somatic growth let alone reproduction. Micro-algae may have a contribution to make in terms of energy intake or nutritional composition, but it is insignificant compared to the larger organic material of animal origin, even for small barnacles. The capture and ingestion of algae and zooplankters

involve different cirri and can occur simultaneously, either by design or incidentally. The measured ingestion rates were stimulated by what were considered unnaturally high prey concentrations and, because of the density dependence of ingestion rates, may be far in excess of the rates in the wild.

Chapter 8

Digestion of natural diets by *Pollicipes pollicipes*

Introduction

The assimilation efficiency* of several barnacle species has been investigated and generally found to be very high. *Balanus balanoides* feeding on *Artemia* nauplii assimilated about 80% and 85% of their dry weight and calorific content respectively (Ritz & Crisp, 1970). Assimilation efficiencies over 90% have been documented in *Balanus improvisus* eating barnacle nauplii (Kuznetsova, 1973), *Balanus glandula* on a diet of *Skeletonema costatum* (Wu & Levings, 1978) and *Pollicipes polymerus* (Page, 1983) feeding on *Artemia*. Crisp & Southward (1961) observed that *Elminius modestus* utilises proteinaceous material readily (particularly that of animal origin), while pure fats and starches usually pass through the gut unchanged. Total digestion and the digestion of individual dietary components have also been studied in other Crustacea (see, Conover, 1966; Forster & Gabbott, 1971; Forster & Beard, 1973; Lasenby & Langford, 1973 and Cosper & Reeve, 1975) generally showing digestive efficiencies are high but vary with diet.

Having established the rates at which *Pollicipes pollicipes* ingests various live diets, it is necessary to assess the ability of the barnacle to digest the nutritional components of each diet if ingestion rates are to be interpreted in terms of useful matter or energy to the animal. Before *P. pollicipes* can be cultured commercially it is essential to know digestive efficiencies and how they may vary with diet. Some indication may emerge as to whether feeding and digestive efficiencies could be increased by the use of diets enriched in particular substances. Such diet enrichment is frequently used in the preparation of live and artificial diets in aquaculture (e.g. shrimp, Fast & Lester, 1992).

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* probably actually digestion efficiency, i.e. what actually crosses the gut wall, and not assimilation which is digestion minus the nitrogen which is never incorporated into the tissues - there is considerable confusion in the literature.

Materials and Methods

Pollicipes pollicipes were fed monocultures of four live diets; *Skeletonema costatum*, *Rhinomonas reticulata*, *Artemia* nauplii and *Brachionus plicatilis*. Food and faecal samples were collected. The growth rates of barnacles feeding on each diet were monitored by measuring rostro-carinal length (RC) every two months (see Chapter 2). After 11 months the animals fed solely on algae were switched to animal diets as their peduncles had become thin and animals had started to die.

Preparation of food and faecal samples for analyses.

Faecal collection and sample preparation.

Between ten and fifty juveniles (5 - 7 mm RC) and adult (>9 mm) *P. pollicipes* (subject to availability) were fed each diet. The animals were attached to slate panels by means of Velcro and were placed in 10 l. plastic tanks. The following diets were given, to a tank containing juveniles and a tank containing adults:

100 ml (~900 ml^{-1}) newly hatched *Artemia* suspension
1000 ml (~2400 cells μl^{-1}) *Skeletonema costatum* suspension
1000 ml (~1000 cells μl^{-1}) *Rhinomonas reticulata* suspension
500 ml (~140 ml^{-1}) *Brachionus plicatilis* suspension

Each tank was topped up to 8 litres with UVFSW. The animals were fed daily but the faeces were removed and the water changed every 48 hours. Faeces produced during the first week were discarded as they may have contained traces of previous diets. Subsequently, faeces were removed, separated from other material using a pipette and thoroughly rinsed with three changes of 3.9% ammonium formate solution to remove salt. Faeces were placed in pre-weighed glass vials and dried at 37°C. The method of sample drying, although not the best, was the most convenient, and adequate when subsequently determining only the gross proportions of the various constituents (see Giese, 1967). Faeces were added to these vials each time faeces were collected.

Food collection and sample preparation.

To obtain representative samples of the diets (allowing for compositional variability) regular samples were taken. Samples were placed in four glass vials, one for each diet. One litre of each algal suspension (*Skeletonema* 2400 cells μl^{-1}, *Rhinomonas* 1000 cells μl^{-1}) was centrifuged at 2600 x g. for 15 min. The supernatant was poured away and the algal material resuspended in a few drops of 3.9% ammonium formate solution. A 20 ml suspension of newly hatched *Artemia* (900 ml^{-1}) was poured into an 80 μm mesh sieve and rinsed with 3.9% ammonium formate solution. A 20 ml suspension of rotifers (~140 ml^{-1}) was treated similarly.

Samples were dried at 37°C to constant weight. When a sufficient quantity of faecal and food material had been collected to perform all the required analyses, the samples were thoroughly crushed and as far as possible homogenised to ensure that each subsample contained a fully representative batch of food or faeces from the whole collection period and that the particles were as small as possible, providing a maximal surface area for reaction.

Unless otherwise specified, all sample weights were obtained using a Cahn C31 microbalance. A Shimadzu UV1201 Spectrophotometer was used for all absorbance readings.

Measurement of the ash weights of food and faeces

Triplicate 1 mg dried food and faecal samples were weighed into precombusted, preweighed foil boats. The samples were placed in a muffle furnace at 450°C for 7 hours and the weight of remaining ash determined.

Calculation of dry matter digestion efficiency of barnacles feeding on different diets

The digestion efficiency of juvenile and adult barnacles feeding on the four diets were calculated from the ash-free dry weights of food and faeces. This method for direct measurement of the organic matter digestion does not require the quantitative recovery of faeces and was described by Conover (1966). It assumes that only the organic component of the food is significantly altered by the digestive process, hence it is only necessary to obtain the ratio of ash-free dry weight to dry weight (proportion of organic matter) of food and faeces. The method of calculating digestion efficiency given in Appendix 4.

Energy and Protein Measurement

The energy content and Kjeldahl protein were measured using the methods described in Chapter 7 and Appendix 3.

<u>Protein determination using a Pierce BCA Protein Assay Reagent.</u>

The BCA Assay combines the reaction of protein with Cu^{2+} in an alkaline medium (producing Cu^{1+}), with a highly sensitive, selective, detection reagent for Cu^{1+} (Bicinchoninic acid).

Standard Preparation

Standard concentrations of Bovine Serum Albumin (BSA) were made up in the diluent (3:3:4, 0.3 M NaOH, 0.3 M HCl and distilled deionised (ddH_2O) at concentrations of 200, 400, 600, 800, 1000, 1200 $\mu g\ ml^{-1}$.

Triplicate 1 mg food and faecal samples were weighed into glass tubes. The protein in the samples was solubilised by the addition of 300 μl of 0.3 M NaOH and incubation at 56°C for 30 minutes (see Waite, 1986). Assay reagents A and B were mixed in the proportion of 50:1 to produce a clear light green liquid. Samples were neutralised by the addition of 300 μl 0.3 M HCl and made up to 1 ml with ddH_2O. 100 μl of each standard and sample were pipetted into labelled test tubes. A blank of 100 μl of the diluent was put into another tube. To each tube, 2.0 ml of the reagent mix were added and the contents mixed. Tubes were incubated in a water bath at 37°C for 30 min then cooled to room temperature. The absorbance of each solution was measured at 562 nm in a 5 mm (path length) cuvette against a water reference. The absorbance of the blank was subtracted from that of each standard and sample and a standard curve of net absorbance against protein concentration was plotted (Appendix 4). The protein concentration of the samples (relative to bovine serum albumin) was calculated from the calibration curve.

Although both methods give only an approximation to the total protein content, the Kjeldahl method consistently gave a higher estimate of the protein content of *Artemia* than BCA. The BCA method measures soluble protein hence its accuracy as a method of estimating total protein depends upon complete solubilisation of protein by NaOH and assumes that the NaOH does not destroy any protein that will then not be detected. To try and increase the degree of solubilisation, a series of protein measurements were made after a sample had been incubated in NaOH for 15, 30, 60 and 90 minutes. There was no difference in the protein released. The volumes of the reagents were then doubled to ensure that the NaOH was not becoming saturated, again with no significant effect. Using 0.5 M or 10 M NaOH rather than 0.3 M also had no effect on the protein measured. The incubation temperature was increased to 100°C for 2 hours (see Holland & Walker, 1975) but this did not increase the protein solubilisation. Either the BCA method consistently underestimated protein content or Kjeldahl nitrogen overestimated the protein content.

The Kjeldahl technique measured total nitrogen in the sample, not all of which came from proteins. To try to elucidate the apportioning of nitrogen within the food and faeces, perhaps obtaining a better estimate of protein content, a series of analyses were performed. Triplicate 1 mg samples of food and faeces were analysed for protein

using the BCA reagent. To triplicate 1 mg samples of food and faeces, 300 μl of 0.5 M NaOH were added and incubated at 56°C for 30 minutes. The mixtures were neutralised with 300 μl of 0.5 M HCl and diluted with 400 μl of ddH_2O. This mixture was centrifuged at 800 x g for 15 minutes. The supernatant was decanted and the particulate material rinsed with diluent. The tube was recentrifuged and the supernatant added to the other. The particulate material that would not dissolve in NaOH was analysed for nitrogen content. To the supernatant, an equal volume of 10% trichloroacetic acid (TCA, a protein precipitant) was added and the tube shaken for 5 minutes. The tube was left to stand for 30 minutes at 4°C, then centrifuged at 800 x g for 10 minutes. The supernatant was collected, the precipitate washed with 10% TCA, recentrifuged and the supernatants combined. The supernatant was evaporated to leave a brown residue. The nitrogen content of the supernatant residue and the pellet were measured by Kjeldahl. A further BCA assay was performed on the supernatant residue.

Determination of chitin content of *Artemia* nauplii and *Pollicipes* faeces.

A similar method to that used by Holland & Walker (1975) and Lucas (1980) was employed to estimate chitin in food and faeces. Replicate 50 - 100 mg samples of *Pollicipes pollicipes* faeces and *Artemia* were homogenised in 5 ml of cold 5% TCA. Samples were left to stand for 30 minutes at 4°C. Homogenates were centrifuged for 30 minutes at 800 x g and the precipitates were washed twice (by resuspension and centrifugation) with 10% TCA at 4°C. TCA enabled the removal of $CaCO_3$ and non-structural carbohydrates. The acid released CO_2 from carbonates and combined with calcium to form soluble calcium trichloroacetate, removed with the supernatant.

Lipids were extracted from the precipitate by washing twice and centrifuging with 5 ml 2:1 chloroform:methanol (v/v). The residues were washed twice with methanol and air dried. Chloroform:methanol was added in excess of 15:1 v/v to the aqueous pellet residue, to prevent the formation of a two-phase mixture which does not form a pellet on centrifugation (Lucas, 1980). The residue, consisting mainly of protein and chitin, was heated at 100°C in a water bath with 10 ml 10M NaOH for 2 hours to hydrolyse protein. The mixture was cooled and diluted with 10 ml of ddH_2O before centrifugation. Without dilution, the high density of 10M NaOH causes the residue to remain as a flocculant in suspension (Lucas, 1980).

The reaction mixture was centrifuged at 800 x g for 30 min. However, a pellet would not form during centrifugation, even following the addition of more ddH_2O, so an alternative method was employed at this stage. Samples were filtered through preweighed, precombusted Whatman 47 mm GFC glass fibre filter papers after the

dilution of the NaOH with ddH_2O. The residues were rinsed six times with ddH_2O, the filters were dried at 35°C for 5 days and then weighed. The remaining material was assumed (Holland & Walker, 1975) to be chitin, perhaps with a little residual protein. The samples were ashed at 450°C for 7 hours to ensure that no inorganic material had remained following the TCA treatment (Lucas, 1980). The weight of any material remaining after ashing would have had to be subtracted from the 'chitin' weight previously obtained.

Analysis of samples for carbohydrate content

The anthone method has been used for the quantitative analysis of glucose, glycogen, maltose, lactose and galactose by Morris (1945), of dextran (Scott & Melvin, 1953; Roe, 1954), for the determination of sugar in blood and spinal fluid by Roe (1955) and the estimation of glycogen by Seifter *et al.* (1950). It was found by Giese (1967) to be preferable to the phenol-sulphuric acid (Dubois *et al*, 1956) or Samogyi (1945) methods for the analysis of total carbohydrate.

The anthrone method (see Appendix 4) relies on the digestion of the sample in hot 5% TCA which liberates glycogen and oligosaccharides; monosaccharides dissolve at once (see Giese, 1967). The sulphuric acid in the reagent dehydrates the sugar to a furfural derivative which then condenses with anthrone to form a blue coloured compound (Sattler & Zerban, 1948). Anthrone reagent is very unstable so must be made up fresh every two days (Roe, 1954). Sample concentrations were read from the calibration curve (see Appendix 4)

Analysis for lipid content

A chloroform-methanol extraction similar to that of Folch *et al.* (1957) was employed (see Appendix 4). Giese (1967) found the method to be both superior to the Soxhlet reflux method and faster than Soxhlet or the Charring method used by Marsh & Weinstein (1966).

Results

Faecal production of *Pollicipes pollicipes* feeding on the experimental diets.

P. pollicipes feeding on algal diets produced very small amounts of faecal material, insufficient for most of the biochemical analyses. Adult animals produced more faecal material when feeding on *Artemia* than on *Brachionus*, whereas juveniles produce similar amounts when feeding on either diet (see Table 8.1).

Table 8.1. The amount of faecal material collected from *Pollicipes pollicipes* feeding on each experimental diet and average faecal production (mg animal $^{-1}$ month^{-1}) are given. N = number of barnacles. * Based on mean number of animals.

Diet	Animal Size	N	Duration (months)	Faecal weight(g)	Production (mg/an./mo.)
Rhinomonas	Adults	6-10	6	<0.001	<0.0208*
Rhinomonas	Juveniles	18	1	<0.001	<0.0536
Skeletonema	Adults	8	12	0.013	0.1354
Skeletonema	Juvenile	8	12	0.009	0.0938
Artemia	Adults	7-12	13	1.199	9.709 *
Artemia	Juveniles	8	13	0.432	4.154
Brachionus	Adults	32	2	0.238	3.719
Brachionus	Juveniles	39	2	0.329	4.218

Ash weights and dry weight assimilation.

The percentage ash was greatest in *Skeletonema* (59%) and at 7%, lowest in *Artemia* (Table 8.2a). The apparent dry matter digestion efficiency (calculated by the ash-ratio method) for *Pollicipes pollicipes* feeding on *Rhinomonas* was unreliable because the foil lid became dislodged during combustion, resulting in contamination of the samples with ash. The large amounts of ash in the combusted sample undoubtedly led to overestimated digestion efficiency (69%). Insufficient faecal material precluded repetition of the analysis. The ash to organic weight ratio is greater in the faeces than in the diets (Table 8.2a). The digestion efficiency of juvenile barnacles is the same as that of the adults (Table 8.2b) while that of barnacles feeding on *Artemia* is around three times that when feeding on *Skeletonema* and about twice that when feeding on *Brachionus*.. There is a strong negative correlation between ash in the diet and digestion efficiency ($r = -0.845$, 4 df, $P = 0.03$).

Table 8.2. The apparent dry matter digestion by *P. pollicipes* feeding on natural diets.

a) Ash weight, organic weight (mg g^{-1} dry weight) and ash/organic weight ratio (A/O) of each diet and faeces. Mean of three replicates (± standard error) for ash weight are given.

	Food			Faeces					
				Juveniles			Adults		
	Ash	Org	A/O	Ash	Org	A/O	Ash	Org	A/O
Artemia	70 (5)	930	0.08	254 (7)	746	0.34	227 (4)	773	0.29
Brachionus	139 (6)	861	0.16	207 (20)	793	0.26	209 (45)	791	0.26
Skeletonema	587 (10)	413	1.42	660 (12)	340	1.94	636 (34)	364	1.75

b) The calculated mean apparent dry matter digestion (and range) for juvenile (5 - 7 mm RC) and adult (>9 mm RC) *Pollicipes pollicipes* feeding on the three diets. Each value is calculated from the mean ash-free dry weight to dry weight ratio of three replicate food and faecal samples. * Average ignoring two zero estimates of efficiency.

Diet	% Mean dry matter digestion efficiency (and range)	
	Juveniles	Adults
Artemia	78 (69.3 - 77.8)	74 (73.1 - 81.5)
Brachionus	38 (13.6 - 51.9)	39 (0.00 - 61.7) 58.5*
Skeletonema	27 (18.9 - 39.5)	19 (0.00 - 39.8) 28.5*

Energy assimilation

Barnacles feeding on algal diets produced insufficient faecal material for the energy content to be assessed. Energy digestion by *P. pollicipes* feeding on *Artemia* and rotifer diets was quite high with little difference between juveniles and adults or between diets (Table 8.3). The efficiency of energy digestion is higher than the apparent dry matter digestion efficiency estimated for a diet of rotifers but very similar for a diet of *Artemia*. Why there should be this discrepancy for a rotifer diet is unclear, but may reflect differences in the digestion of the individual nutritional components.

Table 8.3. The range and mean (in brackets) energy content of rotifer and *Artemia* diets and of the faeces of juvenile and adult *P. pollicipes* feeding on the diets. Energy digested is estimated.

Diet	Sample	Energy content (J/mg dry wt.)	Energy digested (%)
Artemia	food	17.50 - 24.69 (23.76)	
	juvenile faeces	15.36 - 19.54 (17.25)	69 - 83
	adult faeces	12.24 - 15.83 (14.11)	72 - 85
Brachionus	food	16.48 - 22.32 (21.14)	
	juvenile faeces	4.41 - 5.64 (5.16)	77 - 87
	adult faeces	4.58 - 6.74 (5.67)	72 - 86

Protein content of diets and faeces and the problems encountered.

The protein content of each diet and *Pollicipes* faeces measured using the Pierce BCA assay were consistently lower than those calculated using the micro-Kjeldahl method (Table 8.4). The Kjeldahl method measured nitrogen content, from which protein content was calculated using the standard nitrogen to protein conversion factor of 6.25 (see Holland & Gabbott, 1971). Use of the conversion factor assumes that nitrogen is only present in protein and will therefore overestimate protein content if other sources of nitrogen (e.g. chitin) are present. There is obviously a source of non-protein nitrogen in the faeces. The chitin of the cirripede moult contains 80 μg N mg^{-1} (a Kjeldahl equivalence of 49.9% protein). If faeces contained only undigested *Artemia* exoskeletons this could account for the high nitrogen measured. However, the faeces of animals feeding on rotifers contain more nitrogen than the food. The protein content of rotifers and *Artemia* measured by BCA Assay were lower than anticipated (ca 30% and 31% respectively) but the protein content of the faeces was considered more realistic than that measured using the Kjeldahl technique.

Table 8.4 The mean protein content (± SE), as calculated from Kjeldahl nitrogen and Pierce BCA Assay, of each live diet and the faeces of juvenile and adult *Pollicipes pollicipes* feeding on them. N = 3.

Diet	Sample	Protein Content (μg mg^{-1} dry weight) Kjeldahl		BCA	
Artemia	food	574	(64)	314.0	(12.7)
	juvenile faeces	471	(9)	60.0	(7.0)
	adult faeces	471	(21)	54.7	(0.9)
Brachionus	food	553	(65)	297.7	(4.9)
	juvenile faeces	861	(37)	5.7	(5.7)
	adult faeces	867	(86)	0	-

Table 8.5 shows the results of protein and nitrogen analysis of food and faecal samples following various treatments. The nitrogen in the fraction that does not dissolve in NaOH will be primarily chitin and any undissolved protein. The nitrogen in this fraction is higher in the faeces than in the food (ca 22% compared with 12%). The fraction that was precipitated by the addition of TCA were proteins dissolved in the NaOH and this nitrogen in this fraction was lower in the faeces (ca 22%) than in the food (ca 27%). It was anticipated that the nitrogen contribution of the supernatant would be negligible, but in fact most of the nitrogen was found in this fraction (ca 62 and 57% in the food and faeces respectively). Although the primary contributor of the nitrogen in this fraction was not identified, analysis with the BCA Assay reagent indicated that there was 153.25 μg ± 21.3 μg protein mg^{-1} in the food supernatant and 29.77 ± 37.5 μg mg^{-1} in the faecal supernatant. This constitutes an approximate drop

of 81% in the protein content of the supernatant from food to faeces. Assuming all the nitrogen in the TCA precipitatable fraction is protein and that all the protein in the sample is dissolved in the NaOH, by adding the TCA fraction to the protein measured in the supernatant, there is a total of 352.8 μg protein per mg of food (dry weight) and a total of 104.5 μg in the faeces. This constitutes a protein digestion efficiency of 92% and is thought most likely to reflect the true value.

Table 8.5. Mean protein content (A) measured using BCA Reagent, and nitrogen contents measured using Kjeldahl technique (μg mg^{-1}); B is the nitrogen content of precipitate on addition of 5% TCA, C is the non-TCA precipitatable nitrogen and D is the nitrogen contained in the material not dissolved in the 0.5 M NaOH. SE in parenthesis.

Sample	Protein (%)	Nitrogen content (μg/mg)		
	(A)	(B)	(C)	(D)
Artemia	35.87	268.3 (29.7)	615.7 (11.0)	116.0 (19.2)
adult faeces	17.96	218.0 (8.75)	573.0 (156.0)	221.7 (79.4)

Proximal biochemistry

The chitin content of *Artemia* was 23.8 mg g^{-1} (± 1.6 SE, N = 3) and the faeces of adult *Pollicipes pollicipes* feeding on *Artemia* contained 96.3 mg g^{-1}. The value for the faeces was based on one measurement due to the small amounts of sample available for analysis but suggest that there is a four fold increase in chitin concentration from food ingested to faeces egested. Protein digestion efficiencies were high in juveniles and adults (>90%) regardless of diet (Table 8.6) . Carbohydrate digestion was strongly dependent on diet (>83% when feeding on *Artemia* but < 40% on rotifers) but less so on size. Crustacean (barnacle) moults contained 9.1% carbohydrate, hence undigested chitin-like material may account for some of the carbohydrate found in the faeces. The faeces of *P. pollicipes* feeding on *Artemia* contain a higher proportion of lipid than the diet, suggesting inefficient lipid digestion (25 - 65%). However, when feeding on *Brachionus*, lipids are digested with efficiencies in excess of 87% (Table 8.6). It seems likely that there are differences between the lipids present in the two diets.

Table 8.6. The proportion of biochemical constituents contained within each natural diet and in the faeces of juvenile and adult *P. pollicipes* feeding on each. The range and mean (in brackets) % from duplicate (lipid) or triplicate samples (protein and carbohydrate) are given. Calculation of component digestion efficiencies using mean ash contents from table 8.2a.

Sample	Protein	Carbohydrate	Lipid
Artemia			
food	24.2 - 39.9 (31.40)	14.9 - 21.7 (18.30)	9.8 - 14.7 (14.00)
Juvenile faeces	5.3 - 7.4 (5.97)	2.15 - 6.33 (4.83)	18.7 - 23.5 (21.10)
Juv. dig. efficiency	**92 - 96%**	**88 - 97%**	**34 - 65%**
Adult faeces	3.34 - 4.21 (5.47)	3.21 - 7.91 (5.26)	23.1 - 23.7 (23.40)
Adult dig. efficiency	**95 - 97%**	**84 - 95%**	**25 - 52%**
Brachionus			
food	28.9 - 55.2 (29.70)	3.87 - 5.16 (4.63)	5.69 - 6.04 (5.87)
Juvenile faeces	0 0 - 1.7 (0.57)	4.65 - 6.8 (5.73)	0.75 - 0.78 (0.77)
Juv. dig. efficiency	**96 - 100%**	**0 - 39%**	**91 - 92%**
Adult faeces	0.0	5.58 - 8.55 (6.86)	0.99 - 1.09 (1.04)
Adult dig efficiency	**100%**	**0 - 28%**	**87 - 89%**

Growth of *Pollicipes pollicipes* feeding on the experimental diets

Juvenile *P. pollicipes* grew faster than adults and growth rates of barnacles feeding on animal diets were significantly higher than those of the animals feeding on algae. No significant growth could be seen with adults or juveniles fed on either algal diet (RC length vs. time, *Rhinomonas*: juveniles, r = 0.354, 25 df, P = 0.07; adults r = 0.001, 34 df, P = 0.994, *Skeletonema*, juveniles, r = 0.233, 52 df, P = 0.09; adults r = - 0.055, 41 df, P = 0.726). In juveniles there were no significant differences in the growth rates of *P. pollicipes* juveniles feeding on *Artemia* or rotifers. However, the adults on a rotifer diet showed no significant growth within the variability of the measurements. The growth of the adults fed *Artemia* is fairly linear over the first 4 - 5 months then the rate becomes progressively less as the size increases (see Fig. 8.1). Comparisons between growth rates were thus performed over roughly the same time scales by fitting regression slopes to the linear (i.e. early) portions of the growth curves (Table 8.7).

Figure 8.1. Mean rostro-carinal length (± SE) of juvenile and adult *P. pollicipes* eating *Artemia* and juveniles eating rotifers (*Brachionus plicatilis*) over the period of faecal collection. Regression analysis and analysis of covariance performed on all data.

Juveniles, *Artemia* diet RC = 5.54 + 0.928 (time in months)
r = 0.859, df = 16, P <0.001

Juveniles, *Brachionus* diet RC = 5.47 + 0.559 (time in months
r = 0.826, df = 52, P<0.001

Adults, *Artemia* diet RC = 7.90 +0.447 (time in months)
r = 0.771, df = 31, P<0.001

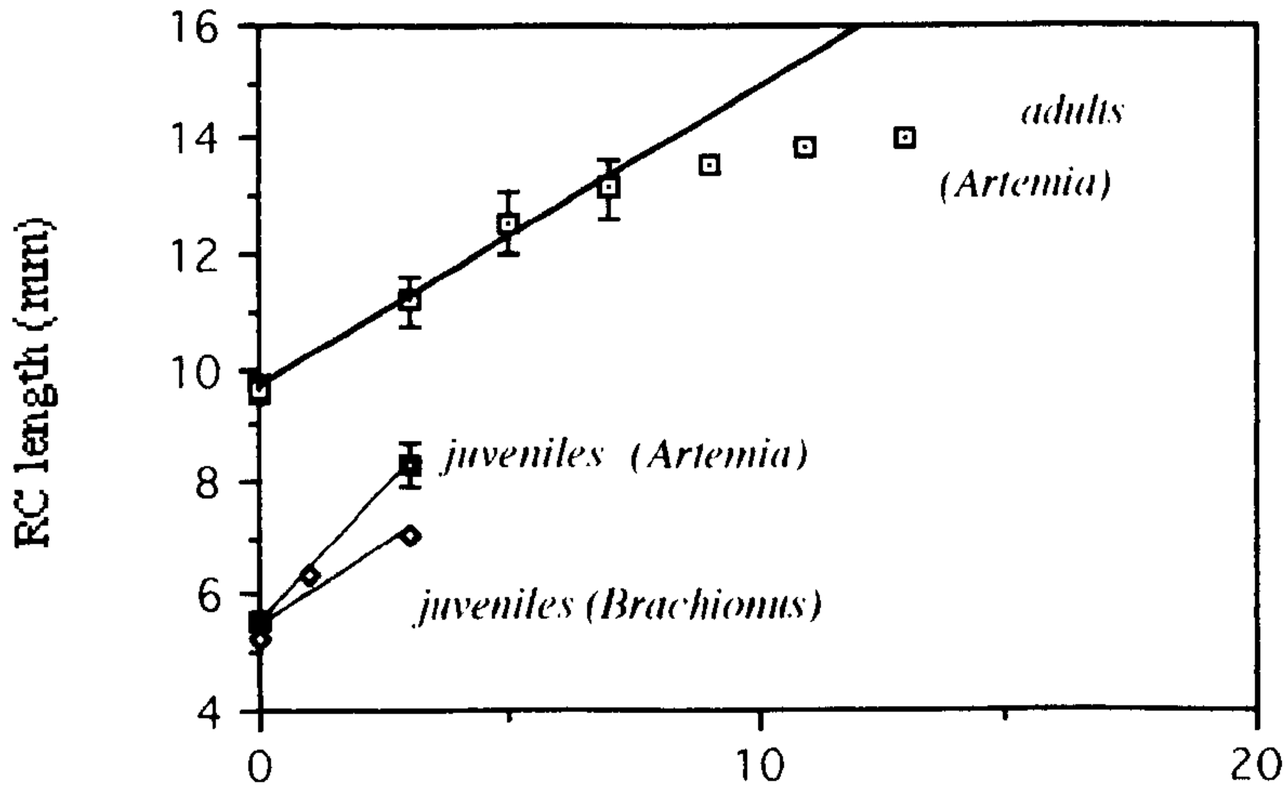

Table 8.7 Analysis of covariance for the rostro carinal length of *P. pollicipes* juveniles and adults feeding on *Artemia* nauplii and juveniles eating rotifers against time.

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
time	1	644.43	94.47	94.47	132.94	<0.001
diet	2	217.14	147.92	73.96	104.07	<0.001
time*diet	2	8.59	8.59	4.30	6.05	0.003
Error	99	70.35	70.35	0.71		
Total	104	940.53				

Growth rates in mm RC month^{-1} (± SE) of *P. pollicipes* feeding on the experimental diets.

Diet	Juvenile		Adult	
Artemia	0.928	(0.095)	0.509	(0.062)
Brachionus	0.559	(0.071)		

Discussion

The highest growth rates were observed in juvenile *P. pollicipes* feeding on *Artemia* (0.93 mm ± 0.095 mm RC month^{-1}), adults grew more slowly (0.509 ± 0.062 mm month^{-1}). These rates were similar to those measured for *P. polymerus* in the wild by Lewis & Chia (1981; 1.4 mm RC month^{-1} in the first year following settlement and 0.17 mm month^{-1} subsequently) and Paine (1974; 0.7 - 1.2 mm RC month^{-1}). Growth rates of barnacles feeding on different diets vary, presumably, according to the food capture efficiency, the energetic content of the food, as well as the ability to digest and assimilate the nutritional components. Food capture efficiency and energy per organism were both highest for *Artemia* (Chapter 7) and digestion efficiencies were also highest on that diet hence the highest rates of growth and faecal production were generally found when feeding on this diet.

Pollicipes pollicipes feeding on algal diets produced little faecal material and showed no significant growth although algae were ingested in large numbers (Chapters 6 and 7). The energy ingested when feeding on algae at even quite high suspension densities is relatively low (for *Skeletonema* 0.0835 J hr^{-1}). Rough calculations based on the ingestion rates measured in Chapter 7 suggest that a 5 mm RC animal feeding on algae ingests about 12 J month^{-1}. To grow at a rate of 0.55 mm month^{-1} (an increase in organic weight of 2 mg) it would need to ingest ca 48 J month^{-1}. However high the assimilation rate, a herbivorous diet cannot provide these barnacles with sufficient energy for growth. The low faecal production precluded most compositional analyses, but the overall dry matter assimilation efficiency was estimated as 20% when feeding on *Skeletonema* compared to around 75% when feeding on *Artemia*. The digestive efficiency of *Balanus improvisus* was also found to be high when feeding on algae (Kuznetsova, 1973).

There were no significant differences in faecal production and growth rates of juvenile *P. pollicipes* feeding on rotifers or *Artemia* whereas, adult *P. pollicipes* produced more faeces and grew significantly faster when feeding on *Artemia*. The dry matter digestion efficiency of both juveniles and adults was higher when eating *Artemia*. The difference between juveniles and adults must reflect the greater ease with which juveniles can handle rotifers and the lower energy requirements of juveniles that may be satisfied by rotifers. Adults are unable to ingest the same amount of energy when feeding on the two diets (Chapter 7).

The dry matter digestion efficiency of *Pollicipes pollicipes* feeding on *Artemia* nauplii, (74% - 78%) is higher than the value of 57% calculated by Castellani (1995) but similar to the 80% measured for *Balanus balanoides* feeding on *Artemia* nauplii

(Ritz & Crisp, 1970). Ritz & Crisp (1970) found that *B. balanoides* digested 85% of the calorific content of *Artemia*, again similar to the 69 - 85% for *P. pollicipes*. Assimilation efficiencies, measured by gravimetric methods, of two other barnacle species feeding on animal food were over 90% (Kuznetsova, 1973 and Page, 1983). Assimilation efficiencies of 78 - 99% have been reported for other Crustacea feeding on animal diets (Lasker, 1966; Kibby, 1971). Such high values have, however, rarely been reported in animals feeding on phytoplankton, e.g. 42% for the oyster, *Crassostrea virginica* (Dame, 1976) and 60% for the copepod *Calanus hyperboreus* (Conover, 1966) although an unusually high value of 99% was measured in *Balanus glandula* eating *Skeletonema* (Wu & Levings, 1978).

There are compelling advantages to the ash ratio method of determining the digestion efficiency as it eliminates the need to estimate accurately food intake or to quantitatively recover the faecal output neither of which is easy for a filter feeding organism (see Chapter 7). However, the ratio method can only be relied on when there is no absorption of inorganic material and if the ingested material remains homogeneously mixed as it passes through the gut, so the collected material is representative of the total faecal output.

Page (1983) compared the assimilation efficiency of *Pollicipes polymerus* using gravimetric methods (92 - 95%) with those from the ash ratio method (24 - 41%). He noted that there was very little difference between the percentage of ash in the food and faeces indicating high absorption of inorganic material by the barnacle. Much larger differences between the percentage of ash in food and faeces were found in the current study, suggesting *P. pollicipes* were absorbing much less inorganic material than the *P. polymerus* studied by Page (1983).

Cosper & Reeve (1975) found that the absorption of inorganic material by the chaetognath *Sagitta hispida* Conant was variable, possibly depending on the animals requirements. Such absorption by experimental animals has lead to problems with the ash-ratio method for other workers. Forster & Gabbott (1971) found the prawn *Palaemon serratus* absorbed ~32% of the inorganic material in its food while a mysid absorbed 74% of the ash in the *Daphnia* it consumed (Lasenby & Langford, 1973). Dry matter digestion efficiencies of 38 - 39% were obtained for *P. pollicipes* feeding on *Brachionus plicatilis*. This is half that found for the diet of *Artemia* nauplii. Whether rotifers are less readily digestible or they contain more readily assimilable inorganic material is unclear, but either would account for the results.

The larger surface area to volume ratio of a rotifer compared to an *Artemia* equates to proportionately more exoskeleton, and twice as much ash, per animal. In the present study there was a strong negative correlation between the digestion efficiency and the ash content of the diet. Conover (1966) found a negative correlation between the ash content of various diatoms and the assimilation efficiency of *Calanus* ingesting them, suggesting that the more inorganic material a diatom contains, the less digestible it is and possibly, that the degree of mineral assimilation is proportional to the amount present in the food. In *Daphnia magna* Strauss (Schindler, 1968) a correlation has been found between the calorific content of the food ingested and the assimilation efficiency. However, no such generalisations have been possible in other species (see Lawton, 1970).

Gravimetric methods always overestimate efficiencies (see, Kurmaly *et al.*, 1989b) because guts are never completely emptied and errors will occur in the measurement of input and output. *Skeletonema costatum* was found to contain ~59% ash. This was probably an overestimate as values of 36% (Kurmaly *et al.*, 1989a) and 40% (Parsons *et al.*, 1961) are documented in the literature so the calculated digestion efficiency may have been underestimated.

The presence of bacteria may have a marked influence on composition of the faeces following evacuation (e.g. see Johannes & Satomi, 1966) and therefore on estimated digestion efficiencies. No assessment was made of the changes in faecal composition occurring between pellet evacuation and collection. Johannes & Satomi (1966) monitored the composition of the faecal pellets of the shrimp *Palaemonectes pugio*. Faeces examined immediately after evacuation were packed with bacteria but, after four days immersion in the dark, showed a marked decrease in protein, slight decrease in carbohydrate but no measurable change in lipid content. Autolysis, dissolution and bacterial respiration masked any concurrent increase in organic matter due to bacterial growth. Newell (1965) showed that the voided faeces of the molluscs *Hydrobia ulvae* and *Macoma balthica* increase in nitrogen and decrease in carbon content over 3 days. However, Forster & Gabbott (1971) estimated that, at water temperatures of 15-20°C, there was a drop of 10% nitrogen and 8% carbon from the faeces of *Palaemon serratus* and *Pandalus platyceros* Brandt after 18 hours of immersion. In the present study, the faeces of *P. pollicipes* were only collected every 48 hours so compositional changes may have occurred. However, Davies (1964) noted only negligible compositional changes in goldfish faeces over a 23 day immersion period at 12.5 and 21.5°C. Conover (1966) estimated that bacteria could lead to a maximum increase of only ~1% in total organic matter present in faeces over 24 hours and that the changes

due to bacteria and solution will cause only small over- or underestimates *providing* the assimilation efficiency is high.

Overestimation of protein in the diets and, more particularly, in *P. pollicipes* faeces when using the micro-Kjeldahl technique probably occurred because not all the nitrogen present was in protein; nitrogen also occurs in nucleic acids, in excretory products and in glucosamine, the building block of chitin. Chitin accounted for 2.4% of the dry weight of the *Artemia* and 10% of the faeces of barnacles feeding on them. Both were probably underestimates because of the incomplete removal of chitin from suspension by centrifugation, and therefore loss in the discarded supernatants. There was no evidence that any chitin had been digested. There was a large amount of non-protein nitrogen or metabolic faecal nitrogen (MFN) present in the faeces of *P. pollicipes* which resulted in very high estimates of faecal protein when 6.25 was used as the conversion from nitrogen to protein.

MFN consists of digestive juice residues (Forster & Gabbott, 1971), bacterial residues (Maynard & Loosli, 1962) and epithelial cells abraded from the walls of the alimentary tract. The peritrophic membrane around the faecal pellets contains mucopolysaccharides and protein (Rainbow & Walker, 1977) which may have further contributed to high faecal nitrogen as it does in prawns (Forster, 1953).

The protein digestion efficiency of *P. pollicipes* feeding on *Artemia* ranged from 92 - 97% and 96 - 100% when eating rotifers. Castellani (1995) found a protein digestion efficiency of 82% (which was incorrectly reported as 99%, by use of the wrong conversion factor) for *P. pollicipes* feeding on *Artemia*. Nitrogen assimilation by the prawns, *Palaemon serratus* and *Pandalus platyceros* varied between 75% and 98% when feeding on a variety of animal, vegetable and microbial diets (Forster & Gabbott, 1971).

The proportion of lipids in the faeces of *P. pollicipes* feeding on *Artemia* increased relative to other constituents and was 1.6 times higher than in the food. Castellani (1995) recorded lipid levels in the faeces that were more than four times higher than in the *Artemia*, which led her to concluded that, like *Elminius modestus* (Crisp & Southward, 1961), *P. pollicipes* was unable to digest lipid. *P. pollicipes* can digest lipid, efficiencies of 87 - 92% were found when feeding on rotifers, although efficiencies were lower when eating *Artemia* (25 - 65%). Rotifers are much smaller than *Artemia* and are ingested by *P. pollicipes* at a similar rate (Chapter 7) so the gut fills more slowly and food will take longer to pass through the gut. The longer residence time in the gut may enable more complete digestion of the lipid in the diet.

Castellani (1995) observed that when feeding rates were high, the contents of *P. pollicipes* faeces "appeared less digested". However, it is also likely that the lipids in rotifers differ from those in *Artemia* and are more readily broken down by the lipases present in *P. pollicipes*.

Carbohydrate digestion efficiencies varied with diet, exceeding 90% on a diet of *Artemia* but only 0 - 39% when feeding on rotifers. The ability of some Crustacea to digest carbohydrate depends on the form it is in. Carbohydrate in the form of wheat starch, glycogen and dextrin was completely digested by the shrimp *Palaemon serratus* whereas structural carbohydrates such as cellulose were not (Forster & Gabbott, 1971). Most carbohydrates in rotifers are structural, in the lorica (Koehler, 1966; Budd, 1989), and may be less digestible than those in *Artemia*. An inability of *Elminius modestus* to digest starch was noted by Crisp & Southward (1961).

Digestion efficiencies are unlikely to be fixed but may vary with food composition, abundance and the rate at which it is ingested. When animals are fed to excess, food passes through the gut more rapidly and nutritional components that are difficult to digest, perhaps lipid, are barely digested. Crisp & Southward (1961) also showed that in a wide variety of barnacle species assimilation efficiency was inversely related to the amount of food ingested. However, the absorption efficiency of *P. polymerus* is unaffected by large fluctuations in food ration (Page, 1983) as is that of the damselfly, *Pyrrhosoma* (Lawton, 1970) and the copepod, *Calanus* (Conover, 1966). In the current study rotifer rations were always lower than the *Artemia* rations due to the means of production. This intrinsic difference between the ration size of barnacles feeding on the two diets may account for differences in the ability of *P. pollicipes* to digest any of the nutritional components.

In summary, the dry weight digestion efficiency of *P. pollicipes* was highest when feeding on *Artemia* and lowest on *Skeletonema*, corresponding to the growth rates and faecal production. The smaller the food and the more refractory material it contains, the less able the barnacle is to digest efficiently the nutrients it might contain. Algae are a very undesirable food for these barnacles. Kristensen (1972) noted that in general, herbivores ingest large amounts of plants while only digesting a fraction and that omnivores prefer animal to plant food. *P. pollicipes* is an omnivore but its limb morphology, cirral activity, gut contents, and feeding rates all suggest that it is better adapted to an animal diet. The findings of the present chapter suggest that *P. pollicipes* is also more able to digest animal food. There was little difference between the digestion efficiency of juvenile and adult *P. pollicipes*.

Chapter 9

The digestive enzymes of *Pollicipes pollicipes* and *Lepas anatifera.*

Introduction

The alimentary canals of both balanomorph and lepadomorph barnacles have been described by Rainbow & Walker (1977) and consist of three sections: a cuticle-lined foregut and hindgut with an intervening, U-shaped midgut. Barnacles have many epidermal glands (Walley, 1967) including the suboesophageal and labial glands (together known as the salivary glands). In stalked forms the labial glands are the larger of the two. The salivary glands in the species examined by Rainbow & Walker (1977) secreted acid mucopolysaccharide or glycoprotein whilst *Lepas anatifera* and *Balanus hameri* secreted both. These glands are thought to be responsible for binding food material prior to ingestion (Gruvel, 1893) although it was considered surprising that two secretions would perform the same role in one animal. *Lepas anatifera* (Thomas, 1944) and *Balanus hameri* (Rainbow & Walker, 1977) both produce two different secretions.

The foregut consists of three regions - the pharynx, the oesophagus and the ventriculus. In stalked barnacles, the foregut, posterior to the oesophagus is expanded to form the "Magen" (see Rainbow & Walker, 1977). A paragnathe - a region of the pharynx wall with very thick cuticle against which the mandibles act - is present, but the pharynx is much shorter than in balanomorphs, such that the mouthparts pass food almost directly into the oesophagus.

The midgut is the longest region of barnacle digestive tract and in most species it gives rise, anteriorly, to midgut caeca, and a pair of pancreatic glands. Midgut caeca are present in adult *Lepas anatifera* (Rainbow & Walker, 1977) but absent in *Pollicipes* (Darwin, 1851). The midgut is often divided into an anterior and posterior portion with only the anterior midgut connected to the caeca and pancreatic glands (Rainbow & Walker, 1977). The pancreatic glands in all barnacles consist of active secretory cells which produce proteinaceous material, probably digestive enzymes including amylase and protease (Rainbow, 1975).

Rainbow & Walker (1977) described the cirripede hindgut as consisting of a folded, muscular region, leading from the posterior midgut which acts as a sphincter, and an

anal chamber from which the anus opens. Stalked barnacles have a smaller sphincter region separating the mid and hindgut. The histochemical characteristics of hindgut and foregut are similar and both play little part in digestion and absorption. Cells of the midgut are capable of absorption with the highest concentration of such cells in the anterior portion and, when present, the caeca (Rainbow & Walker, 1977).

Mechanical processes, such as peristalsis and trituration, contribute to the digestion of food in the Crustacea (Vonk, 1960a). Peristalsis is the process responsible for propelling material through the gut. In *Nephrops norvegicus* there is peristalsis in the oesophagus, midgut and hindgut, in addition to antiperistaltic movements (Yonge, 1924). Antiperistaltic movements are necessary as absorption occurs in the anterior midgut while digestion occurs in the posterior part. Many Crustacea, particularly the malacostracans have a well developed gastric mill which is responsible for squeezing food (see Vonk, 1960a). Barnacles have no gastric mill and highly toothed mandibles are responsible for breaking food up sufficiently to pass through the mouth.

Although food may be broken up by mechanical processes it is chemical means that are employed by barnacles for digesting consumed food. Little work has been done on the physiology of digestion in barnacles. In the more thoroughly investigated decapods and Malacostraca, the digestive juice is produced almost entirely by the cells of the hepatopancreas and transported to the stomach (Vonk, 1960a) where digestion occurs. Absorption occurs in the midgut. However, barnacles represent a more primitive situation possessing no hepatopancreas.

The pH of the gut is of primary importance to digestion, because different enzymes act optimally at different hydrogen ion concentrations. *Potamon martensi* Woodmason, for example, like other crustaceans (see Hasler, 1935; Vonk, 1955; Aragawal *et al.*, 1967), has a very weakly acidic medium in the alimentary canal, except in the rectum where it is weakly alkaline. The freshwater prawn *Macrobranchium dayanum* (Henderson) has a weakly acid medium in the mouth, oesophagus and pyloric stomach, a distinctly acidic fluid in the hepatopancreas and cardiac stomach and an alkaline medium in the intestine and rectum. In *Orchestia gammarella*, the caeca and midgut are acidic and the foregut and hindgut are neutral (Aragawal, 1964). Fiddler crabs have more alkaline gastric juice than many other crustacea (Vonk, 1960a). In several crustaceans, the pH of gastric juice is lower in starved animals than it is in recently fed animals (Vonk, 1960a; Aragawal, 1964; Aragawal *et al.*, 1967; Tyagi & Prakash, 1967) although the converse was true in *Ligia* (Nicholls, 1931).

Proteolytic enzymes have been extensively studied in Crustacea, particularly decapods (Vonk, 1960a). Crustacea and many other invertebrates are thought to hydrolyse protein using similar enzymes to those of vertebrates except for the lack of a peptic enzyme (Vonk, 1960b). Trypsin is the primary protease in Crustacea (Ceccaldi, 1990). The distribution of the tryptic enzymes within the Crustacea was investigated by DeVillez & Buschlen (1967) who determined a tryptic component to proteolysis in amphipods, isopods and decapods but not in *Balanus nubilus* Darwin. All the extracts examined demonstrated alkaline proteolytic activity as indicated by casein hydrolysis. However, the gastric juice and digestive gland extracts of the barnacle *Balanus nubilus* possessed less activity in alkaline medium than did the amphipod, isopod and decapod extracts. Gastric juice extracts of the barnacle hydrolysed casein at a pH optimum of 4.0 compared to all the other gastric juice extracts which showed optima over 7.0.

The reviews of Vonk (1960a) and Huggins & Munday (1968) and the work of Ceccaldi (1990) give detailed descriptions of crustacean carbohydrases. Strong carbohydrase activity has been demonstrated in a number of Crustacea, including the primarily carnivorous freshwater crab *Potamon martensi* (Aragawal *et al.*, 1967) and in the mainly herbivorous prawn *Macrobrachium dayanum* (Tyagi & Prakash, 1967). The omnivorous *Palaemon serratus* is able to digest efficiently a variety of carbohydrates and the assimilation of a small amount of cellulose (ca 21%) indicates the presence of weak cellulase activity in the gut (Forster & Gabbott, 1971) although it is unclear whether this enzyme is secreted by the prawn itself or released from gut bacteria. Cellulase activity has been found in the digestive juices of the crayfish, *Astacus fluviatilis* and the lobster *Homarus vulgaris* (Kooiman, 1964). The possession of a cellulase would be advantageous to *Palaemon serratus* in the digestion of various marine algae which Forster (1951) reports form a considerable part of its natural diet. Wheat starch was less efficiently assimilated by *Pandalus platyceros* than by *P. serratus*. The digestive gland of *Potamon martensi* was found to secrete carbohydrases such as amylase, maltase and lactase, with invertase, maltase, lactase, amylase and raffinase present in oesophageal extract. All the enzymes were present in the midgut while none were found in the extracts of the midgut caecum, hindgut caecum or rectum (Aragawal *et al.*, 1967). Similar enzymes were found in *Macrobrachium dayanum* (Tyagi & Prakash, 1967). Although most carbohydrates were digested by enzymes secreted by the hepatopancreas, such enzymes were found in the oesophagus and stomach juices. Few enzymes were found in the intestine and none in the rectum. *Balanus crenatus* was found by Kristensen (1972) to possess the lowest number of carbohydrases in any of the 22 crustaceans he studied. Harnden (1968) found that *Balanus nubilus* had very few carbohydrases (maltase, laminarinase,

chitinase and glycogenase), the same enzymes found to be significantly active in *Balanus crenatus*.

In vitro studies on several crustacean species have demonstrated lipase activity (Vonk, 1960a; Aragawal *et al.*, 1967; Tyagi & Prakash, 1967; Huggins & Munday, 1968; Ceccaldi, 1990). To date no information is available on lipase activity in barnacles although it is clear that lipid biochemistry is a crucial element in determining the viability of barnacle cyprids (Lucas *et al.*, 1979) and storage lipid is certainly laid down in cirripede eggs (Achituv & Barnes, 1978).

The current study aims to compare the gut pH and enzyme complement of two species of pedunculate barnacles, *Pollicipes pollicipes* and *Lepas anatifera*, to identify differences that might relate to diet or ecology. The determination of the pH in all the parts of the digestive tract is a prerequisite for understanding digestive physiology since different enzymes act optimally under different hydrogen ion concentrations. An *in vitro* survey of the major digestive enzymes present in the gut tissue was undertaken. The total protease, trypsin and amylase activities in the guts of both juvenile and adult *P. pollicipes* were compared, and the effect of diet on enzyme activity investigated.

Materials & Methods

Measurement of gut pH

Six adult *Pollicipes pollicipes*, that had previously been feeding on *Artemia* nauplii and the rotifer *Brachionus plicatilis*, were selected. The body was removed from the capitulum and the gut was carefully sliced open along the bottom, between the two rows of filamentary appendages, using a scalpel blade (see Fig. 6.1). The contents of the gut were rinsed away using deionised distilled water. The pH of the foregut, midgut and hindgut was measured using BDH indicator paper strips (pH 4-7, 5.2-7.2 and 6.5-10). The procedure was repeated using animals that had previously been starved for 72 hours and again for previously starved and fed *Lepas anatifera* animals.

Preparation of gut samples for qualitative enzyme analysis.

Both the methods of sample preparation and subsequent analyses are similar to those used by Aragawal (1963; 1964), Aragawal *et al.* (1967) and Tyagi & Prakash (1967). Twenty five animals were dissected and their guts removed. After being thoroughly washed UVFSW the guts were ground in a mortar with a little thymol and a few drops of glycerol to produce a fine emulsion. The resultant homogenate was diluted with

with 50% glycerol and UVFSW to ca 10% concentration. The tube was then filled with toluene and the extracts kept at room temperature for 48 hours before being tested for the presence of different enzymes.

Qualitative assessment of enzymes present in the gut

All experiments took place in test tubes incubated at 30°C, unless otherwise specified. Each enzyme-containing tube had a control, identical but containing boiled, rather than fresh, extract.

Carbohydrase Enzymes.

Test reagents:

i) Benedict's reagent (BDH).

A test for reducing sugars including all monosaccharides and certain disaccharides. These sugars reduce blue Copper (II) sulphate to red copper (I) oxide. Equal volumes of sample to be tested and reagent were mixed and placed in a water bath (at 100°C) for 5 min, shaking occasionally (see Toole & Toole, 1994). Negative result: solution remains blue. Positive results: green, yellow, brown or red precipitate, with increasing quantities of reducing sugar.

ii) Barfoed's reagent

(see CRC Handbook of Chemistry and Physics 1986 - 87). A test for reducing monosaccharides only. To 2.5 ml of glacial acetic acid in distilled deionised water, 16.5 g cupric acetate were added and dissolved. This solution was diluted to 250 ml. Equal volumes of sample and reagent were placed in a test tube and heated to 100°C for 5 min. A red precipitate of Cu (II) oxide was formed in the presence of monosaccharide sugars. Disaccharides produce a negative result as they are weaker reducing agents.

iii) Iodine in potassium iodide solution

Test for the presence of starch. Ten drops of test solution were placed into a test tube with one drop of reagent. Negative result: reagent retains its yellow-orange colour. Positive result: Blue black colour produced.

In each case, 2 ml of extract was incubated with 1 ml of substrate solution. Two ml of toluene was placed into the tube after the test solution and extract, to prevent contamination by micro-organisms. Tubes were incubated and tested after 12, 24, 48 and 96 hours for signs of hydrolysis using the stated tests. The carbohydrate substrate solutions used are summarised in Table 9.1.

Table 9.1. Summary of substrate solutions used in the survey of carbohydrases in *Pollicipes pollicipes* and *Lepas anatifera*.

Enzyme	Substrate	Solution strength	Test
	Disaccharides		
maltase	maltose	2 %	Barfoed's *
lactase	lactose	2%	Barfoed's *
(ß-galactosidase)			
invertase	sucrose	5%	Benedict's
salicinase	salicin	1%	Benedict's
fucase	alpha L-galactose	1%	Benedict's/Barfoed's
	Polysaccharides		
glycogenase	glycogen (type II oyster)	Saturated	Benedict's
amylase	starch. (soluble potato)	1 % boiled	Iodine/Benedicts
cellobiase	cellobiose	2 %	Barfoed's
ß-1,3-glucanase	laminarin	2 %	Benedict's
mannitolase	mannitol	2 %	Benedicts
	carrageenan (type II)	2 %	Benedict's
	agar agar	2 %	Benedict's
	alginic acid	1 %	Benedict's

*Benedict's test cannot be used for maltose or lactose as both these disaccharides are reducing sugars and will give a positive result.

Protease

1 ml of 10% saturated gelatine solution was incubated with 1 ml of extract in a boiling tube. A positive reaction was denoted by the liquefaction of the gelatine.

Lipase

Two tests were used to provide different lipid substrates, one of animal origin, the other of plant.

a) Twenty-five ml of a 10% solution of condensed milk were prepared, to which ten drops of bromo-thymol blue indicator (BDH) were added. To this was added 1% NaOH solution, drop by drop, until the colour changed to light blue. One ml of this solution was incubated with five drops of extract. The presence of lipase was indicated by a yellow coloration.

b) Ten drops of olive oil were dissolved in 4 ml of ethyl alcohol and 4 ml of hot water were then added. When cool, ten drops of phenol red (BDH) were added, followed by a few drops of 0.01 M NaOH until the mixture became light pink. Two ml of this mixture was incubated with 1 ml of extract and the presence of lipase was indicated by the mixture turning yellow.

Measurement of enzyme activities in juvenile and adult *Pollicipes pollicipes* feeding on animal and algal diets.

General protease activity was assayed, as were amylase and trypsin-like activity. For each assay about ten juvenile and ten adult *P. pollicipes* were maintained for two to three weeks on monospecific diets of *Artemia* nauplii or *Skeletonema costatum*. The animals were then dissected, their gut tissue excised, gut contents rinsed away, tissue weighed and four extracts made from their guts, i.e. juveniles and adults fed algae, juveniles and adults fed *Artemia*.

Preparation of extracts and protease assay

Total protease activity was assayed using azocasein as a substrate as described by Sarath *et al*. (1989). Gut tissue samples were weighed then homogenised in ice-cold Tris-HCl buffer (to a 100 mM Tris solution, HCl was added drop by drop to give a pH of 7.5 (measured using a Jenway 3020 pH meter). Tissue homogenates were centrifuged at 8000 x g for 5 min and the supernatant was assayed for protease activity at 25°C. A 2% azocasein solution was prepared in the same buffer and centrifuged at 12000 x g to clarify. Substrates and enzyme solutions were equilibrated at 25°C in a waterbath. To 250 μl of substrate in an Eppendorf microcentrifuge tube, 150 μl of sample supernatant were added and incubated for 60-90 minutes. A reagent blank was prepared by replacing the enzyme solution with buffer. The reaction was stopped by the addition of 1.2 ml of 10% trichloroacetic acid (TCA). Enzyme blanks were made for each extract by mixing enzyme, TCA, and substrate in that order. The contents were thoroughly mixed and tubes were allowed to stand for 15 min to ensure complete precipitation of the remaining azoprotein and azoprotein fragments. Precipitated protein was removed by centrifugation (8000 x g for 4 min) and 1.2 ml of supernatant was transferred to a test tube containing 1.4 ml of 1.0 M NaOH. The absorbance of the resulting solution was measured at 440 nm on a Jenway 6100 spectrophotometer against the blanks. One unit of protease activity was defined to be the amount of enzyme required to produce an absorbance change of 1.0 in a 1 cm cuvette, under the conditions of the assay. Triplicate samples of each extract were assayed.

Preparation of extracts and assay for trypsin-like activity

Gut tissue was excised, weighed and homogenised in ice cold Tris-HCl buffer (prepared as before, but with the pH adjusted to 8.1). Homogenates were centrifuged at 8000 x g for 5 min and the supernatant was assayed for trypsin-like activity at 25°C using 1 mM N-alpha-p-toluenesulphonyl-L-argenine methyl ester (TAME) (see LeVay, 1994), prepared in the same buffer. 100 μl of substrate solution and 700 μl of buffer were equilibrated at 25°C in 1.5 ml Eppendorf tubes and 200 μl of sample were

Measurement of enzyme activities in juvenile and adult *Pollicipes pollicipes* feeding on animal and algal diets.

General protease activity was assayed, as were amylase and trypsin-like activity. For each assay about ten juvenile and ten adult *P. pollicipes* were maintained for two to three weeks on monospecific diets of *Artemia* nauplii or *Skeletonema costatum*. The animals were then dissected, their gut tissue excised, gut contents rinsed away, tissue weighed and four extracts made from their guts, i.e. juveniles and adults fed algae, juveniles and adults fed *Artemia*.

Preparation of extracts and protease assay

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centrifuged at 8000 x g for 5 min and 375 μl of the supernatant was added to 375 μl of the starch solution. Samples were mixed and incubated at 25°C for 10 min as was a blank containing only 375 μl of substrate. To each sample tube and to the 375 μl enzyme sample, 750 μl of dinitrosalicylic acid reagent was added. All tubes were stoppered, mixed and heated in a water bath at 100°C 5 min. Tubes were cooled in cold water for 20-60 minutes and the extinction was measured at 546 nm in a 1cm cuvette. To prepare a maltose standard curve (maltose released when starch is broken down), 75 - 375 μl maltose standard solution were diluted with water to 375 μl in 1.5 ml microcentrifuge tubes and a blank prepared with 375 μl water only. To all tubes 375 μl of substrate were added together with 750 μl of dinitrosalicylic acid reagent. Tubes were treated as before. Extinctions were plotted against mmol maltose per reaction mixture. Results were expressed as mmol of maltose equivalents released per animal (and per gram wet weight) per minute.

Results

Measurement of gut pH

The foregut and hindgut of *P. pollicipes* were more alkaline than the midgut (Table 9.2). The hindgut was most alkaline in all animals. Those animals that had been previously starved had a more acidic foregut (Two-factor analysis of variance, $F = 12.64$, $P = 0.003$) and hindgut ($F = 21.64$, $P < 0.001$) than those animals that had been fed but there was no significant change in the pH of the midgut (Table 9.2) ($F = 1.93$, $P = 0.186$).

Table 9.2 Measured pH in foregut, midgut and hindgut of previously fed (n = 6) and starved (n = 10) *Pollicipes pollicipes*. SE = standard error.

	Previously fed		Previously starved	
Region	**Mean pH**	**SE**	**Mean pH**	**SE**
Foregut	7.18	0.05	6.89	0.06
Midgut	6.90	0.03	6.79	0.06
Hindgut	7.70	0.11	7.18	0.06

The pH of the various parts of the gut of *Lepas anatifera* were all more acidic than the equivalent regions in *P. pollicipes* guts. The foregut and hindgut of *Lepas anatifera* also were more alkaline than the midgut and the hindgut was most alkaline in all animals (Table 9.3). Those animals that had been previously starved had a more acidic midgut than those animals that had previously been fed ($F = 18.22$, $P = 0.001$)

but there were no changes in the pH of the other regions of the alimentary canal (Table 9.3) ($F = 1.58$, $P = 0.226$; $F = 0.09$, $P = 0.767$ for fore and midgut respectively).

Table 9.3. Measured pH in foregut, midgut and hindgut of previously fed (n=10) and starved (n = 8) *Lepas anatifera*. SE = standard error.

Region	Previously fed		Previously starved	
	Mean pH	SE	Mean pH	SE
Foregut	6.62	0.04	6.69	0.03
Midgut	6.51	0.06	5.78	0.17
Hindgut	6.79	0.05	6.81	0.05

Qualitative assessment of enzymes present

The presence of various enzymes in the extract of *P. pollicipes* were inferred from substrate degradation (Table 9.4). There is clear indication of the presence of glycogenase, amylase and invertase and possibly 1, 3-glucanase, mannitolase though here the activity is much weaker.

Table 9.4 The presence or absence of particular enzymes in the gut of *Pollicipes pollicipes*, as indicated by substrate degradation over a period of four days. ++ indicates a strong reaction; + a positive reaction, ± very weak reaction, - no reaction.

Enzyme	Substrates	Duration of reaction (hours) & extent of digestion							
		Experiment				Control			
		12	24	48	96	12	24	48	96
lactase	2% lactose	-	-	-	-	-	-	-	-
maltase	2% maltose	-	-	-	-	-	-	-	-
invertase	5% sucrose	-	±	+	+	-	-	-	-
salicinase	1% salicin	-	-	-	-	-	-	-	-
fucase	1% fucose †	++	++	++	++	+	+	+	+
		-	-	-	-	-	-	-	-
amylase	1% starch solution	+	+	+	+	-	-	-	-
glycogenase	saturated glycogen	+	++	++	++	-	-	-	-
cellobiase	2% cellobiose	-	-	-	-	-	-	-	-
mannitolase	2% mannitol	-	-	±	+	-	-	-	-
1,3-glucanase	2% laminarin	±	±	±	+	-	-	-	-
	2% carageenan	-	-	-	+	-	-	-	-
	2% agar agar	-	-	-	-	-	-	-	-
	1% alginic acid †	±	±	±	±	±	±	±	±
		-	-	-	-	-	-	-	-
	methyl cellulose	-	-	-	-	-	-	-	-
protease	10% gelatine	-	-	-	-*	-	-	-	-
lipase	condensed milk	-	-	+ (33 hours)		-	-	-	-
	olive oil	-	-	-	-	-	-	-	-

† First results from Benedict's test the second from Barfoed's.
* Solid after 150 hours

It is, however, unclear how concentration dependent these tests are, so negative results may reflect the high substrate concentrations and low enzyme concentration.

The presence of various enzymes in the guts of *Lepas anatifera* inferred from substrate breakdown are indicated in Table 9.5. The findings are very similar to those for *P. pollicipes* although, the *Lepas* extracts caused some liquefaction of the gelatine and some weak hydrolysis of agar agar.

Table 9.5. The presence or absence of particular enzymes in the gut of *Lepas anatifera*, as indicated by substrate degradation over a period of four days. ++ indicates a strong reaction; + a positive reaction, ± very weak reaction, - no reaction.

Enzymes	Substrates	Duration of reaction (hours) & extent of digestion							
		Experiment				Control			
		12	24	48	96	12	24	48	96
lactase	2% lactose	-	-	-	-	-	-	-	-
maltase	2% maltose	-	-	-	-	-	-	-	-
invertase	5% sucrose	-	±	+	+	-	-	-	-
salicinase	1% salicin	-	-	-	-	-	-	-	-
amylase	1% starch solution	+	+	+	+	-	-	-	-
glycogenase	saturated glycogen	+	+	+	++	-	-	-	-
cellobiase	2% cellobiose	-	-	-	-	-	-	-	-
mannitolase	2% mannitol	-	-	±	+	-	-	-	-
1,3 glucanase	2% laminarin	±	+	+	+	-	-	-	-
	2% carageenan	±	±	±	+	-	-	-	-
	2% agar agar	-	-	-	±	-	-	-	-
	1% alginic acid †	+	+	+	+	+	+	+	+
		-	-	-	-	-	-	-	-
protease	10% gelatine	-	-	+	+*	-	-	-	-
lipase	condensed milk	-	+	++(30 hours)		-	-	-	-
	olive oil	-	-	-	-	-	-	-	-

† First results from Benedict's test the second from Barfoed's.
* Two thirds of gelatine liquefied after 96 hours.

Measurement of the digestive enzyme activities in juvenile and adult *Pollicipes pollicipes* feeding on animal and algal diets.

General protease activities

There was a significant difference between the gut protease activity of one group and at least one other (Table 9.6, Kruskal-Wallis H = 9.50, 3 df, P= 0.024). Dunn's method of pairwise comparison indicated that the protease activities differed significantly between the groups of juveniles fed *Artemia* and adults fed on algae and between juveniles and adults fed on *Artemia*. The enzyme activity of animals feeding on algae is much lower than animals feeding on *Artemia*. Juveniles feeding on

Artemia have protease activities over three times higher than those feeding on algae, while in the adults the difference is forty fold. The activity of juveniles is much higher than adults. Juveniles feeding on algae have a protease activity over 180 times greater than the adults feeding on the same diet. This disparity is not so great in animals feeding on *Artemia* although the protease activity of juveniles is nearly 14 times greater than that of the adults.

Table 9.6. Mean protease activity in juvenile and adult *Pollicipes pollicipes* feeding on monospecific diets of *Skeletonema costatum* and *Artemia* nauplii. Activity is expressed in units of activity (U) per animal and per gram excised gut tissue wet weight, per minute, where one unit is defined to be the amount of enzyme required to produce an absorbance change of 1.0 in a 1 cm cuvette under the assay conditions. SE for each are given from 3 replicates. * effectively no activity.

Animal	Diet	Protease activity ($x10^{-5}$)			
		U/an/min	SE	U/g/min	SE
Juvenile	*Skeletonema*	29.63	1.47	2963.30	146.70
Juvenile	*Artemia*	102.88	8.59	9270.00	784.00
Adult	*Skeletonema*	1.24	9.64	17.60	137.88*
Adult	*Artemia*	42.52	1.37	675.50	21.70

Trypsin-like activity

There were no significant differences between the trypsin-like activity of juveniles and adults nor between diets (Table 9.7, Kruskal-Wallis H = 5.07, 3 df., P = 0.167). The variability of the assay for juveniles feeding on *Skeletonema* was, however, particularly high. The weight-specific rates consistently show higher trypsin-like activity for animals eating *Artemia*.

Table 9.7. Mean trypsin activity in juvenile and adult *Pollicipes pollicipes* feeding on monospecific diets of *Skeletonema costatum* and *Artemia* nauplii. Activity expressed in terms of μmol substrate cleaved ($x10^{-3}$) per minute per gram wet weight of tissue and per animal under the assay conditions), SE for each are given as are the number of replicates (N). * effectively no activity.

Animal	Diet	Trypsin-like activity ($x10^{-3}$)				
		N	μM an/min	SE	μM/g/min	SE
Juvenile	*Skeletonema*	3	5.00	5.00	45.00	45.00*
Juvenile	*Artemia*	3	1.65	0.24	74.30	10.70
Adult	*Skeletonema*	3	2.33	0.44	52.30	9.96
Adult	*Artemia*	2	3.77	0.75	81.00	16.00

Amylase activity

Amylase activity in the guts of juvenile and adult *P. pollicipes* fed on algal and animal diets are given in Table 9.8. Weight specific amylase activity was much higher in juveniles than in the adults where activity could only be demonstrated for animals which were fed on *Skeletonema*. Little difference in specific activity was exhibited between juveniles feeding on *Skeletonema* or *Artemia*. Within the variability of the experiment and small sample size no significant differences between specific activities could quite be demonstrated (Kruskal-Wallis H = 5.65, 2 df., P = 0.06, ignoring adults fed on *Artemia*).

Table 9.8. Total mean amylase activity (n = 3) in juvenile and adult *Pollicipes pollicipes* feeding on monospecific diets of *Skeletonema costatum* and *Artemia* nauplii (expressed in terms of mM maltose equivalents released per animal and per gram wet weight per minute under the assay conditions). SE = standard error.

Animal	Diet	Amylase activity (x 10^{-3})			
		mM/an/min	SE	mM/g/min	SE
Juvenile	*Skeletonema*	13.00	0.58	1325.00	59.60
Juvenile	*Artemia*	20.00	0.88	1356.00	63.00
Adult	*Skeletonema*	53.00	2.00	704.00	133.00
Adult	*Artemia*	0.00	–	0.00	–

Discussion

The pH of the various parts of the gut of *Pollicipes pollicipes* was broadly similar to those of other crustaceans, with foregut mildly alkaline, midgut slightly acidic and the hindgut alkaline. In general, both *P. pollicipes* and *Lepas anatifera* show the range of enzyme capabilities for digesting a wide range of food. Both exhibit carbohydrase, protease and lipase enzymes with perhaps minor differences obvious in the carbohydrase compliment. *Lepas* shows activity towards carageenan and agar which is not seen in *Pollicipes* extracts. Protease activity was not demonstrated for *Pollicipes* in response to a gelatine substrate yet was clearly measurable with azocasein. The fact that *Lepas* extracts showed activity with gelatine perhaps reflects greater enzyme activity in their extracts hence stronger reactions for the other carbohydrases rather than a genuine lack in *P. pollicipes*. Attempting to assess the likely food habits of an organism based on qualitative enzyme tests is likely to be futile when major differences in general complement are not apparent (e.g. in the present study). Although phytoplankton are thought to play an insignificant role in the

nutrition of *Hyas araneus*, for example, the larval stages do possess the enzymes necessary to process carbohydrate-rich phytoplankton (Hirche & Anger, 1987).

The results suggest that *L. anatifera*, although omnivorous, is better able to digest algal food than *P. pollicipes* (an assertion also supported by limb and mouthparts, see Chapter 4). *L. anatifera* live in the open ocean which is a less food rich environment (Parsons, 1980) than the coastal water inhabited by *P. pollicipes*, possibly necessitating more active digestive enzymes permitting efficient digestion of food ingested. The difference in activity between the extracts of *Lepas* and *Pollicipes* might well be expected given the same amount of gut tissue extracted.

Several of the enzymes in the qualitative study required several days before their action could be detected whereas the food *Pollicipes pollicipes* ingests is retained in the gut for only 12 hours or so (Chapter 6). The extracts were made not only from all the gut tissue but probably more besides (when digestive enzymes in most crustacea are produced and secreted by a small portion of the gut), but were also diluted to 10% strength (more including the substrates), so in vitro digestion would occur less rapidly than in the gut. The volume and concentration of enzymes were so low relative to those of the substrates, that the amount of products formed is likely to be small, requiring very sensitive tests. It appears that Barfoed's test was relatively insensitive and that negative results could have been obtained even if some hydrolysis of disaccharides occurred.

According to Yonge (1937), there is a correlation between the feeding habits of an animal and the nature and relative strength of the digestive enzymes it possesses. Herbivores generally exhibit much higher levels of carbohydrase activity and lower levels of protease activity than carnivores (Gaudy & Boucher, 1983) and higher amylase activity was measured in the more "herbivorous" copepods by Boucher & Samain (1974). However, the carbohydrase activity of *Pollicipes pollicipes* was found to be considerably higher than the activity of the trypsin which suggests that *P. pollicipes* is herbivorous.

Sather (1969) examined six species of decapod crustaceans of known dietary habit and compared amylase and protease activities. He found that the omnivores generally had higher activities than either carnivores or herbivores, although he suggested that the results may reflect differences in the pH optima of the enzymes or could be an effect of substrate on enzymatic hydrolysis. The larvae of *Hyas*, a spider crab, are generally considered carnivorous but amylase and trypsin activities were found by Hirche & Anger (1987) to be in the range quoted for herbivorous calanoid

copepods and the herbivorous cladoceran *Daphnia* (Hirche, 1981). The evidence is clearly contradictory so digestive enzyme activity alone is insufficient to allow classification of an animal as carnivore or herbivore. *P. pollicipes* possesses a variety of enzymes with activities neither typical of herbivore nor carnivore. It seems likely that this species is equipped to digest a variety of foods, both algal and animal.

It was anticipated that diet may affect digestive enzyme activities regardless of whether an animal is intrinsically a herbivore, carnivore or omnivore. Many studies have shown that dietary substrate does have an impact on digestive enzyme activity, although again, the effect is variable. Van Wormhoudt *et al.* (1980) noted that enzyme activity diminishes if the protein and carbohydrate content of an experimental diet varies from the optimal. Significant positive correlation between potential food and amylase activity was reported by Mayzaud & Conover (1975), Mayzaud & Poulet (1978) and Hirche (1981). Mayzaud & Poulet (1978) observed that five out of six enzymes they studied showed significant correlation with the particular carbohydrate or protein. In *Penaeus vannamei* larvae, the presence of protein in the diet causes a decrease in trypsin activity (LeMoullac & Van Wormhoudt, 1994). Studies with *Artemia* have shown no correlation, positive correlations and negative correlations between food and enzyme activity (Samain *et al.*, 1983;1985).

Pollicipes showed higher protease activity in animals feeding on the higher protein diet (*Artemia* - see Chapters 7 & 8) but generally higher amylase activity in adults when feeding on *Skeletonema costatum*. Although *S. costatum* does not store starch it is conceivable that the presence of algal carbohydrate triggers the production and/or secretion of all carbohydrases, including amylase. Kumlu *et al.* (1992) found that an unidentified substance in phytoplankton triggers production of digestive enzymes in larval penaeid shrimps. It is also likely that the starch-hydrolysing enzyme possessed by *P. pollicipes* is not specific in its action and can act on other carbohydrates present in the diatom. Indeed, there have been many reports of highly purified enzymes breaking down a number of substrates with identical linkages between molecules (see Kristensen, 1972). *Artemia* do contain carbohydrates (Chapter 8) although again, it will not be starch but is more likely to be glycogen the presence of which may not affect amylase activity. Harris *et al.* (1986) provided copepods with two algal foods one rich in starch and the other rich in laminarin and measured the ratios of amylase to laminarinase. The enzyme ratio was not greatly changed by the two diets suggesting that digestive enzymes were not completely dependent on the presence of a particular substrate.

Although *Pollicipes* are omnivorous (Chapters 6 and 7), their cirral morphology (Chapter 4), cirral activity (Chapter 5), gut contents (Chapter 6), feeding rates (Chapter 7) and estimated dry weight assimilation efficiencies (Chapter 8) suggest they rely more heavily on the more energy rich animal component of their diet. Protease activity is higher when feeding on *Artemia* and may reflect the higher protein content of the diets. Perhaps when fed for prolonged periods on low energy diets (algae), these barnacles reduce enzyme production to conserve energy.

Diet is not the only factor that can influence enzyme activity. There may also be ontogenetic changes in the activity of digestive enzymes such as those observed in the white shrimp, *Penaeus setiferus* (Lovett & Felder, 1990). In *Pollicipes pollicipes*, the total protease and amylase activities (per wet weight, excluding shell) were much higher in the juveniles, regardless of diet, although trypsin activity was marginally higher in the adults. The disparity between juvenile and adult enzyme activities was most marked when feeding on algae. Juvenile *P. pollicipes* may be better equipped than adults to digest algae, but even their enzyme activities were higher when feeding on *Artemia*. Both qualitative and quantitative changes in enzyme activities were observed during crustacean larval development (Galgani & Benyamin, 1985; Lui *et al.*, 1991). Age-related changes in digestive amylase and protease activities have been demonstrated in *Palaemon serratus* (Van Wormhoudt, 1973) and *Penaeus japonicus* Bate (see Galgani & Benyamin, 1985) and for proteases in *Artemia salina* (Osuna *et al.*, 1977; Garesse *et al.*, 1980). Changes in the protease/carbohydrate ratio during ontogenetic development of crustacea are related to changing nutritional requirements (Hirche & Anger, 1987). *P. pollicipes* juveniles are perhaps more capable of capturing and retaining smaller particles than adults (see Chapter 4). Most small particles are algae and bacteria and juveniles had a larger proportion of algal material in their guts than the adults (Chapter 6). Most large particles are animals and these dominated the guts of adult *P. pollicipes*. It seems likely that *P. pollicipes* juveniles are more able to digest algal material than the adults.

High amylase activity was found in the gut extract of *P. pollicipes*. The presence of a range of carbohydrases and strong amylase activity in particular, are in conflict with the observation of Ritz & Crisp (1970) that barnacles are unable to digest starch but high amylase activity has been observed in many crustaceans such as *Balanus crenatus* (Kristensen, 1972), the white shrimp, *Penaeus setiferus* (Lovett & Felder, 1990) and copepods (see Harris *et al.*, 1986). Starch is rarely stored by marine phytoplankton and is only found in rather small cells belonging to groups which are relatively uncommon (Cryptophyceae and Chlorophyceae). High amylase activity suggested to Harris *et al.* (1986) and Samain *et al.* (1983) the possible importance of

small starch-containing flagellates for copepod production and the optimisation of the assimilation of algae containing starch at low concentrations. However, it could just be indicative of low enzyme specificity. Samain *et al.* (1985) found peaks in amylase activity in copepods occurred when there was starch depletion, which suggested that the high levels of amylase found in copepods and other crustaceans are necessary because starch concentrations in particulate material in the ocean are relatively low. The result of higher amylase activity in *P. pollicipes* feeding on algae indicates the opposite, i.e. that enzyme secretion may be triggered by the presence of algal carbohydrates. High amylase activity in *P. pollicipes* and in most other crustaceans indicate that carbohydrates may be digested. *P. pollicipes* was able to digest the carbohydrate found in *Artemia* but unable to digest rotifer carbohydrates (Chapter 8). However, there was no measurable amylase activity in adult *P. pollicipes* feeding on *Artemia*. It appears that the digestibility of carbohydrates is variable and that amylase activity varies with diet.

At the enzyme and substrate concentrations used, there was no measurable hydrolysis of maltose, lactose or salicin by either *Pollicipes pollicipes* or *Lepas anatifera* gut extracts. These are sugars that would rarely, if ever, be encountered by either species in their natural diet. However, such enzymes are present in other crustaceans where the ecological significance is equally puzzling (Nicholls, 1931; Kooiman, 1964; Aragawal *et al.*, 1967; Tyagi & Prakash, 1967; Harnden, 1968; Telford, 1970; Kristensen, 1972) and can perhaps only be explained by non-specificity of the carbohydrases involved. The storage carbohydrates of many marine algae (laminarin and mannitol) were broken down by both extracts as was carrageenan. Laminarin is an important reserve in diatoms (Meeuse, 1962) so laminarinase activity indicates that the diatoms, or at least the laminarin stored there, that form part of the *P. pollicipes* diet (see Chapter 6), may be digested.

There was no cellobiase activity detected in either *P. pollicipes* or *L. anatifera* extracts. Many invertebrates have strong cellobiase activity (Kristensen, 1972) but none was found in *Balanus crenatus*, the only barnacle he tested. Pentreath (1969) and Telford (1970) found that often cellobiose was strongly hydrolysed even when degradation of cellulose could not be demonstrated by the same crustacean extracts. Again, the ecological significance of cellobiase activity is unclear because cellobiose is only known to occur free in the last step of cellulose breakdown so will rarely occur in natural foods. The main carbohydrate storage product of animals is glycogen and *Balanus crenatus* exhibited high glycogenase activity (Kristensen, 1972). Both *P. pollicipes* and *L. anatifera* had glycogenase in their gut extracts, suggesting that they are able to breakdown animal storage products.

Little digestion of *Artemia* lipids by *P. pollicipes* was measured (Chapter 8) although rotifer lipids were more completely digested. Both *P. pollicipes* and *L. anatifera* possess a lipase. The lipase acted on condensed milk releasing sufficient fatty acids to give a positive result but did not act on olive oil. This probably reflects a difference between the lipid present in the two substrates or perhaps the sensitivity of the two indicators. The presence of lipases again conflicts with the observation of Ritz & Crisp (1970) that barnacles are unable to digest fats and that these pass through the digestive tract unaltered. Lipid is also a reserve product of diatoms.

Digestion of the protein in *Artemia* and rotifers by *P. pollicipes* was found to be high (Chapter 8) but the proteases present in *Pollicipes* extract were unable to liquefy solid gelatine. There was a protease that could break down casein in the guts of both juvenile and adult *P. pollicipes*, but with higher activity in the juveniles. There was also a trypsin-like enzyme found in the gut extracts of *P. pollicipes*. DeVillez & Buschlen (1967) found no trypsin-like component in the gastric juice of *Balanus nubilus* and Degkwitz (1957) claimed that barnacles and shrimps had only catheptic proteases. However, trypsin plays an important role in the digestion of proteins in most crustaceans (Galgani & Benyamin, 1985; Galgani *et al.*, 1985). In *Penaeus japonicus*, trypsin contributes up to 40% of the proteolysis in the hepatopancreas (Galgani *et al.*, 1984). Abundant trypsin activity has also been identified in different parts of the digestive tracts of several other species of shrimp (Tsai *et al.*, 1986), contrary to the findings of Degkwitz (1957).

Chapter 10

General discussion

Over-harvesting of natural populations of *Pollicipes pollicipes* for human food was a stimulus for the present work. If these barnacles are to be cultivated commercially, far more knowledge of their ecology, diet, feeding and growth is needed.

The requirements for successful cultivation are diverse: firstly, the barnacles must be 'seeded' onto a suitable substratum in sufficiently high numbers and the substratum must offer secure attachment. Secondly, the barnacle must feed or be fed on a suitable diet that can sustain a rapid growth rate. Thirdly, it must remain relatively free of epiphytes and pathogens so that mortality rates are low and the end product is acceptable to consumers. Fourthly, it must be easy to harvest without damaging the organism and finally, all these requirements must be met economically so that the organisms are delivered to market at an attractive price.

There are no obvious ways to increase the natural populations or to enhance growth rates on the shore. Stock replenishment could only be achieved by restricting access to the shore for the present artisanal harvesters through an integrated management strategy which might be difficult to enforce. To increase the overall production therefore, *P. pollicipes* would have to be cultivated, either in tanks or on rafts or ropes in the sea. The requirements for any such system are that it is cheap, non-labour intensive and that the barnacles are easy to harvest.

Before commercially rearing a species it is desirable to know the proportion of the food energy it ingests that goes into the production of new somatic tissue. This is particularly important when the feeding regime is going to be controlled. An energy budget has been tentatively established for sexually mature *P. pollicipes*.

I	=	Eg	+	Gr	+	R	+	Rn
29.9 $J\ day^{-1}$	=	8.71 - 12.3 $J\ day^{-1}$	+	6.2 $J\ day^{-1}$	+	8.14 $J\ day^{-1}$	+	2.8 $J\ day^{-1}$
100%	=	29.1-41.1%	+	20.7%	+	27.2%	+	9.4%

where I = ingested energy, Eg = egested energy, Gr = growth, R = reproduction and Rn = respiration. No estimates are available for the energy lost through exuviation and excretion.

The energy budget above is based on several assumptions, some which are more reasonable than others. Firstly, it was assumed that the barnacles on the shore would be submerged for 14 hours per day and during that time feed constantly at the rate measured in the laboratory when feeding on *Artemia* nauplii. Prey concentrations are much lower in the field (perhaps more than ten times lower) and although over the range of concentrations examined in the present study *Artemia* ingestion rates were not density-dependent, they may be at very low densities. The ingested energy may therefore be an overestimate. Secondly, the energy allocated to growth was calculated assuming growth rates equal to those of adult *P. pollicipes* eating *Artemia* in the laboratory, organic weight was calculated from the equation: log (organic weight - g) = -4.05 + 2.77 log (RC length). The growth rates of animals measured on the shore (Cruz, 1993) were lower than this, hence the estimate of energy allocated to somatic growth is also likely also to be an overestimate. Thirdly, the respiration rate was calculated using the relationship proposed for *P. pollicipes* in the laboratory by Castellani (unpublished), Rn (in μl O_2 hr^{-1}) = 0.91 (organic weight)$^{0.52}$ and converted to joules assuming 1ml O_2 is equivalent to 20.1 J (Crisp, 1984). Reproductive output was measured for *P. pollicipes* in the field by Cardoso & Yule (1995) and the figure used in this budget is based on three broods per season (183 days) and the energy content of the eggs being ca 20 J mg^{-1} dry weight (A.B. Yule, pers. comm.). Although there are queries regarding aspects of the energy budget, it does give an indication of the proportion of the ingested energy that is used for growth of somatic tissue (ca 21%).

P. pollicipes on the shore were found to orientate into, and presumably filter food from, the wave backwash that flows over the rocks after a wave has broken. Both juveniles and adults exhibit prolonged cirral extension in conditions of water flow so it seems likely that this will be the predominant cirral activity of *P. pollicipes* on the shore. Cirral beating was only displayed in conditions of no or very low flow and the frequency was unaffected by the presence of food, providing no evidence for the juvenile to adult shift in feeding mechanisms proposed for *P. polymerus* (Lewis, 1981) and *P. pollicipes* (Hui, 1983). The results of the present study suggest that cirral beating, such as it is, serves a respiratory function and is not involved in feeding in either juveniles or adults. As water flow is a requirement for efficient feeding by cirral extension, it will also be necessary for rapid growth so a culture system must provide water flow, be it provided naturally or artificially and a method of secure attachment for the barnacles.

Cruz (1993) found growth rates of 3 mm RC $year^{-1}$ of *P. pollicipes* on the shore. Goldberg (1984) measured a similar amount of growth in just 50 days in animals that

were maintained on a raft - the higher growth rates perhaps attributable to the constant immersion and therefore greater opportunity to feed. However, in the laboratory, *P. pollicipes* were found by A.C. Cardoso (pers. comm.) to grow more rapidly when emersed with tidal periodicity than when continually immersed. The maximal growth rates measured in the laboratory during the current study were higher than those measured on the shore by Cruz (1993) but were only half those measured by Goldberg (1984). Again, it appears that the lower rates on the shore reflect the uncovering by the tide. The fact that animals on the shore feed omnivorously while those in the laboratory feed on *Artemia* alone is unlikely to be responsible for the lower growth rate as no significant difference was found between the growth rate of animals feeding on *Artemia* alone and those feeding on a mixed diet in the laboratory. The growth rates measured by Goldberg (1984) were probably highest because the animals are constantly immersed and therefore able to feed and are subject to water movement. Although the measurements were made during the summer months when growth rates are highest (Cruz, 1993), therefore perhaps giving an overestimate of annual growth rates.

Rough estimates made from the growth rates of *P. pollicipes* on the shore measured by Cruz (1993) suggest that these barnacles may take between one and four years from settlement and metamorphosis to an edible size. However, the results of Hoffman (1984) although based on only 44 animals give some cause for optimism that this time can be reduced in a suspended culture system.

The requirements for rapid growth will also include an ample food supply, few competitors and low epizoite infestation. The present study indicates that culturing this species in tanks is not ideal. Animals rapidly became fouled with algae, anemones and bryozoans and were prone to fungal infection. Such infestations may be greatly reduced in the wild by intermittent aerial exposure.

Culturing in the sea has both advantages and disadvantages. Flowing water of an appropriate temperature is freely available although, structures to which the barnacles are attached must withstand water movement. The barnacles could be continually submerged attached to ropes on offshore rafts or buoys. Many marine organisms are cultivated on ropes (e.g. mussels, seaweeds, pers. obs.) because ropes are cheap, have low drag and therefore survive at exposed sites, and are easily hauled in for harvesting. If placed on structures on the shore, the barnacles would be subjected to more suitable hydrodynamic conditions for feeding. However, the animals would perhaps be subject to theft and the structures could be vandalised or washed away.

Harvesting animals from low down on wave-battered shores would also be far more difficult than from floating structures offshore.

However, natural plankton densities and therefore food supply vary seasonally. Research into the factors limiting growth rate during the winter months is required to assess whether food supplements could improve barnacle growth. Diet has a strong influence on energy intake, digestion efficiency and growth rate. Although *Pollicipes pollicipes* eats phytoplankton in the wild and the laboratory, the feeding appendages are not well adapted for such small prey so feeding rates are insufficient for weight maintenance and growth when a monospecific algal diet is eaten. Growth rates were not enhanced by feeding *Artemia* in conjunction with algae so enrichment with algae of the water surrounding the barnacles would be fruitless. The diet required by *P. pollicipes* is one of predominantly zooplankton and for prey the size of *Artemia*, the maximum ingestion rate is 60 animals hr^{-1} which would ensure reasonable growth rates as long as hydrodynamic conditions were conducive to feeding.

Digestion of animal prey was more efficient than of phytoplankton. Dry matter and total energy digestion efficiencies are quite high, as they are for other species of barnacle (Kuznetsova, 1973; Wu & Levings, 1978). Protein digestion is high (92 - 100%) but carbohydrate and lipid digestion is very variable (from 0% to 97% and from 25 - 92% respectively) depending on the diet consumed. *P. pollicipes* has an enzyme complement similar to the other barnacles documented in the literature (for example, Kristensen, 1972) and one able to handle an omnivorous diet so it should be possible to culture *P. pollicipes* on a number of artificial diets. However further research into lipid and carbohydrate digestion by this species would be required to aid diet formulation. Diet development would be very worthwhile if the barnacles were cultivated in tanks, but there is perhaps less scope for enhancing feeding and assimilation efficiencies of filter feeding animals cultured in the sea. Fish farmed in sea pens can be fed on formulated diets because they are predatory feeders (pers. obs.). If farmed in the sea the barnacles would not capture most of the food that was given to them perhaps making feeding expensive.

Whatever system of culture was used, it would be desirable to exploit natural larval settlement, tapping the reservoir of larvae that would not normally settle successfully and is therefore wasted. The numbers of *P. pollicipes* cyprids in the coastal waters has not been established. They are however, difficult to settle in the laboratory, and offshore, the hydrodynamic conditions may be unattractive to the larvae. On the shore, there is evidence that large numbers of larvae settle on the peduncles of adults then migrate onto the rock substratum (see Kugele & Yule, 1993 and for *P. polymerus*

Hoffman, 1984). Thus the adults are essential as a nursery for the juveniles and indeed, no resettlement of *P. polymerus* occurred during the seven years following a particular adult harvest (Bernard, 1988). The presence of adults on the culture ropes might be sufficient to induce natural settlement offshore, otherwise settlement could be induced on ropes on the shore and the ropes then be moved offshore. *P. pollicipes* reproduce from about April to the end of September (Cardoso & Yule, 1995) hence larvae are only likely to be present in the plankton during these months.

The transplantation of juvenile barnacles from natural populations onto offshore structures for 'growing on' does not seem a sensible alternative. Even if they could be dislodged without damaging their peduncles, which seems unlikely, transfer from the shore to a 'farm' does not increase production or protect stocks. The combination of collection for ongrowing on rafts and increased pressure of local fishermen may cause the destruction of *Pollicipes pollicipe*s populations completely, so both culture and collection become untenable. Some of the artisanal harvesters could become barnacle farmers that tend the cultivation.

If commercial cultivation utilises settled cyprids (either from the wild or commercially cultured) rather than transplanted animals, the impact on natural populations would be reduced and, with proper marketing, it might be possible to greatly increase the demand for *P. pollicipes* by introducing more people to the delicacy. The only sustainable means of culturing *P. pollicipes* is by settling cypris larvae on ropes and ongrowing the metamorphosed barnacles offshore. To ensure the long term survival of *P. pollicipes*, some regulation of collection is necessary. Future work on this species should concentrate on ways of inducing larval production and settlement as this is currently the main obstacle to sustainable farming.

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Appendices

Appendix 1: Distribution and orientation (Chapter 3)

Appendix 1.1: Calculation of confidence intervals for orientation angles

This gave an indication of the deviations caused by chance fluctuations. This procedure required the mean vector length (r) and the mean angle of orientation (Ø) and the selection of a confidence coefficient (Q). Q = the probability that the unknown parameter will lie within the confidence interval (= 0.95).

Calculation of the confidence intervals were made using tables (see Batschelet, 1981). These give an angle of deviation (∂) in degrees.

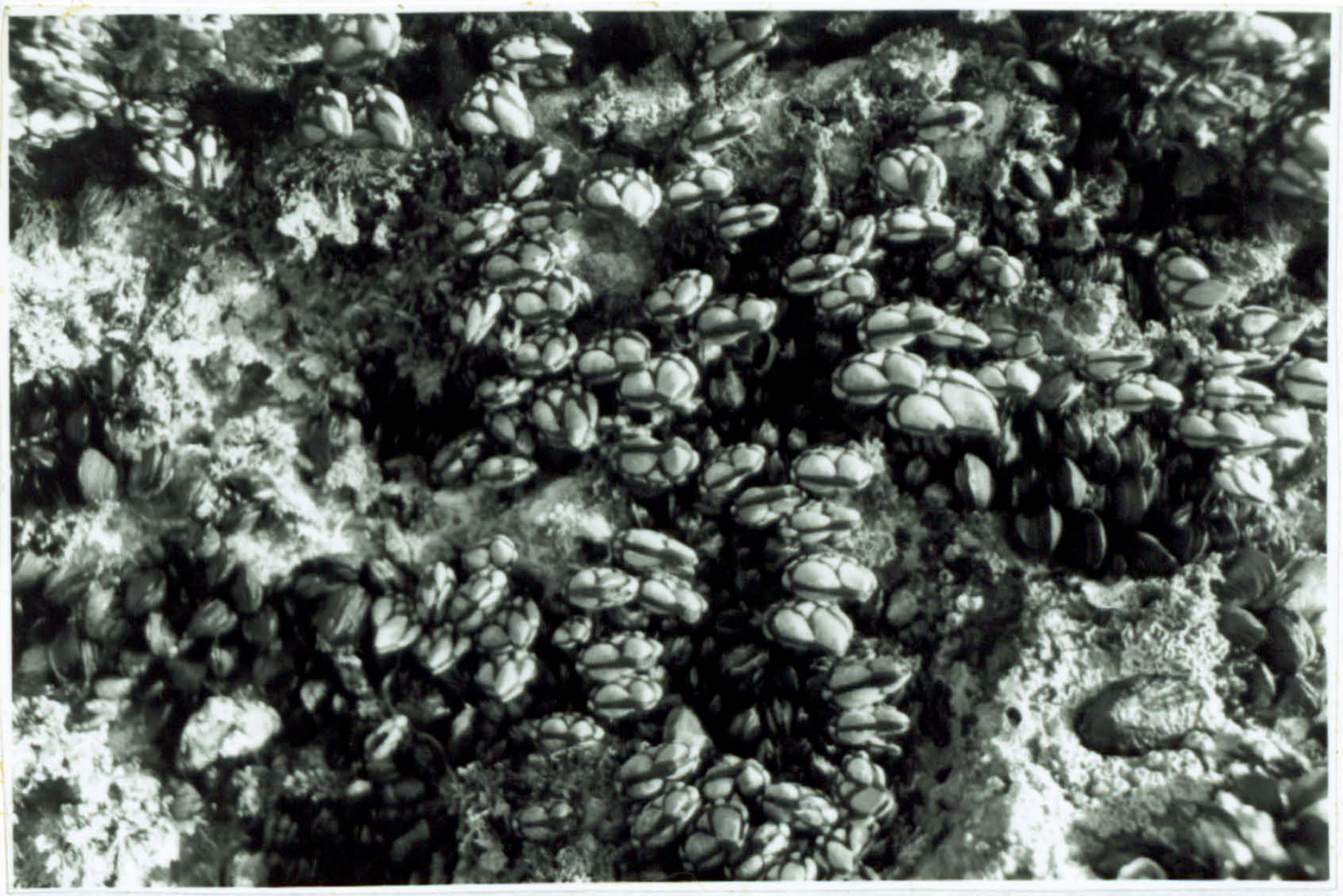
$\text{Ø} \pm \partial$ = the confidence limit

If the angle designated to indicate full orientation into water flow (ie.180°) lies outside the limits, the mean sample orientation Ø is significantly different from 180° and the sampled animals are not orientating into the water flow.

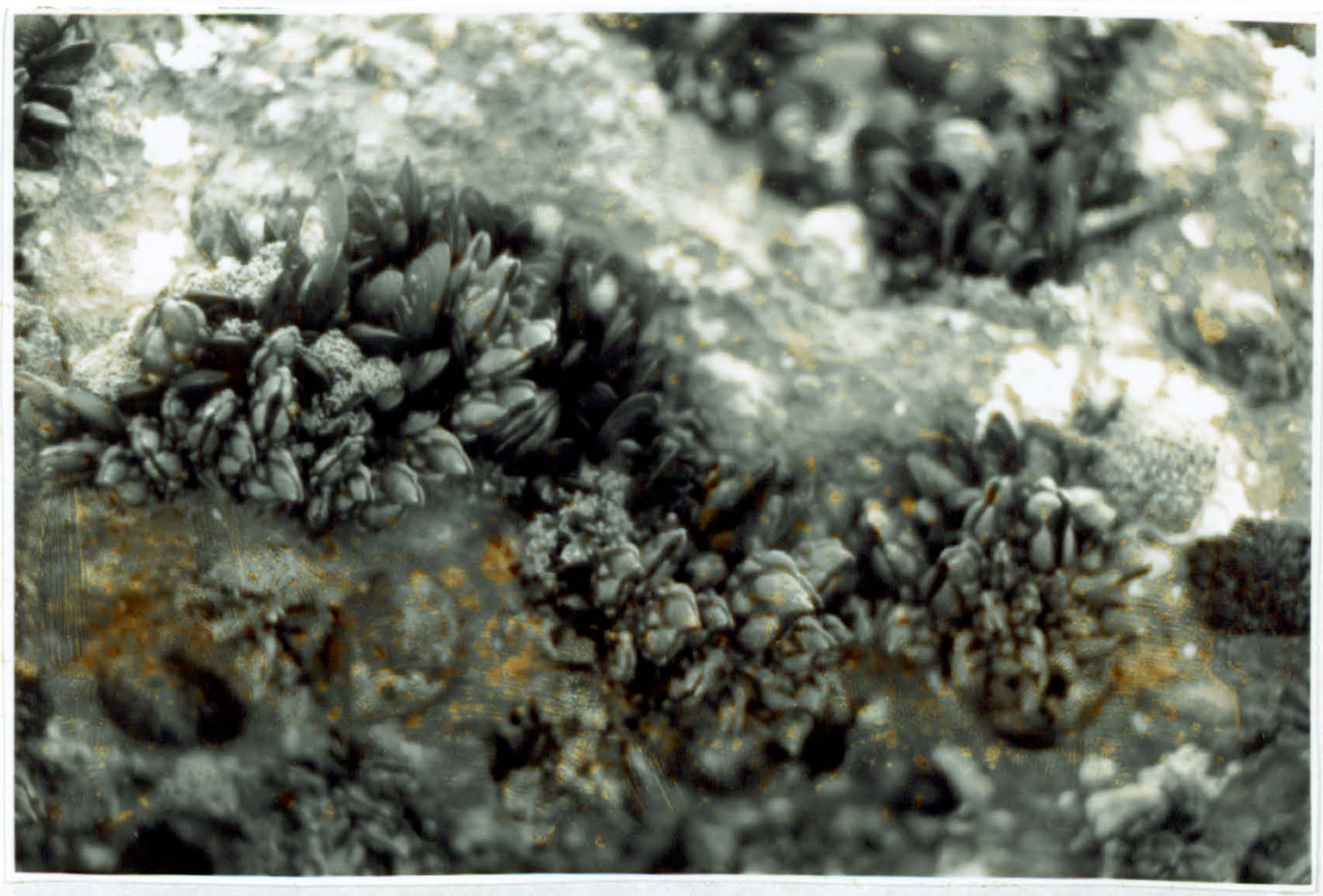
Appendix 1.2 a - k. Photographs of *P. pollicipes*, from which orientate measurements were expressed in Table 3.2.

Appendix 1.2 (a). *P. pollicipes* in a crack at Ponta da Fisga orientating into the incoming waves. Scale: 1 cm = ⟵⟶

Appendix 1.2 (b). ***P. pollicipes*** **in a shallow crack in the rock at Ponta da Fisga also orientating into the inflowing water. Scale: 1 cm = ——**



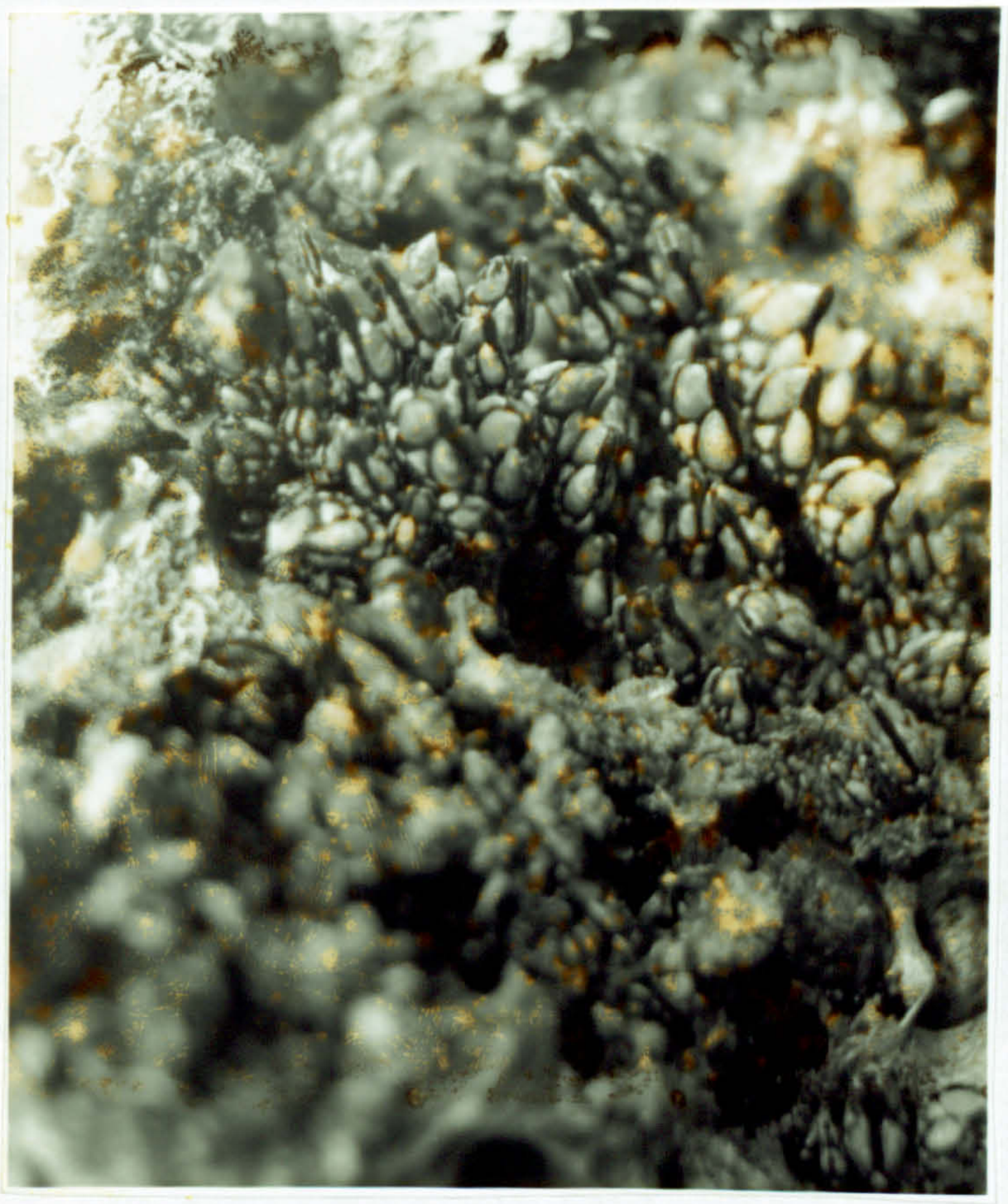
Appendix 1.2 (c). ***P. pollicipes*** **on the leading edges of two boulders at Site 3, in both cases orientating into the upsurge generated by incoming waves. Scale: 1 cm = ——**



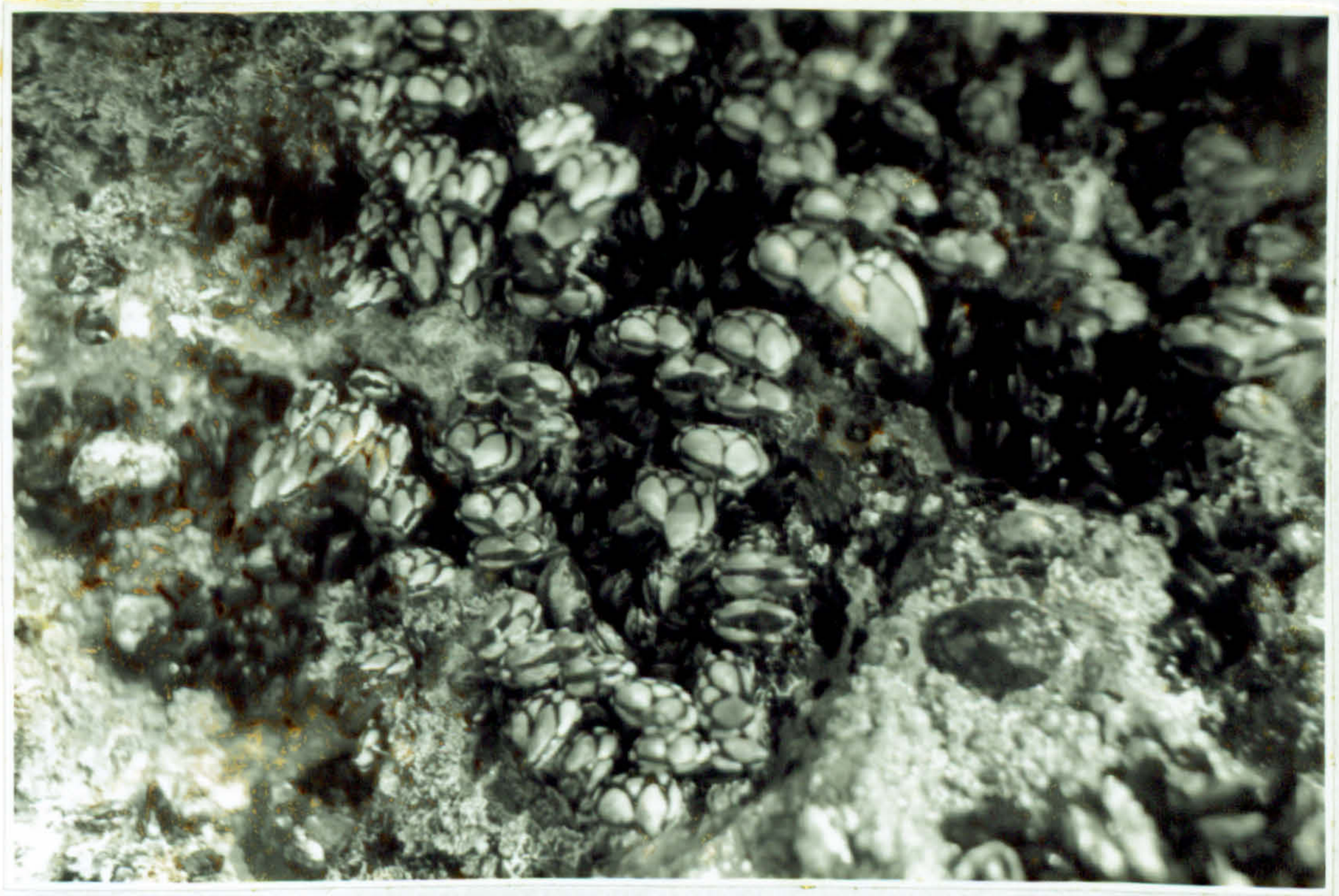
Appendix 1.2 (d). ***P. pollicipes*** **on the leading edges of two boulders at Site 3, in both cases orientating into the upsurge generated by incoming waves. Scale: 1 cm = ⌊——⌋**

Appendix 1.2 (e). ***Pollicipes pollicipes*** **in a crevice at Ponta da Fisga, orientating into incoming water. Scale: 1 cm = ⌊——⌋**

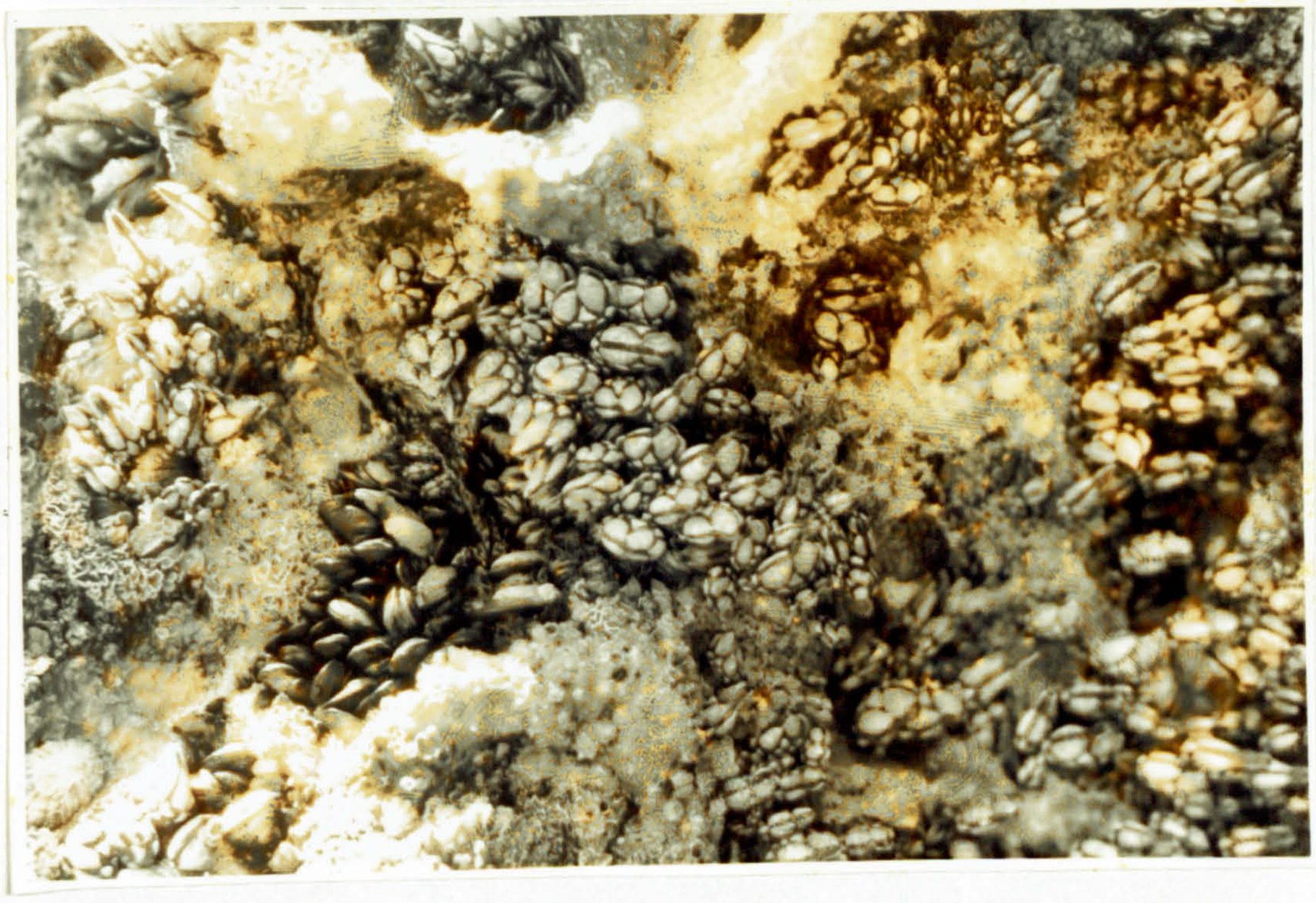
Appendix 1.2 (f). A boulder at Site 3, near Sagres. *P. pollicipes* orientating into the wave generated upwelling of water. Scale: 1cm = ——



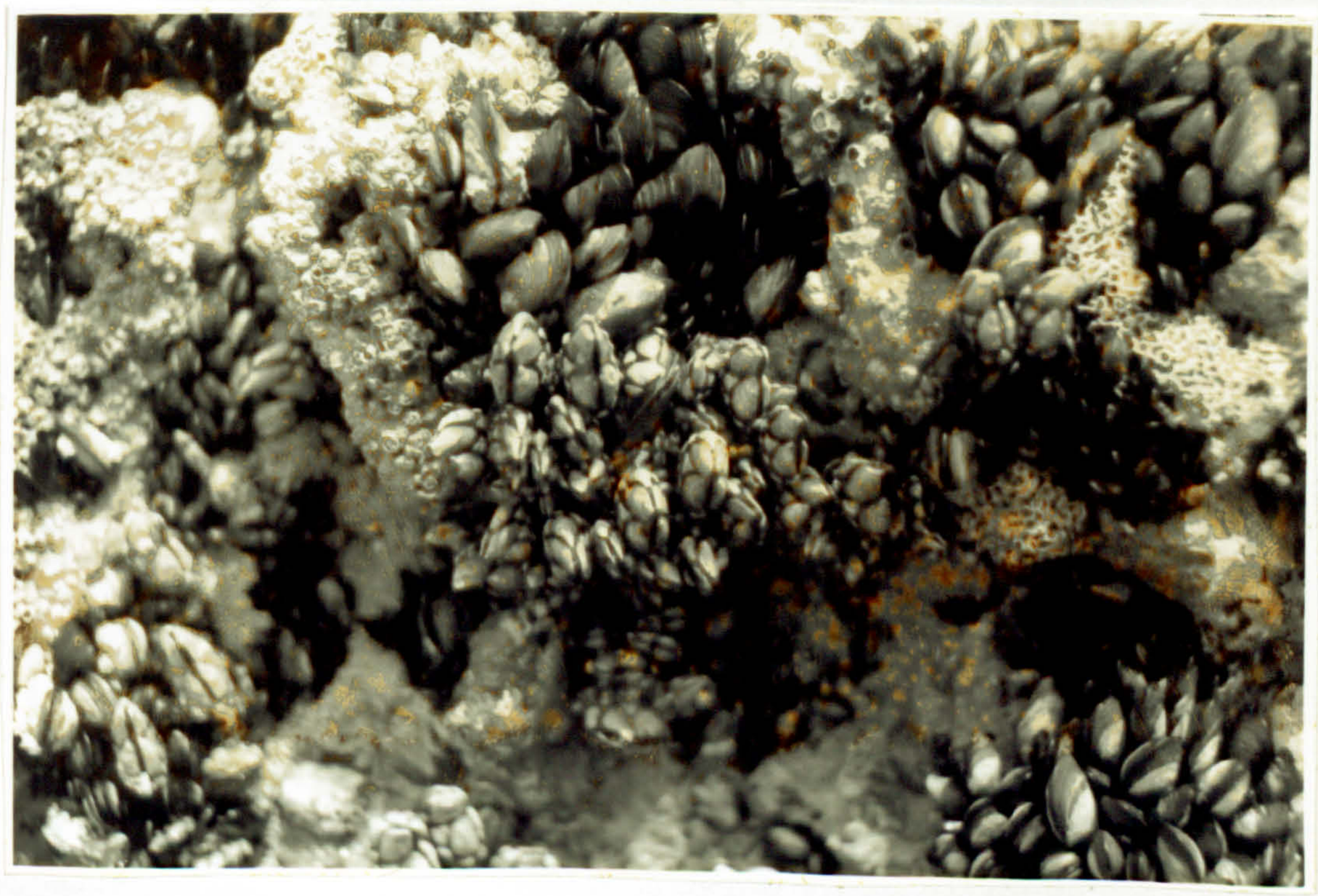
Appendix 1.2 (g). Rock surface at Ponta da Fisga showing *Pollicipes* orientating into the incoming water. Scale: 1 cm = ——



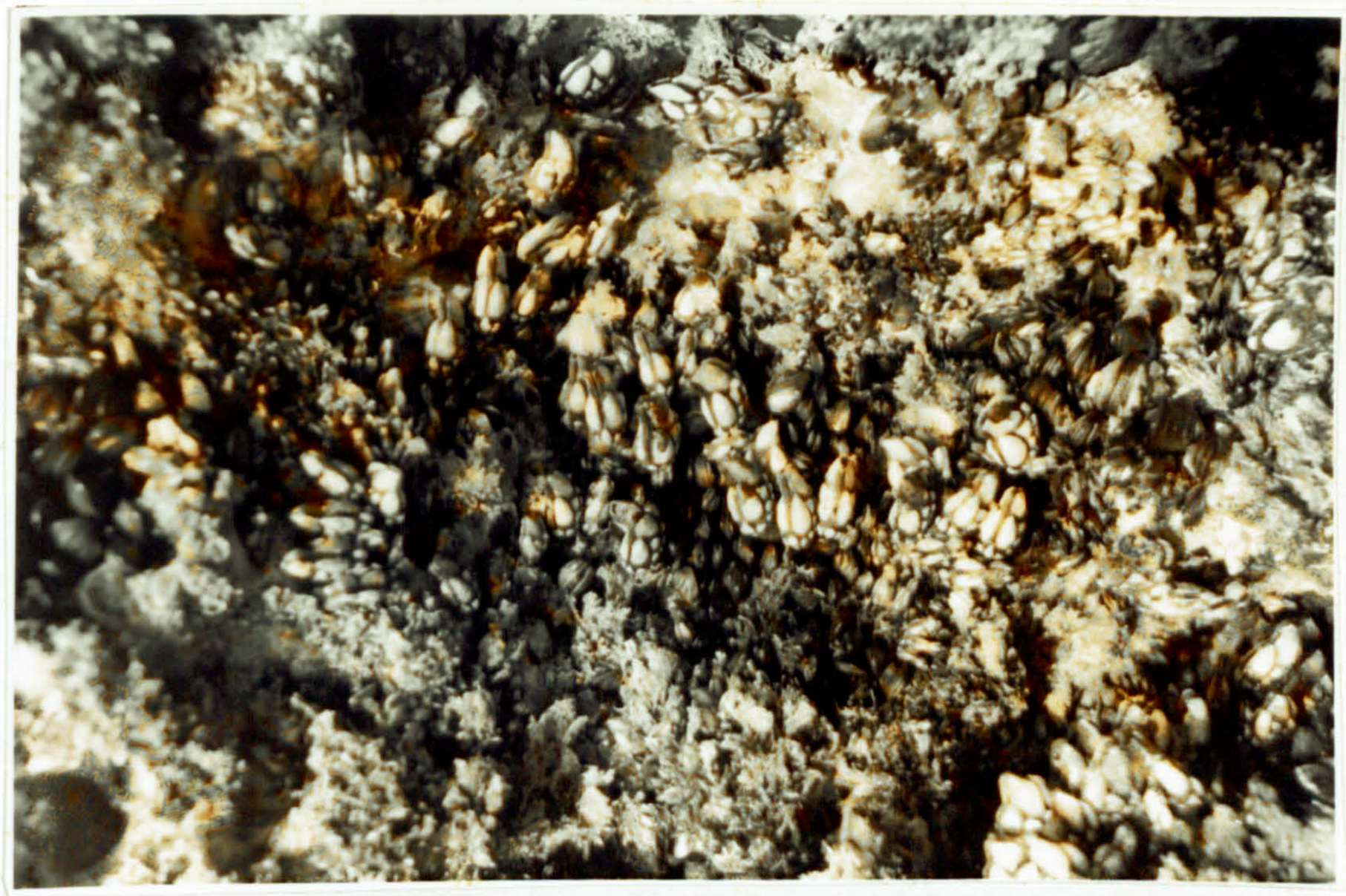
Appendix 1.2 (h). Rock surfaces at Ponta da Fisga showing *Pollicipes* orientating into the incoming water. Scale: 1 cm = ⟵



Appendix 1.2 (i), showing the rocks at Ponta da Fisga with *P. pollicipes* orientating into the incoming wave water. Scale: 1 cm = ⟵



Appendix 1.2 (j), showing the rocks at Ponta da Fisga with *P. pollicipes* orientating into the incoming wave water. Scale: 1 cm = ——



Appendix 1.2 (k). The seaward face of a boulder at Site 3, near Sagres. *P. pollicipes* are orientating into the inflowing water. Scale: 1 cm = ——

Appendix 2: Cirral Activity (Chapter 5)

Appendix 2.1. Calculation of flow rates from flow meter readings

The flow meter gave readings in Hertz (rotations of the propeller per minute) and each reading was the mean of ten readings taken over the previous ten seconds. During each period of observation, five such readings were taken and the mean calculated. The frequency of propeller rotation was converted to a flow rate using the following equation:

$$V = 0.60139\,(H) + 1.84861$$

where V = Velocity in cm s^{-1}

H = mean frequency in Hertz.

Appendix 3: Feeding (Chapter 7)

a) Wet oxidation technique for the measurement of energy content.

The following reagent was prepared:

Oxidation mixture. 2.5 g potassium dichromate mixed with 10 ml distilled water and made up to 500 ml with concentrated sulphuric acid.

2 ml of the 0.5 % dichromate solution was diluted with 50 ml distilled water to provide the dichromate standard.

Triplicate 1 mg food and faecal samples were weighed out into digestion tubes. One mg of benzoic acid was weighed into a tube as a standard (benzoic acid has 6.319 calories mg^{-1}) and a further tube containing 0.5 ml distilled water was used as a control. Two ml of digestion mixture were added to each tube on a hot block and heated at 100°C for 1 hour. The mixtures were cooled, each diluted to 50 ml with distilled water and the absorbance read at 347 nm in 1 cm cuvettes. The zero point was set with 4% sulphuric acid. Comparison of the control and standard dichromate solution indicated any colour loss in the absence of food or faeces.

Calculation of energy content.

The absorbance of the control was 0.64 relative to the blank. The presence of a sample reduced the absorbance towards zero. If this value is E, the fraction of dichromate removed is **(0.64-E) / 0.64.** Of the 10 mg potassium dichromate ($K_2Cr_2O_7$) originally present in 2 ml of reagent, the amount removed is **(0.64-E) / 0.64 x 10 mg.** Since 3 mg of $K_2Cr_2O_7$ is equivalent to 0.489 mg oxygen and a mean

oxycalorific for carbohydrate, fat and protein is 3.38 calories, then 1 mg of $K_2Cr_2O_7$ is equivalent to 0.552cals. Hence the energy in the original 1 mg sample is **(0.64 - E) / 0.64 x 5.52 cals.** Only about 60% of the protein in the sample reacts in the process (Forster, 1970) so a correction must be made once the protein content is measured. Protein typically contains 5.65 calories mg^{-1}, so a value of **0.4 (protein content mg) x 5.65 calories** must be added to the energy calculated above.

b) Measurement of protein content using Kjeldahl method.

The following reagents were prepared:

Digestion mixture - 300 g selenium dioxide were dissolved in 15 ml distilled deionised water (ddH_2O) mixed with 85 ml nitrogen-free H_2SO_4. The resulting solution was made up to 200 ml with ddH_2O.

Phenol reagent - 4 g of phenol and 20 mg of sodium nitroprusside were made up to 400 ml with ddH_2O.

Hypochlorite reagent - 2 ml of fresh sodium hypochlorite were added to 80 ml of 2.5% NaOH and made up to 200 ml with ddH_2O.

Standards of 3, 6, 18, 24, 30 and 36 $\mu g ml^{-1}$ ammonium sulphate were prepared in 2 % diluted digestion mixture. All glassware was washed with 10% HCl prior to use to remove ammonia.

Triplicate 1 mg dried samples of food and faeces were placed in digestion tubes and together with a control tube were heated with 1 ml of digestion mixture on a recessed hot plate. Samples were incubated for 16 hours at 120°C and then for a further 8 hours at 260°C. Each sample was diluted to 20 ml with ddH_2O. One ml of each diluted mixture was transferred to a clean tube. One ml of each standard was placed in a further 6 tubes. To each were added 2 ml of 2.5% NaOH, 4 ml phenol reagent, 2 ml of Hypochlorite reagent and 1 ml of ddH_2O. The blue colour was allowed to develop for 20 minutes at room temperature. The absorbance was read at 635 nm in a 1 cm cuvette and the nitrogen concentration of samples was calculated from the calibration curve. Calculation of protein content:

% protein = {(|conc. dig NH_4 / 4.680| x 20 x 6.25)/wt of sample} x 100

4.680 = μg nitrogen / μg of ammonium sulphate equivalent measured

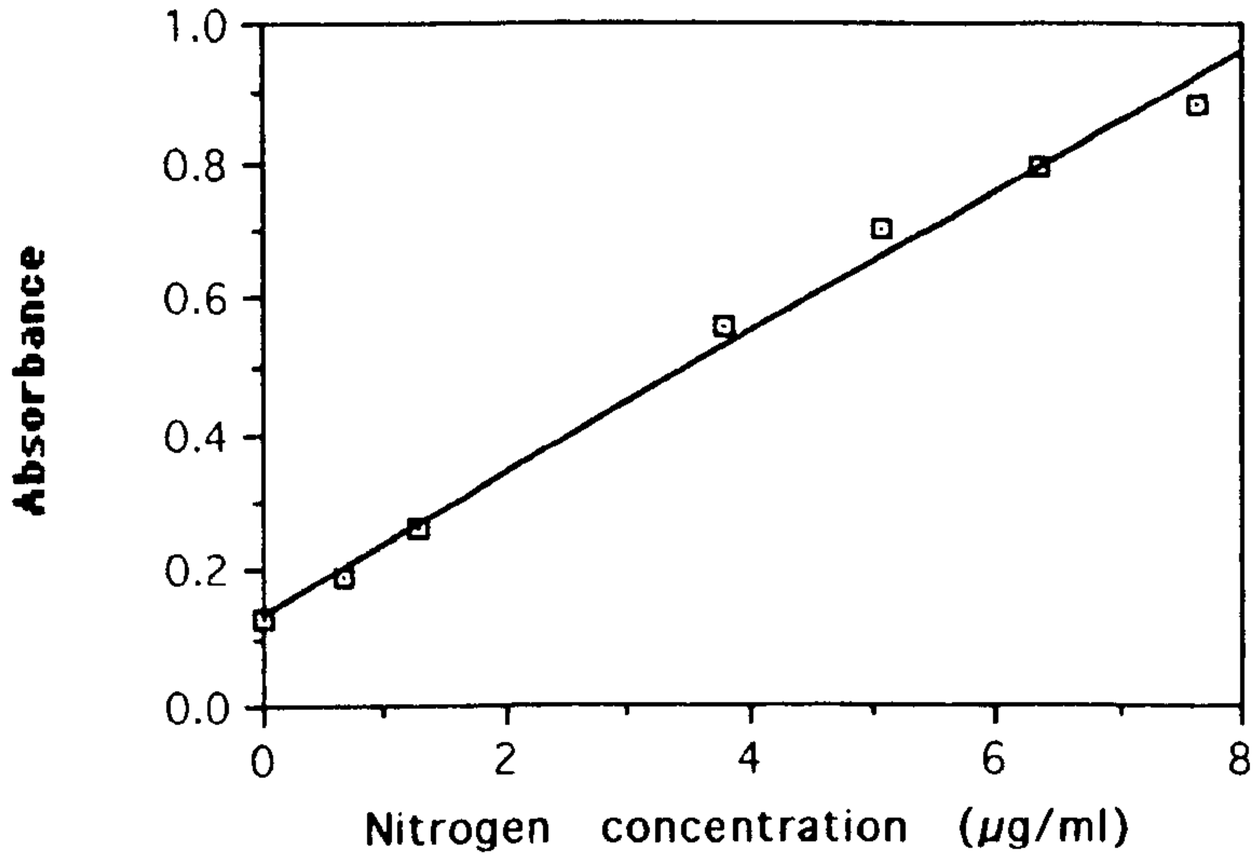
6.25 = μg protein equivalent to every μg nitrogen.

20 = volume of diluted sample.

Appendix 4: Digestion (Chapter 8)

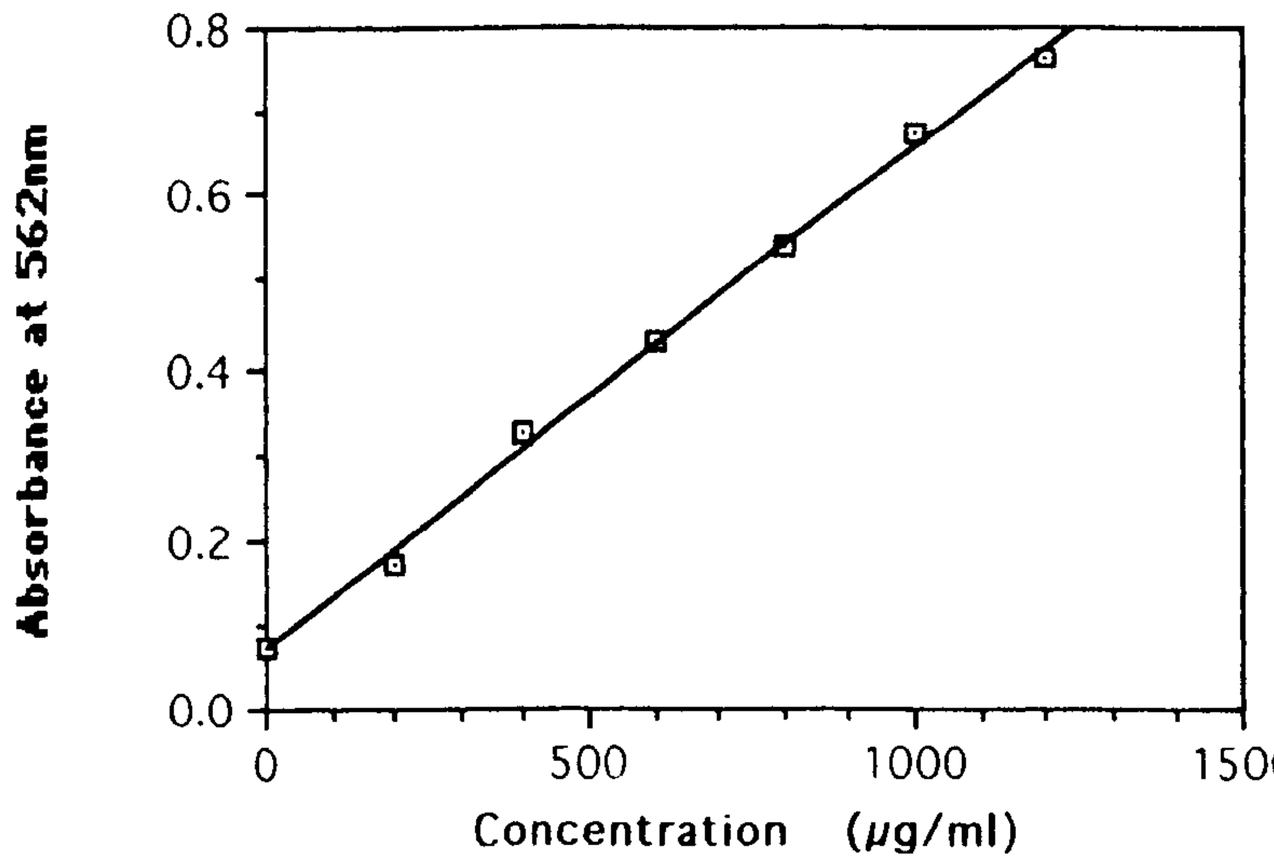
Appendix 4.1. Calibration curve for nitrogen concentration against absorbance in Kjeldahl procedure read at 635 nm in a 10 mm cuvette.

absorbance = 0.1358 + 0.1028 (concentration) r = 0.996, df = 5, P < 0.001



Appendix 4.2 Calibration curve for bovine serum albumin (BSA) protein concentration against absorbance read at 562 nm using a 5 mm cuvette. Absorbances obtained for protein samples following a BCA Protein Assay were read off the curve.

absorbance = 0.074 + 0.00058571 (concentration) r = 0.999, df = 5, P <0.001



Appendix 4.3. Method for calculating apparent dry matter digestion efficiency.

The % digestion was calculated from:

$$\mathbf{U' = [(F' - E') / (1-E')(F')] \times 100}$$

where

U' = % digestion

$\mathbf{F' = (F - A_f) / F}$ and $\mathbf{E' = (E - A_e) / E}$

F = dry weight of food A_f = ash weight of food

E = dry weight of faeces A_e = ash weight of faeces

Appendix 4.4. Anthrone method for measurement of carbohydrate.

The following reagents were prepared and were sufficient for 60 samples:

Anthrone - 0.4 g of recrystallised anthrone was added to a glass beaker containing 16 ml ethyl alcohol and 60 ml ddH_2O. The beaker was placed in a cold water bath, in a fume cupboard, and 200 ml of sulphuric acid was gradually stirred in with a glass rod. The resulting clear yellow-green solution was allowed to cool. The reagent was transferred to a dark bottle and stored in a refrigerator.

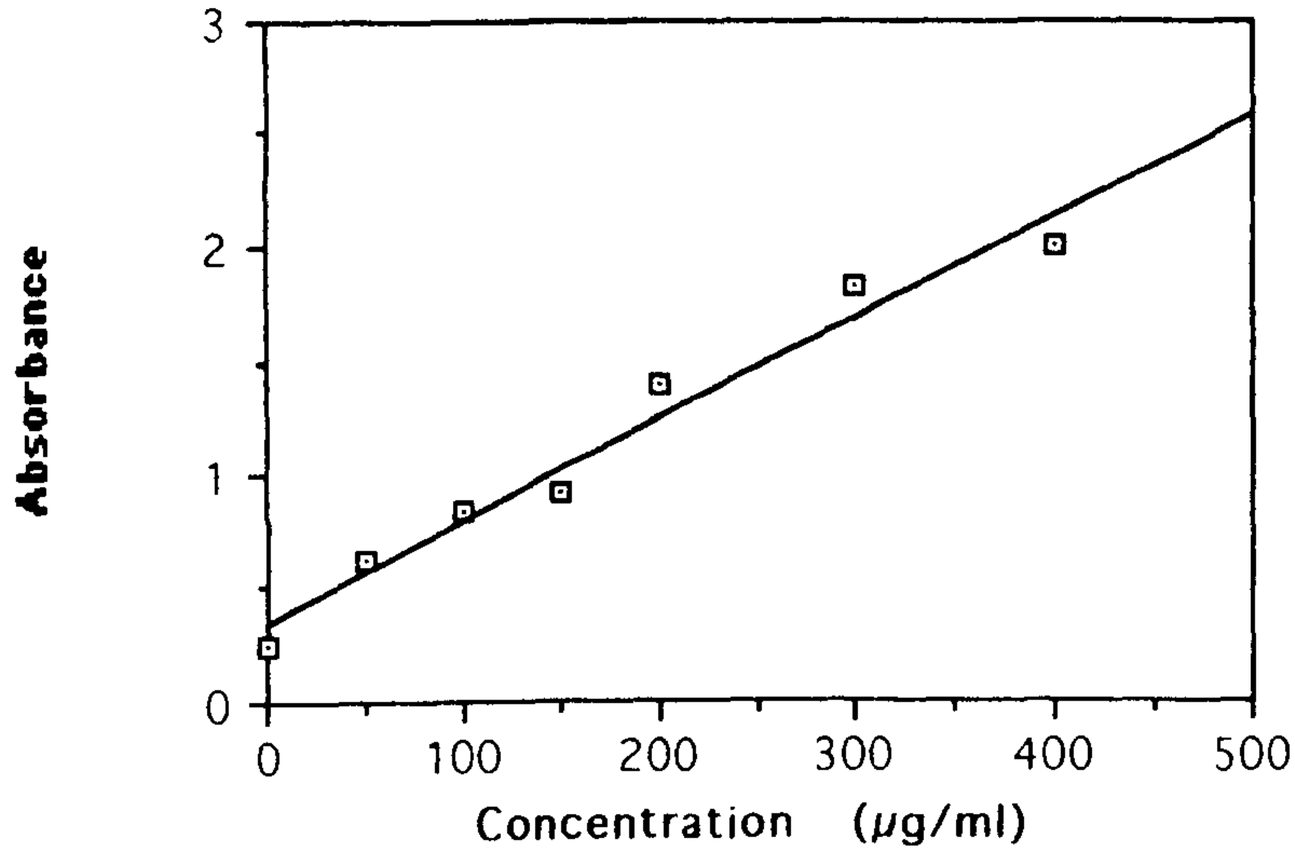
5% TCA - 25 g of Trichloroacetic acid crystals were dissolved in 500 ml of distilled water in a volumetric flask. This was stored in the refrigerator.

Glucose standards - 1.0 g of D-glucose was dissolved in 100 ml distilled water in a volumetric flask and mixed well. It was made up fresh on the day of analysis. Standards containing 50, 100, 150, 200, 300, 400 μg ml^{-1} glucose were made by diluting the solution with distilled water.

Triplicate 2 mg samples of food and faeces were placed into numbered 10 ml conical glass centrifuge tubes. To each tube 2 ml of 5% TCA were added. Tubes were covered with foil to reduce evaporation and placed in a 90°C water bath for 1 hour. A 0.5 ml subsample of TCA supernatant, containing the extracted carbohydrate, was pipetted from each tube and placed in individual numbered thick glass boiling tubes. 0.5 ml of glucose standards were pipetted into further tubes and 0.5 ml of ddH_2O into two tubes for reagent blanks. 4 ml anthrone was added to each tube and the contents vortex mixed. All boiling tubes were placed in a 100°C water bath for 7 minutes, then cooled for 10 minutes in a cold water bath. The samples and standards were transferred to plastic cuvettes and the absorbance read at 620 nm. Sample concentrations were read from the calibration curve (see Appendix 4.5).

Appendix 4.5: Calibration curve of absorbance vs glucose content

absorbance = 0.36211 + 0.0044485 (concentration) r = 0.982, df = 5, P < 0.001



Appendix 4.6. Method for analysis of lipid content.

The following reagent was prepared: 2:1 chloroform-methanol - 300 ml chloroform was mixed with 150 ml methanol and one crystal of BHT (butylated hydroxytoluene), an antioxidant, was added.

Glassware and filter papers were rinsed with solvent prior to use to remove any lipid. Triplicate 20-30 mg samples of food and faeces were placed, together with the foil weighing boats, into 20 ml glass test tubes. About 10 ml of reagent were added to each sample. The tubes were stoppered, shaken and left for 20 minutes for the lipid to extract. The samples were filtered through Whatman No.4 filter papers into 20 ml measuring cylinders. The extraction tubes were rinsed twice with solvent which was added to the cylinders. To each extract 0.2 volumes of 0.017% aqueous magnesium chloride were added. The cylinders were stoppered and shaken briefly. The contents of each cylinder was transferred to a glass centrifuge tube. The cylinder was rinsed with chloroform, which was added to the tube. The tubes were covered with foil lids and centrifuged at 2000 rpm (800 g) for 5 minutes. in an MSE Centaur 1. The upper phase was removed by aspiration. The lower phase was washed with Folch's upper phase (3:48:47 chloroform:methanol:water), which was removed by aspiration and the washing step was repeated. The contents of each tube was passed through a filter containing anhydrous sodium sulphate, to remove any remaining water, into a thick-walled pear shaped flask and was rinsed through with a little chloroform. Each flask was fitted to a Buchi RE120 Rotavapor rotary evaporator, (30°C, vacuum 530mm Hg) to remove the solvent. The lipid, dried onto the inside of the flask, was dissolved in 1 ml of chloroform and pipetted into a preweighed, labelled glass vial. This was repeated twice to remove any residual lipid. The chloroform was removed by standing

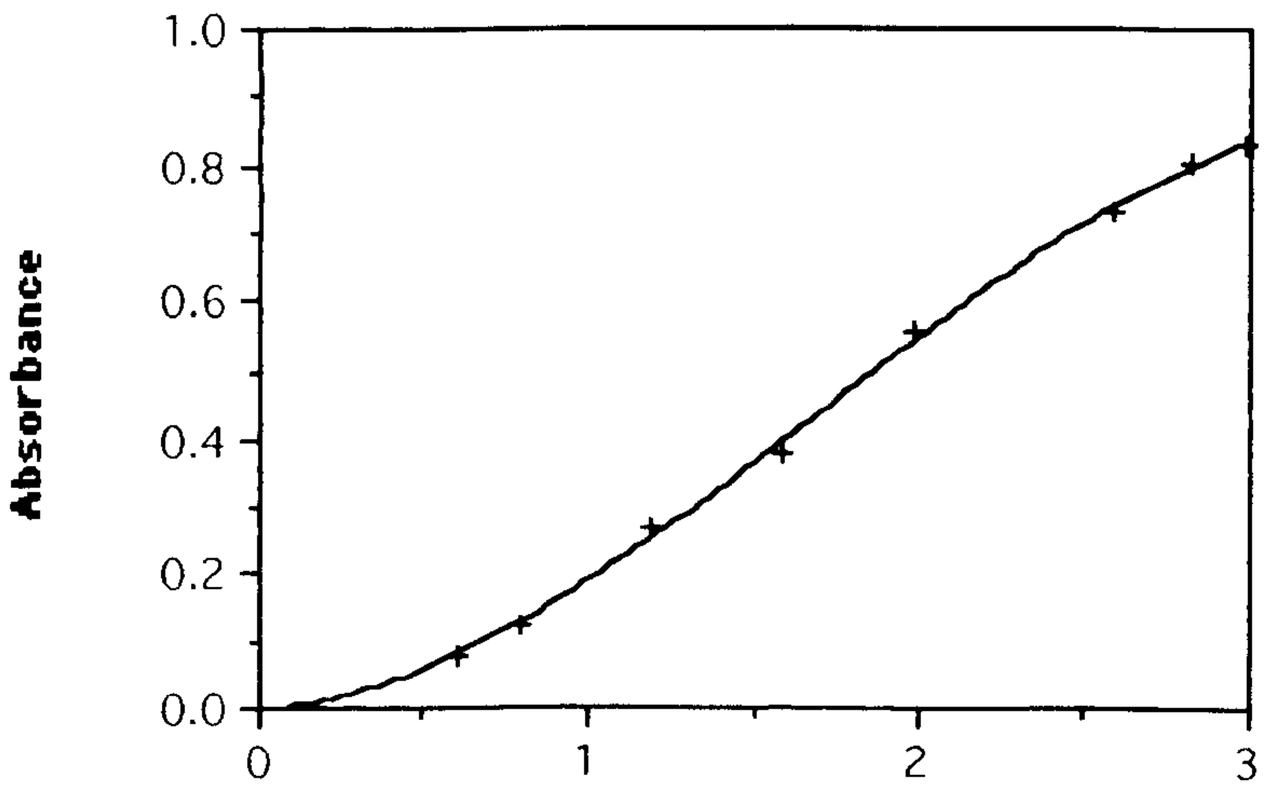
the vial in a warm heating block (30°) under a stream of oxygen-free nitrogen. When the sample was dry, it was weighed on a Oertling four figure balance.

Appendix 5. Digestive enzymes (Chapter 9)

Appendix 5.1. Calibration curve of absorbance at 564 nm of maltose standards used in the assay for amylase activity in *Pollicipes pollicipes*, feeding on monospecific diets of *Skeletonema costatum* and *Artemia* nauplii.

$$Abs = -9.5780 \times 10^{-4} + 3.1610 \times 10^{-2} X + 0.19981 X^2 - 3.9169 \times 10^{-2} X^3$$

$r = 0.999$, $df = 6$, $P < 0.001$.



Appendix 6: Suppliers list

BDH

Ammonium formate
Benedict's reagent
Barium sulphate
Bromothymol blue (0.04%)
Lactose
Phenol red
Sodium chloride
Sodium Hydroxide pellets
Sucrose
Sulphuric acid Analar Grade

Sigma Chemicals Company

alginic acid
Carrageenan Type
Cellobiose
Glycerol
Glycogen (Type II oyster)
Laminarin
Maltose
D-Mannitol
3, 5-dinitrosalycilic acid
Potassium dihydrogen phosphate
Salicin
di-Sodium hydrogen phosphate
Sodium potassium tartrate
Soluble starch
N alpha-p-toluenesulphonyl-L-argenine methyl ester (TAME)
toluene
Thymol
Trichloroacetic acid TCA
Trizma Base