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## **DOCTOR OF PHILOSOPHY**

**The population biology of the gooseneck barnacle *Pollicipes pollicipes* (Gmelin, 1790) in the Algarve, southwest Portugal.**

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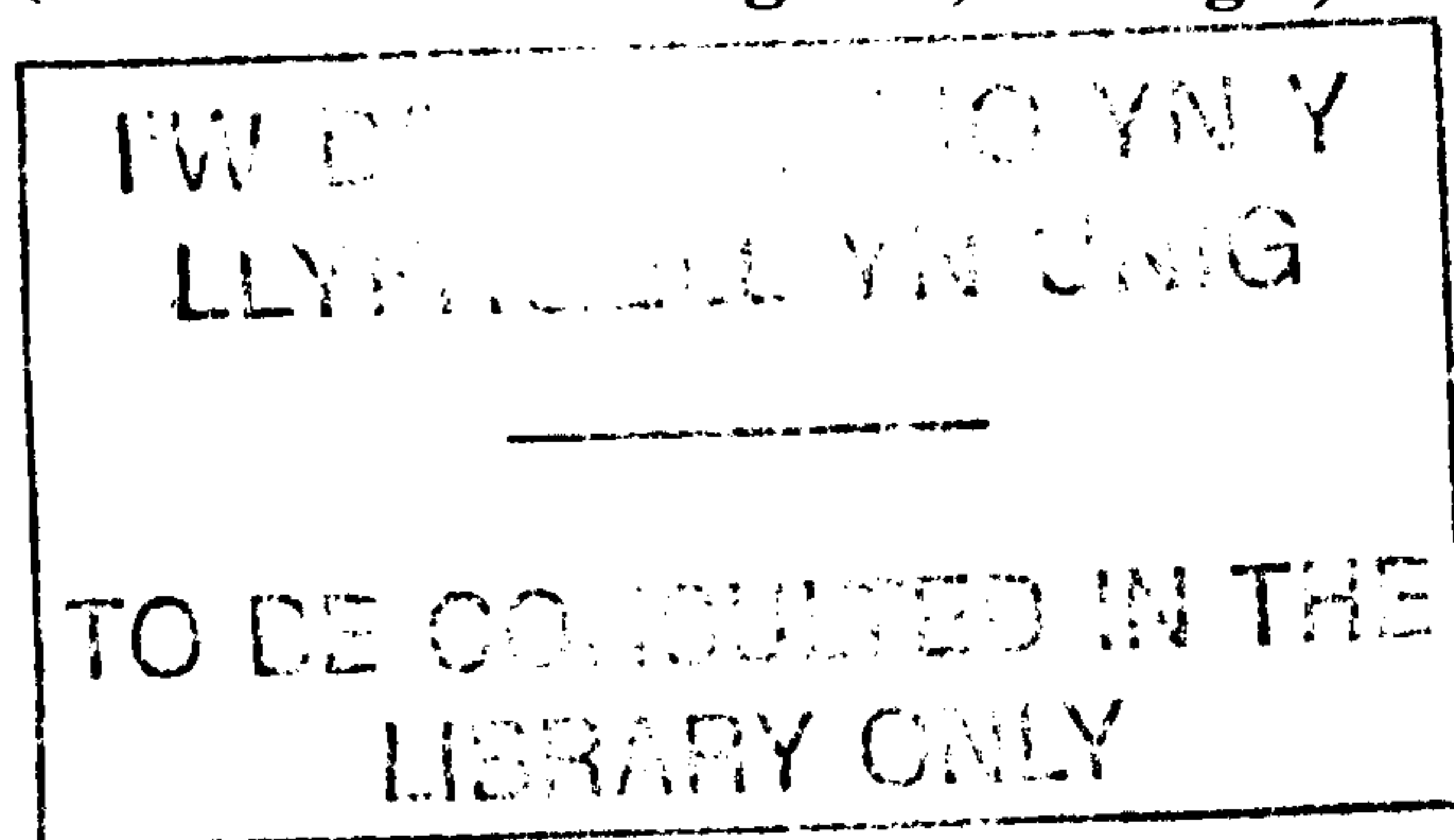
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**THE POPULATION BIOLOGY OF THE GOOSENECK  
BARNACLE *Pollicipes pollicipes* (Gmelin, 1790) IN THE  
ALGARVE, SOUTHWEST PORTUGAL**

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**A thesis presented in partial fulfilment of the requirements for the degree  
of Philosophiae Doctor in the University of Wales**

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## SUMMARY

The pedunculated cirripede *Pollicipes pollicipes* (Gmelin) is of considerable economic importance in Portugal, particularly along the southwest coast. The current knowledge of its biology is poor, as evidenced by the paucity of available literature. Over a period of two years, *P. pollicipes* populations at Castelejo and Zavial were sampled from different tidal levels at approximately monthly intervals. Populations at two other sites, Ponta- da- Fisga and Sagres, were also sampled but at irregular intervals. The current study has provided definitive information on the annual reproductive cycle of *P. pollicipes* that is essential for any attempt at fisheries management. It is shown conclusively that shell-banding techniques are easily applicable to age and growth studies on these lepadomorph barnacles and has provided initial information on the growth rates and factors influencing shell deposition in experimental situations. An exhaustive data set provides a complete set of morphometric tools for the conversion of length measurements into dry and ash-free weights and demonstrates little differences between shapes of *P. pollicipes* on the different shores sampled. The demonstration that *P. pollicipes* is an obligate cross-fertilising barnacle has subtle implications in managing the fishery since it is imperative that any adopted policy incorporates aggregates of the barnacle as a unit necessary to ensure continuing recruitment.

*P. pollicipes* when grown under simulated semi-diurnal conditions of emersion and immersion lay down clearly defined bands with almost an exact coincidence with the number of emersions. However, barnacles from the field showed no clear banding periodicity since band widths were very similar throughout the shell length with no regular "check" marks that could provide age estimation. Differences in the reproductive patterns between Castelejo and Zavial were minor and none statistically significant hence differences in environmental conditions between the two sites do not appear to materially affect the species reproductive patterns. Adult barnacles carried fertilised eggs from April throughout September, and the percentage of individuals with egg masses correlated positively with air but not seawater temperature. Brooding success was greater with average air temperature in excess of 14 °C during months of strong upwelling and presumed food availability. Functional ovaries and testes are available in the population all year round but fertilisation does not appear to occur during the winter months. Reproduction of *P. pollicipes* on the southwest coast of the Algarve appears closely linked to the upwelling phenomenon with the rate of deposition of gonadal tissue probably determined by environmental temperature. Laboratory and field observations permitted to conclude that *P. pollicipes* is an obligate cross-fertiliser. Isolated and communal individuals were observed to carry ripe ovaries and seminal vesicles filled with sperm at the same time but fertilisation took place only on communal individuals.

Increasing numbers of isolated individuals are found on the southwest coast of Portugal, probably as the result of an intense fishery. The assessment of the fishery impact on the natural population and the development of management policies for the fishery are imperative future measures in order to maintain the recruitment levels. Enforcement of reserve areas may prove a practical solution for *Pollicipes* management. Protected populations could serve as stocks for recruitment to other areas. A series of protected areas around the coastline would need to be established in areas that could easily be monitored and, if needed, physically protected. It might also be feasible to rotate areas every 3-4 years resulting in a better quality and increased quantity of *P. pollicipes* entering the fishery.

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## 1. GENERAL INTRODUCTION

The sea has been regarded as an infinite reservoir of food to mankind. Today we are aware that its resources can be depleted if not carefully and rationally exploited. Considerable scientific effort is directed towards a better understanding of the biology and ecology of exploited species, such that the acquired information can be used in formulating legislation to protect and manage natural stocks. Management of fish stocks has a long history and efforts to formulate a scientific basis for rational exploitation presently continue. Management of invertebrate fisheries is in its infancy and it is becoming clear that consideration of the more unusual resources such as edible barnacles must not be overlooked if the necessary exploitation of the marine environment is to continue for the foreseeable future.

There are five extant species of the Lepadomorph genus *Pollicipes*: *P. polymerus* found on the Pacific coast of North America (NEWMAN and ABBOT, 1980); *P. elegans*, a tropical Pacific species (BERNARD, 1988); *P. spinosus*, found in New Zealand (BATHAM, 1945); *P. mitella*, occurring around the Philippines Archipelago and China Sea (DARWIN, 1851); and *P. pollicipes* (Gmelin, 1790), found on the Atlantic coast from France, Spain, Portugal to North Africa, from the Gulf of Gascogne to Dakar (FISCHER *et al.*, 1987) (see Fig. 1).

*P. pollicipes* has been exploited by European and North African peoples for generations (BERNARD, 1988), both as food source and even as terrestrial fertiliser in places where its abundance was high. The species is captured all along its geographic range but it is in the Iberian Peninsula that its consumption predominates and where it is considered a delicacy (BERNARD, 1988).

The intensive, unregulated, exploitation of *Pollicipes* in Northern Spain led to its virtual disappearance from the coast (DURA, 1988). To prevent total eradication of the species, areas of the Spanish coast have had severe fishery restrictions on the capture of *P. pollicipes* since 1970.

In Portugal, the barnacle is also regularly eaten and has a high commercial value (up to 6000 escudos = 24 pounds sterling per Kg in Faro restaurants).

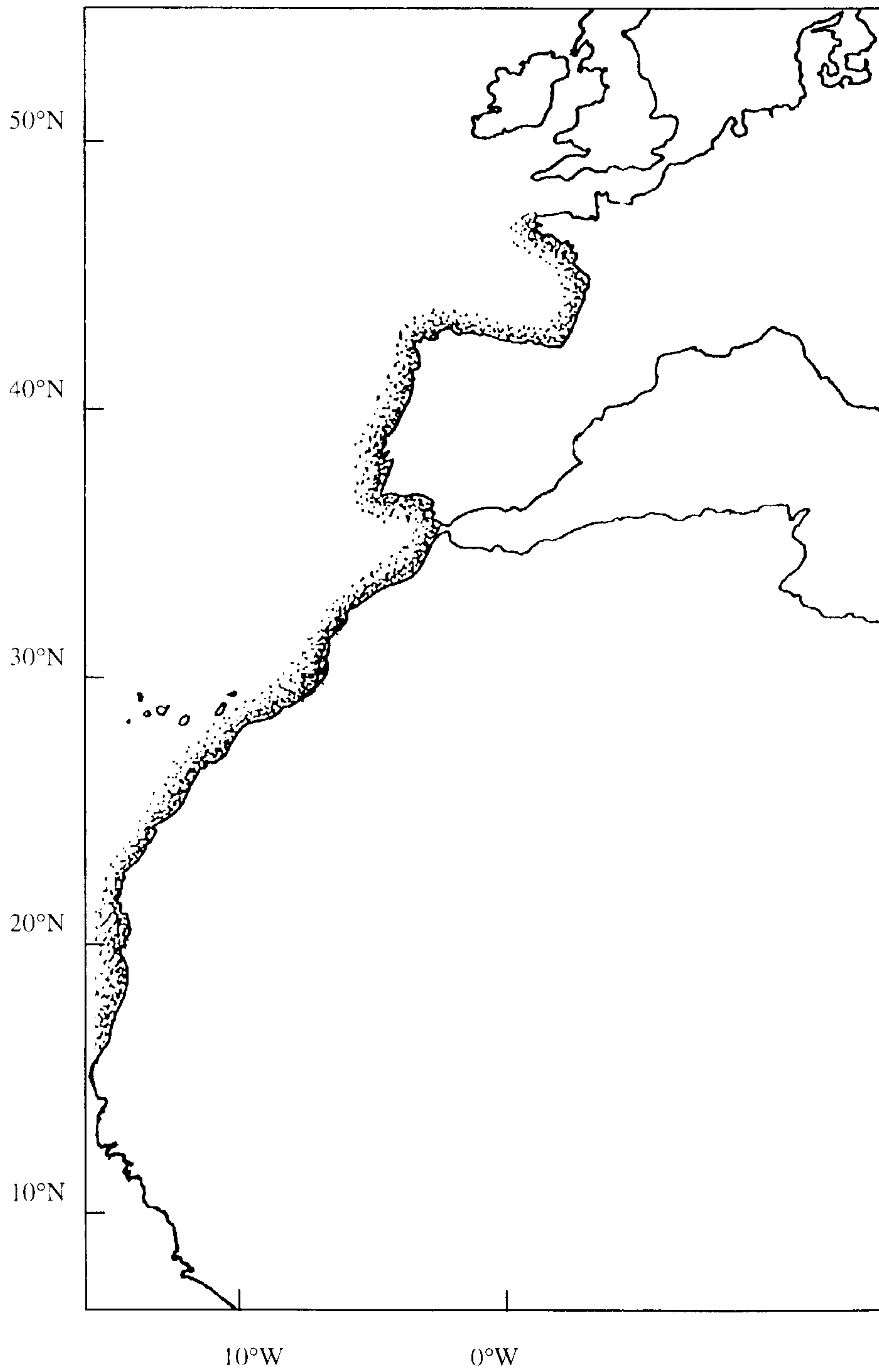


Fig. 1 Geographical distribution of *P. pollicipes* (according to Fischer *et al.*, 1987), represented as a shade lining along the coastal areas where the species is present.

Due to increasing market demand, an highly intensive fishery for *Pollicipes* has developed which is an integral part of the fishing culture around Portuguese coasts.

The most intensive fishery is centred around the western Algarvian coast, with its high cliffs and exposure to the open Atlantic Ocean presenting an ideal habitat for the barnacle. The coastal area in the southwest adjacent to Vila-do-Bispo, is typical of such ideal habitats. Local fisherman turn to the *Pollicipes* fishery only when conditions allow, calmer seas and spring tides, hence the fishery is largely “part-time” but none the less intensive.

### 1.1 GENERAL FEATURES

*P. pollicipes* Gmelin is a cirripede crustacean of the suborder Lepadomorpha, family Scalpellidae. As with other cirripedes it is characterised by an intense modification of the body, as a result of its adaptation to a sessile adult life. In fact the Cirripedia are so highly modified that they were originally included amongst the molluscs rather than the Crustacea, a classification maintained until 1830 when the larval stages were discovered and their relationship with the Crustacea became clear (THOMPSON, 1830). It was, however, Darwin (1851) with his monograph on the Lepadidae who laid the solid foundation for all subsequent work on their biology.

*P. pollicipes* adults are characterised by a flexible muscular peduncle, attached to the substratum at one end, carry the bulk of the body at the other as the capitulum. The integument of the capitulum incorporates at least 18 calcareous shell plates, being most numerous at the junction with the peduncle (FISCHER *et al.*, 1987) (see Fig. 2). It has paired terga and scuta, a carina, a rostrum, several latera and two whorls of plates beneath the carina and rostrum.

The peduncle is, in general, longer than the capitulum (DARWIN, 1851), and is covered in calcareous spicules that increase in size from the base up to the junction with the capitulum. The peduncle represents the preoral end of the animal and contains the ovaries at the top, the adult cement apparatus towards the base and even conceals the vestiges of the larval antennules underneath the base (see Fig. 3).

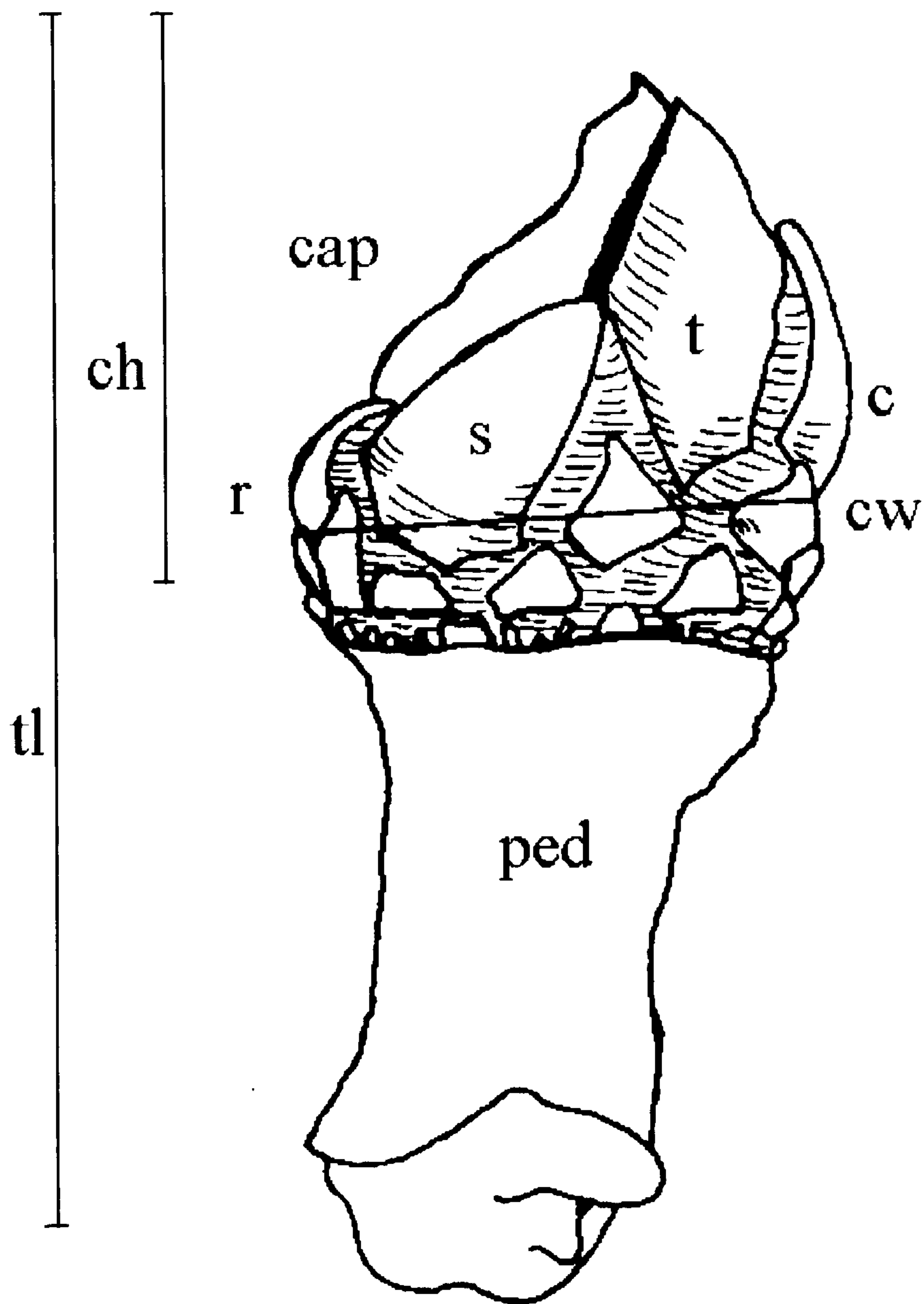


Fig. 2 Diagram to show the measurements taken from *P. pollicipes*. c, carina; cap, capitulum; ch, capitulum height; cw, capitulum width; ped, peduncle; r, rostrum; s, scutum; t, tergum; tl, total length.

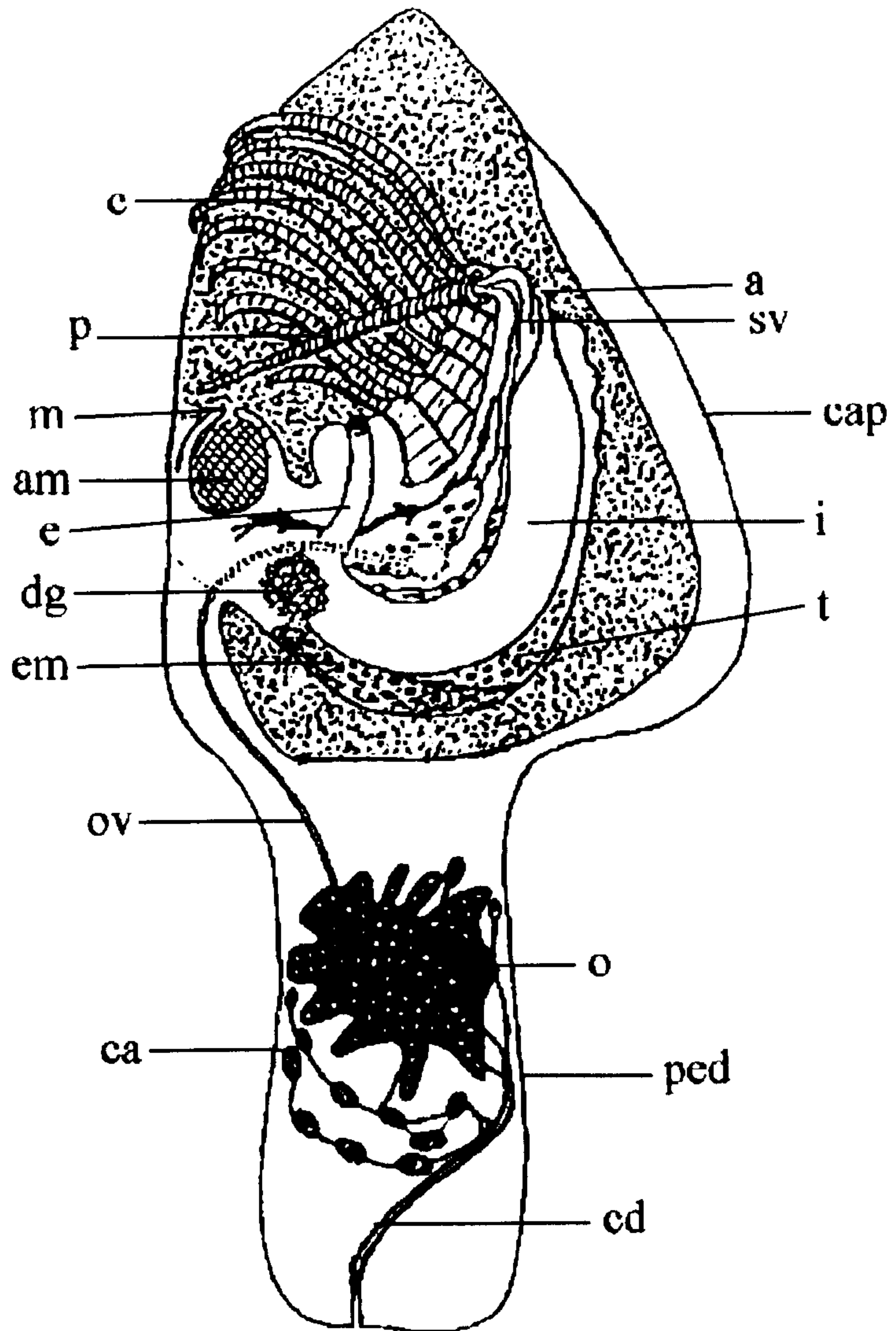


Fig. 3 Schematic diagram of a section of *P. pollicipes* showing general anatomic features. (After Meglitsch, 1978, modified after Walker, 1992). a, anus; am, adductor muscle; c, cirri; ca, cement apparatus; cap, capitulum; cd, cement duct; dg digestive gland; e, oesophagi; em, egg mass; i, intestine; o, ovary; ov, oviduct; p, penis; ped, peduncle; sv, seminal vesicle; t, testis.



The capitulum bears the remainder of the body. The body consists of a cephalic region and the anterior trunk (thoracic) region. There are six pairs of long, biramous thoracic appendages, called cirri, each covered with setae. The cirri can be projected upwards through the capitular opening by haemolymph pressure (CRISP and SOUTHWARD, 1961; WAITE and WALKER, 1986; WALKER and ANDERSON, 1990) and have three main functions: captorial feeding by means of the extended cirri, filtration of small organisms from the water flow through the mantle cavity generated by general movements of the soma, and gas exchange for respiration (WALKER, 1992). *Pollicipes* is omnivorous, feeding on microplankton by microfiltration through the setae, and larger organisms by capture of individual particles with the cirri (BARNES and REESE, 1959; ANDERSON and SOUTHWARD, 1987).

Thoracican barnacles are mostly hermaphroditic (DARWIN, 1854; CHARNOV, 1987) although self-fertilisation is rare, however, *P. pollicipes* (= *P. cornucopia*) has never been seen either cross-fertilising or self-fertilising (BARNES, 1992).

In *Pollicipes* the ovaries are located in the peduncle, and paired oviducts open at or near the basis of the first pair of cirri (WALKER, 1992). The mechanism of egg deposition has not been described in *P. pollicipes*. In other thoracican barnacles, e.g. *Semibalanus balanoides* and *B. hameri*, just before reaching the gonopore, each oviduct dilates as an oviducal gland that secretes a thin elastic ovisac at the time of egg deposition (WALLEY, 1965; WALKER, 1980). In *S. balanoides*, as the ovisac receives eggs, it swells and stretches, emerging from the gonopore and coming to lie within the mantle cavity (WALKER, 1977).

The paired testes are located in the thoracic region, and the two sperm ducts unite within a long penis, which lies in front of the anus (DARWIN, 1851). The thoracicans are able to protrude the penis from one individual into the mantle cavity of another individual (pseudo-copulation) for the deposition of sperm (KLEPAL, 1990), however this behaviour has never been observed for *P. pollicipes*. The functional males of *S. balanoides* and *B. hameri* can recognise functional females (WALLEY, *et al.*, 1971; WALKER, 1980), which may be inseminated by more than

one male (CLEGG, 1955). The sperm in these species is deposited as a mass near the first pair of cirri and must penetrate the ovisac to reach the eggs.

The fertilised eggs of *P. pollicipes* are brooded within the ovisac in the mantle cavity. The eggs hatch as free living nauplius larvae which develop through 5 further naupliar stages before moulting into the settling cypris stage (MOLARES *et al.*, 1994).

The cyprid is a non-feeding larva with its entire body enclosed within a cuticular bivalve carapace (DARWIN, 1851). It possesses 6 pairs of thoracic swimming appendages providing limited manoeuvrability in the water column and numerous sense organs such as the paired compound eyes, chemosensors on the antennules and, mechanosensors distributed about the carapace and limbs (WALKER and LEE, 1970). The cyprid attaches temporarily to suitable substrata with the aid of a protein secretion on the attachment disc of the antennule, and then permanently, with secretions from the permanent cement glands (WALKER and YULE, 1984).

Barnacle larvae tend to settle in close proximity to other individuals of the same species (*e.g.* KNIGHT-JONES, 1953; BARNETT *et al.*, 1979; CRISP, 1979; LEWIS and CHIA, 1981), which they are able to recognise through chemical cues (KNIGHT-JONES, 1953). This gregarious behaviour has several advantages, the most obvious being the increased chance of reproductive success through cross-fertilisation, and assurance of a suitable habitat with surviving adults (CRISP, 1979).

## 1.2 FISHERY

The commercial collection of *P. Pollicipes* is a difficult and dangerous activity carried out by a relatively small number of fisherman. Big barnacles with high commercial value, are found in the most inaccessible places requiring the descent of 70m cliffs, or diving in wave swept rocky areas in extreme currents.

The animals have to be prised from the rock, without damage, using a tool resembling a knife or with a cruder wooden handled iron bar (see Plate 1). The necessity for the collection of intact individuals is essential since damaged animals rapidly decompose and spoil the rest of the catch around them.

Plate 1

In Vila-do-Rapto as indicated in the text, they collect between 10 and 40 kilos per spring tide (equivalent to 100-200 kg of barnacles). Once captured the

A.



B.



Collection of *P. pollicipes*: A. Detail of the collecting activity. B. View of a fishing site at Castelejo beach.

In Vila-do-Bispo an individual fisherman may collect between 10 and 40 kilos per spring tide (equivalent to 800-4000 individual barnacles). Once captured the animals are transported to market or sold directly to the consumer. Market values have increased in recent years and anecdotal evidence from fishermen suggests that *Pollicipes* abundance is decreasing. Estimated catches are particularly inaccurate since the majority of landings are never collated in the official statistics because they are sold directly to the consumer, providing the fisherman with a better price.

In Portugal, official statistics on *Pollicipes* catches in 1990 (January to September) indicate that catches were least in the north and steadily increased towards the south (Table 1). The south, the Algarvian region, is the principal supplier of *Pollicipes* with a production 80 times greater than the northern region. Quarteira and Fuzeta are the areas of lower production in the south probably due to the predominance of sandy shores over cliffs. The commercial value of *Pollicipes* is higher in the Algarve which reflects the gastronomic tastes of the inhabitants and possibly an increasing curiosity value to tourists.

### 1.3 OBJECTIVES

Fishing for *P. pollicipes* is done in Portugal with no restriction on season or size. Information available on the fishery for the same species in Spain, and fishery ecology studies of the remarkably similar *P. polymerus* on the Pacific coast of the USA, leads one to fear for the depletion of the species stocks and possible extinction in Portugal, particularly on the southwest coast.

The efficient management of an exploited marine species is dependent on the extent of the knowledge of the species' biology, in particular its population dynamics. Size/age structure, growth rates, reproduction and recruitment are among the most important population parameters needed to monitor fishery performance and establish management practices.

Limited literature on the population dynamics and reproduction of intertidal cirripedes is available (*e.g.* MOORE, 1934; BARNES and BARNES, 1968; HINES, 1978; CIMBERG, 1981; LEWIS and CHIA, 1981; PAGE, 1984; BERNARD, 1988; BERTNESS *et al.*, 1991; BURROWS *et al.*, 1992) reporting the impact of

environmental variability, phenotypic plasticity, and genetic differences on population dynamics. Even less information is currently available on the biology of *P. pollicipes* (AZEVEDO and CORRAL, 1982; BARNES, 1992; CARDOSO and YULE, 1995; CARVALHO, 1983; GOLDBERG, 1984; CRUZ, 1993; MOLARES *et al.*, 1994; CRUZ and HAWKINS, 1998) and, if direct fishery management is to be established clearly more information on the species population dynamics and reproduction need to be acquired.

The species life cycle, generally described above, can be divided in two phases, a sessile - juvenile to adult forms - and a planktotrophic larval phase. The current study focuses mainly on the adult phase and juvenile recruits.

The main objectives are the following:

- 1) to develop reliable ageing techniques for fisheries monitoring and management;
- 2) to study the relative growth of *P. pollicipes* from four sites in Algarve and to compare it in a temporal and spatial scale;
- 3) to ascertain population age and size structure from time series sampling at four sites in Algarve;
- 4) establish the breeding season, reproductive condition of the population, and potential fecundity, and
- 5) to ascertain the capability of *P. pollicipes* to self-fertilise.

**Table 1**

Landings of *P. pollicipes* in Portugal 1986-1993 (Ministério do Mar, Direcção Geral das Pescas, unpublished data).

Year	Area	Captures(Kg)	Total value (\$)	Price/Kg (\$)
1986	Portugal	770	609 375	791
1987	Portugal	1 004	1 033 423	1 029
1988	Portugal	2 902	3 669 981	1 265
1989	Portugal	16 990	21 488 240	1264
	North area	128	50 625	396
1990 (Jan. to Sep.)	Centre	1 447	678 633	469
	Algarve Barlavento	11 699	18 156 848	1 552
	Algarve Sotavento*	74	82 214	1 111
1990 (total)	Portugal	15 300	21 634 200	1 414
1991	Portugal	21 100	31 565 600	1 496
1992	Portugal	23 700	32 042 400	1 352
1993 (Jan. to Nov.)	Portugal	23 800	28 012 600	1 177

\* Quarteira to Fuzeta

\$ Portuguese currency, escudos

## 2. GENERAL MATERIALS AND METHODS

The materials and methods described here are those common to most of the practical work. Whenever more specific methodology was adopted it will be detailed in the appropriate chapters.

The collection of data included field and laboratory work. The field work was carried out in Portugal while the most of the laboratory work was carried out at the School of Ocean Sciences, University of Wales, Bangor.

### 2.1 FIELD WORK

During July and August 1991 a pilot survey was carried out by boat along the southwest coast of Portugal, from Carrapateira down to, and around, Cape St. Vincent (see Fig. 4). The survey led to a better working knowledge of the area and helped in the planning of the future sampling strategies.

The southwest coast of Portugal is a very exposed rocky area of inaccessible cliffs alternating with sandy bays. The intertidal of Portugal is characterised by the typical dominant communities of barnacles and mussels, and a broad littoral fringe as the result of spray being carried far up the beach. However, the climate is classed as temperate, since seasons are identifiable but environmental parameters change little from one season to another. Monthly average temperature varies from 13 to 20 °C and the water temperatures remain within a similar range (13 to 22 °C, COELHO, 1991). There is little rainfall but the winds are frequent and strong.

A significant upwelling feature occurs along the west coast of Portugal and Spain (from Cape St. Vincent to Cape Finisterre), typically from April through to October (FRAGA, 1981; FIUZA, 1983). Surface coastal waters are driven offshore in response to northerly winds, reaching 30-50 Km from the coast in calm conditions and extending to 100-200 Km during, and shortly after, strong north winds (FIUZA, 1983). Thus, the southwest coast of the Algarve experiences an upwelling of nutrient rich, colder water making the area very productive. The upwelling is virtually a permanent feature throughout the summer in the sea of Castelejo but its occurrence is much more variable off the south coast (*e.g.* Zavial). The Algarvian south

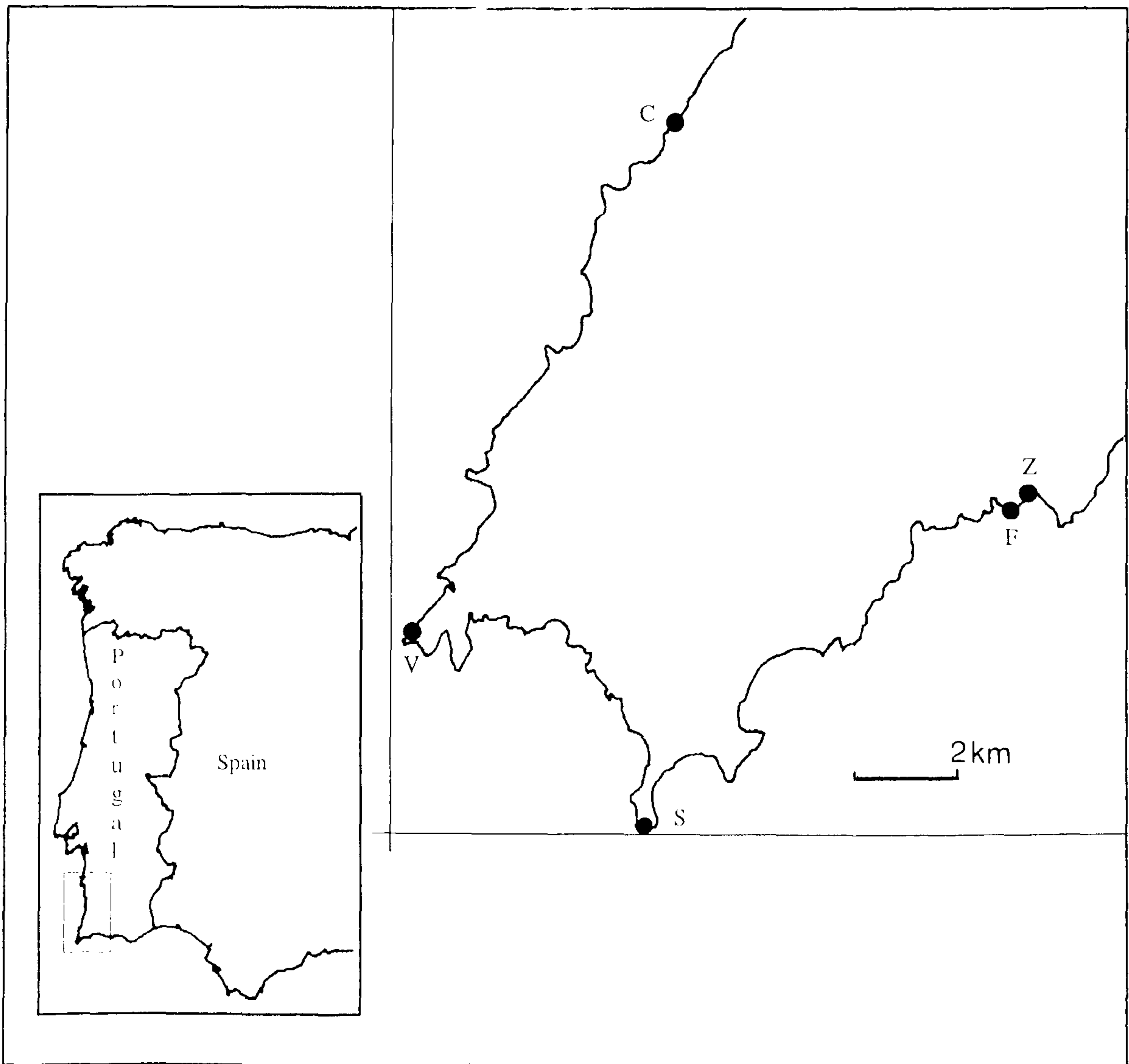


Fig. 4 Map of the sampling sites: C, Castelejo; F, Ponta-da-Fisga; S, Sagres; V, Cape St. Vincent; Z, Zavial.



coast experiences direct upwelling only during westerly winds which blow occasionally. However, during cycles of moderate to strong north winds over the west coast, the upwelling waters turn around Cape St. Vincent and flow eastward along the south coast (FIUZA, 1983).

*P. pollicipes* in this area are particularly abundant between the mean high water neaps (MHWN) and mean low water neaps (MLWN) (Plate 2), similar to the distribution of the closely related *P. polymerus* in the USA (BARNES and REESE, 1960; HILGARD, 1960). Rosette-shaped clusters of *Pollicipes* cover the exposed rocky regions (exposed rock faces, sheltered overhangs, and crevices) attached to rock, other *Pollicipes*, *Mytilus* shells and other barnacle species (e.g. *Balanus perforatus* and *Chthamalus stellatus*). Occasionally solitary animals can be found. They may be, as Barnes and Reese (1960) suggested for *P. polymerus*, the remnants of an extensive population where abrasion by sand, and in the case of *P. pollicipes* also human intervention, took place.

Considerable phenotypic variation exists amongst *Pollicipes* populations. In exposed situations, individuals tend to have short and robust peduncles, while in less exposed locations the peduncles are often elongated and ramify throughout the clump. Animals in the lower shore display a bright crimson red colour (DARWIN, 1851) to the mantle border, whereas those in the upper levels have mantle edges with a greenish-brown colour (Plate 3).

Examination of groups of animals shows that over a restricted area most of the individuals have their capitula oriented in the same direction (Plate 3). The orientation seems to be, as noted by Barnes and Reese (1960) for *P. polymerus*, not fixed relative to the general wave direction but largely determined by the microtopography of each local situation, varying with any irregularities and their position with respect to the breaking and particularly receding waves (NORTON, 1996).

Zavial is a relatively sheltered beach facing south. The samples were taken from large shale/sandstone boulders standing on the east side of the beach (Plate 4).

Plate 2

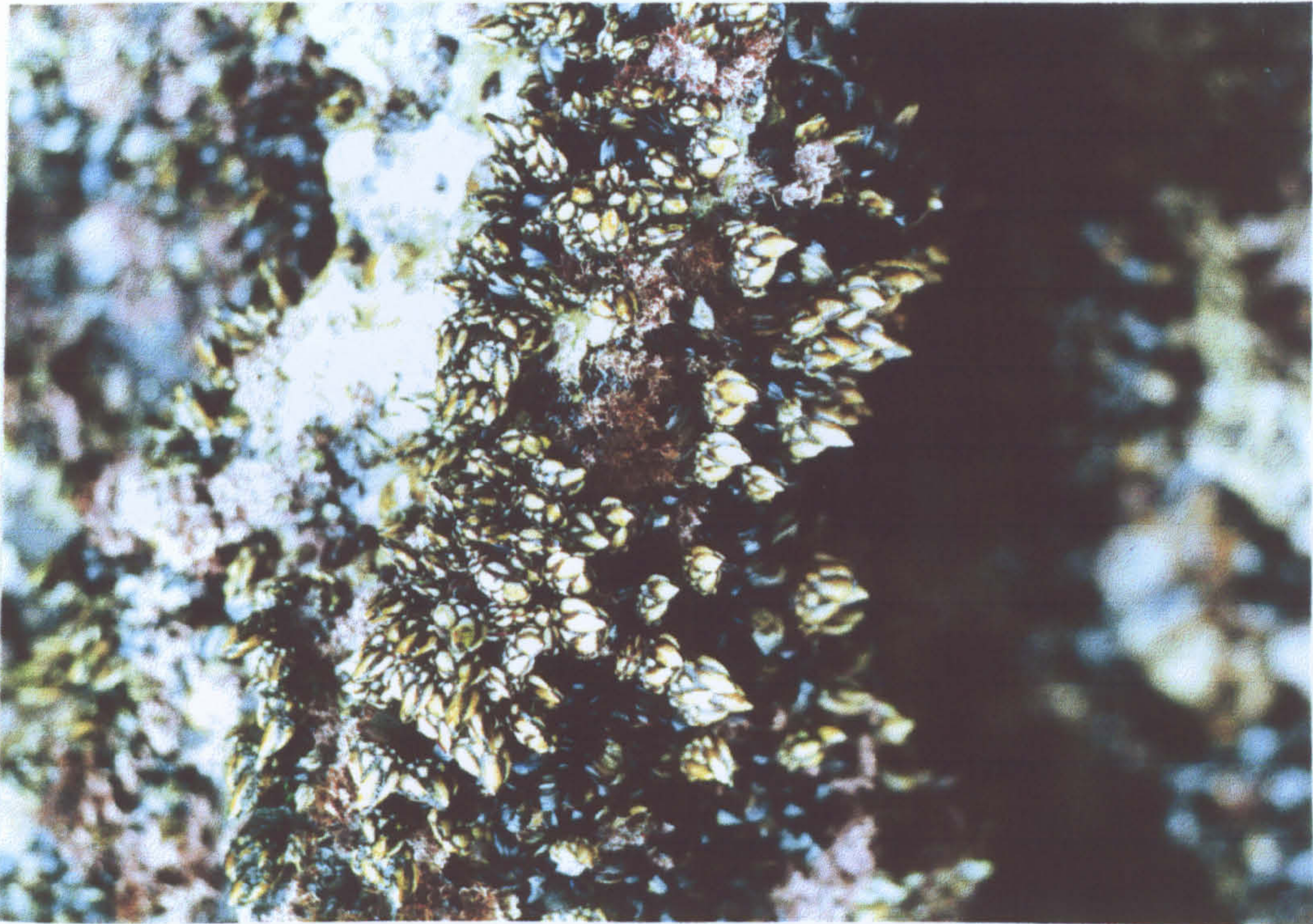


Vertical distribution of *P. pollicipes* (arrow) at Castelejo beach. MHWN, mean high water neaps; MLWN, mean low water neaps.

*P. pollicipes* at Castelejo beach. A and B. Views of dense aggregates of *P. pollicipes* associated with *Mytilus* and displaying distinct mantle edges colours.

Plate 3

A.



B.



*P. pollicipes* at Castelejo beach: A and B. Views of dense aggregates of *P. pollicipes* associated with *Mytilus* and displaying distinct mantle edges colours.

General view of two sampling sites (A, B) at Castelejo beach. Arrows indicate the sites from where samples were collected.

Plate 4

A.



B.



General view of two sampling sites: A. Zavial beach. B. Castelejo beach. Arrows indicate the sites from where samples were collected.

Ponta-da-Fisga, as the name indicates, is a pointing spit of rock projecting seawards. It is more exposed than Zavial and located between Zavial and Cape St. Vincent. The rocks are mostly composed of shale. Castelejo is located on the west coast and is frequently exposed to severe wave action. The coast is rocky, with high cliffs, composed of shale and sandstone. The sampling site was located among large boulders on the right hand side of the beach on a westward facing rock directly exposed to the sea and experiencing the full force of the Atlantic waves (see Plate 4).

Due to the wide geographic distribution of *Pollicipes* along the Portuguese coast, and the generally inaccessible and dangerous coastline, the sampling area was restricted to provide adequate populations of the barnacle to study.

The main sampling programme took place between September 1991 and December 1993. From March 1992 onwards sampling was conducted at roughly monthly intervals. Two sites located either side of Cape St. Vincent were extensively sampled: Castelejo and Zavial (see Fig. 4). There is a gap in the sampling programme from October 1992 to May 1993 at Zavial. During this period experiments were carried out at the School of Ocean Sciences and, sampling could only be conducted at Castelejo. Another site, situated between Zavial and Cape St. Vincent was occasionally sampled: in September 1992 and 1993, and in October 1992.

Sampling was very dependent on the weather and sea conditions, which render the sites inaccessible even during appropriate tides. All samples were taken at spring low tides by random sampling at different tidal levels. Several quadrats with an area proportional to barnacle abundance were cleared of animals for later measurement. The total number of animals in each quadrat was counted and their capitulum lengths measured.

Attempts to mark individuals or groups of individuals with enamel paint so that the same individuals could be sampled on successive occasions were thwarted, either because the enamel paint was sand blasted from the shells during subsequent wave action or, more often, the individuals were collected by one of the many fisherman who visit those sites.

The majority of the samples were preserved in a freezer before analysis, although, occasionally preservation in 8% formalin in seawater was resorted to.

## 2.2 LABORATORY WORK

For all the animals collected the following measures were taken (see Fig. 2): 1) capitulum height, measured from the junction of the capitulum shell plates and the peduncle to the tip of the tergum, following a line perpendicular to the capitulum/peduncle junction; 2) capitulum width, measured as the distance between the rostrum and carina from their junction with the tips of their underlying supplementary shell plates; 3) capitulum depth, measured as the greatest distance across the capitulum (from right to left) at the capitulum/peduncle junction; and 4) total length, measured as the maximum length from the base of the peduncle to the tip of the tergum. All the dimensions were measured to the nearest 0.01 mm with vernier callipers. Juveniles were measured under a dissecting microscope using a calibrated eye piece graticule.

The specimens were dissected and the presence of (a) ripe ovaries with large quantities of yolk material (b) fertilised egg masses and (c) seminal vesicles filled with seminal fluid was recorded for each specimen.

Each specimen (whole animal without egg masses) was then dried to constant weight at 60 °C and weighed. Dried animals were ashed at 450 °C for 3 h and the organic (ash free dry) weights determined. When present egg masses were removed, dried and weighed. All weights were measured to the nearest 0.1 mg with a Sartorius balance (Standard SX).

An examination of shell (mostly tergal plates) cross sections was made in an attempt to identify and quantify internal growth bands. Growth increments were measured from acetate peels largely following the methods described by Richardson *et al.* (1979) and Kennish *et al.* (1980). The procedure can be summarised as follows:

1. Embedding - In order to protect the shell from fracture during sectioning each plate to be analysed was embedded in clear Metaset resin (Buekler, U.K. Ltd.).

2. Sectioning - Embedded shell plates were cut along the maximum axis of growth using either a hacksaw or a diamond saw.

3. Grinding - Shell sections were ground smooth with a series of silicon carbide papers of decreasing coarseness from 100-1200 carborundum grit. The grinding was finished off using a fine alumina abrasive powder (Alloxite no. 50) and water, as a lubricant, on a piece of glass.

4. Polishing - When the surface was completely smoothed it was polished with a cloth soaked in 'Brasso' (a household metal polish). Shell sections were cleaned with detergent and washed thoroughly with water to remove all the metal polish residues.

5. Acid-etching - Dry and polished sections were etched in 1% Decal for 20 min or, when Decal was unavailable 0.01% HCl for 10 min, to decalcify the microstructure of the shell.

6. Washing and drying - Immediately after etching the shells were washed with tap water and allowed to air dry.

7. Acetate peel replication - Strips of acetate sheet (Agar Scientific Ltd.) were submerged in ethyl acetate until molten, then carefully laid along the dry etched surface of the shell and peeled off when hardened.

Peels, mounted between slide and cover slip, were examined under a light microscope and all measurements made with a calibrated eye piece graticule.

**CHAPTER I: RHYTHMS OF BAND FORMATION IN THE SHELL OF THE  
STALKED BARNACLE *POLLICIPES POLLICIPES***

Part of the results in this chapter were presented in a poster, under the title “Rhythms of band formation in the stalked barnacle *Pollicipes pollicipes*”, at the Biomineralization Symposia held in the Oceanographic Museum of Monaco on the 15 to 20 of November 1993.



## 1.1 INTRODUCTION

Information about the gross dynamics of an organisms physiology in relation to its environment is capable of being preserved as structural, morphological, or chemical change in the skeletal parts of the body. For a change in the external environment to be recorded in the skeleton, changes in the organisms physiology may promote a biochemical response which alters the depositional dynamics of the hard structures forming the skeleton.

Many physiological changes are preserved in the shells of bivalves or gastropods, and in the shell plates of barnacles as growth increments, growth discontinuities, or changes in shell morphology, structure, mineralogy or chemistry. These skeletal features can be observed on the whole shell or studied from preparations of shell sections (DILLON and CLARK, 1980; RHOADS and LUTZ, 1980). A specific environmental change may involve one or more of these variables and most skeletal growth research relates observed changes in shell variables to specific environmental factors.

Microgrowth increments, observed in sections of shell material, are thought to be delimited by changes in the ratio of calcium carbonate to organic material and can be observed as changes in the optical density of the shell (RHOADS and LUTZ, 1980). Acetate peel replicas of sectioned and acid-etched shells preserve the patterns as topographical features which can be readily observed under the microscope (RICHARDSON *et al.*, 1979; KENNISH *et al.*, 1980). Boundaries to discontinuities in the shells can be gradational or abrupt. Abrupt discontinuities may represent periods of non-deposition or an erosion of the inorganic material produced through shell dissolution at a lowered pH (CRENSHAW, 1980). These growth interruptions are sometimes called "breaks", "biochecks", or "checks". They can be periodical, as in the case of shell resorption at each low tide (*e.g.* EVANS, 1972; RICHARDSON *et al.*, 1979; RICHARDSON, 1991) or aperiodic and related to random events such as predator attacks or disturbance caused by storm turbulence (*e.g.* RICHARDSON *et al.*, 1980; RICHARDSON and WALKER, 1991).

A great deal of the work on shell growth-lines has been conducted on bivalves, and it is generally agreed that when the valves are open and the organism is actively

pumping, shell material, rich in calcium carbonate, is deposited but deposition does not take place when the valves are tightly shut (RHOADS and LUTZ, 1980). Patterns may arise as a result of one of the following categories: 1) diurnal and tidal; 2) fortnightly; 3) monthly; 4) annual and 5) random events.

Accretive skeletons are in contact with living tissues only along a single surface called the surface of deposition and any interruptions or variations in growth will be registered along this surface (DILLON and CLARK, 1980). Any perturbation in deposition can be viewed directly by sectioning the skeleton and the patterns of shell deposition become visible as internal growth lines. The outer skeletal surface, often called the "surface of accretion", can also bear witness to variations in the rate of shell deposition through the appearance of surface growth lines or rings.

Although, there is often a good correlation between internal and external growth lines, this is not always the case and numerous examples from a wide range of invertebrate taxa show there is a surprising lack of correlation between the two kinds of patterns (*e.g.* BARNES, 1972; CLARK, 1975; LUTZ and RHOADS, 1977).

Most of the available information on barnacle skeletal growth concerns the Balanomorpha (*e.g.* BARNES and POWELL, 1953; BARNES, 1956; COSTLOW and BOOKHOUT, 1957; CRISP, 1960; BOURGET and CRISP, 1975a, b) and relates to the length or weight of the shell plates (see also reviews by BOURGET, 1980, 1987; CRISP and BOURGET, 1985; SIMKISS and WILBUR, 1989). Barnacles are unique among the arthropods because so many cirripede species possess permanent calcareous shells in addition to the cuticular exoskeleton (CRISP and BOURGET, 1985; WALKER, 1992). Unlike other crustaceans that possess a cuticle impregnated by calcium carbonate, barnacles exhibit a complete separation of the outer cuticle from the underlying accretion of calcite as true shell. The accretion of calcium carbonate is continuous leading to a relative increase in all shell dimensions with somatic growth. Bourget and Crisp (1975b) proposed that shell growth in the Balanomorpha starts with cuticle formation and secretion of calcium carbonate into the newly formed cuticle at moulting. During intermoult (and only when immersed) the new fold of cuticle is stretched as it becomes filled with calcite secreted from the hypodermis underneath it. Each burst of growth manifests itself externally as a growth ridge at the edge of the wall plate. These ridges are totally

independent of the hirsute ridges which can be produced, but are not necessarily produced, at each moult (BOURGET and CRISP, 1975b).

CRISP (1960) noted that both regional and local factors affected the somatic growth rate of barnacles. Regional environmental factors include the temperature and salinity of the water, and availability of food, whilst local environmental factors include the flow of water over the barnacle, orientation of the barnacle in relation to the water flow, the population density of surrounding individuals and the presence of parasites. Variation in the factors affecting growth may be predictably periodic in the short or long term, *e.g.* tidal, daily, lunar or annual cycles or, they may be episodic and generally random, *e.g.* a lowering of salinity through local flooding or anthropogenic contamination.

It was mainly as a result of the work of Rhoads and Pannella (1970) and Farrow (1971, 1972) that the potential of shell banding patterns for the analysis of short term growth and the prediction of population age structure was first recognised. Several studies are today available which report on the rhythms of formation of the growth increments within sections of bivalves shells (*e.g.* EVANS, 1972; RICHARDSON *et al.*, 1979; RICHARDSON *et al.*, 1980; RICHARDSON, 1988, 1991; BOURGET and BROCK, 1990; BERARD *et al.*, 1992), gastropods (*e.g.* CRISP *et al.*, 1990), corals (*e.g.* RUNCORN, 1966), cephalopods (*e.g.* ARKHIPKIN and BIZIKOV, 1991) and, fish (*e.g.* PANNELLA, 1971).

Crisp and Bourget (1985) noted that the cyprid of the primitive thoracican *Lepas* already contained the uncalcified primordia of the five major calcareous valves, scuta, terga (both paired), and the carina. Almost immediately after settlement calcification begins, and the mature shell is laid down around the primordial shell marks that indicate the position of the growing edge at each progressive growth episode. Using date-marked animals, tidally deposited growth lines (microgrowth bands) were demonstrated in the barnacles *Semibalanus balanoides* and *Elminius modestus* (BOURGET and CRISP, 1975b; CRISP and RICHARDSON, 1975, respectively). To date no documentation of the pattern of microgrowth bands in lepadomorph barnacles has been made.

Age determination and growth in the genus *Pollicipes* has largely been assessed by length-frequency analysis and through marking the shells of individuals

(e.g. LEWIS and CHIA, 1981; GOLDBERG, 1984; BERNARD, 1988; CRUZ, 1993). Bernard (1988) claims to have determined the age of *P. polymerus* individuals using the patterns observed in the carinal shell plate, however, no details are given of the method employed, the patterns of shell deposition, or the factors affecting calcification. Bernard (1988) merely claims that age was successfully ascertained using the annual layers in the carina, utilising methodology developed for fish otoliths. This work probably refers to the outer shell plate rather than to the internal structure.

The current study is the first step in a major effort to determine age in the stalked barnacle *Pollicipes pollicipes* by shell plate band microgrowth analysis. The reliability of growth rings and band/lines as a basis for determining growth and age depend largely on the regularity of their formation. Therefore, the approach used was to determine the average frequency at which bands were produced by influencing experimentally the frequency of banding formation with a view to assessing the reliability of the technique for estimating the age and growth of wild *P. pollicipes* populations.

## **1.2 MATERIALS AND METHODS**

*Pollicipes pollicipes* for the first experiment were collected from the intertidal rocky shore of Castelejo beach, on the 24 of December 1992. They were immersed in an aquarium before transportation to the School of Ocean Sciences (S.O.S.) 4 days later. They were transported wrapped in absorbent paper soaked in sea water. *Pollicipes pollicipes* for the second experiment were removed from an aquarium in the S.O.S., where they had been maintained ever since they had been transported from the coast of Portugal.

Prior to the experiments, each barnacle was carefully separated from their natural clumps, cleaned, measured and then fastened by the base of the peduncle to 5×2 cm slate panels, using superglue. Smaller animals with capitulum heights ranging between 1.0-8.3 mm (average=4.5 mm) were chosen for the first experiment since they would grow rapidly. Animals for the second experiment were slightly longer (range 2.3-10.3 mm, average=7.9 mm in capitulum height).

At pre-determined times, prior to or during the experiments, the barnacles were removed from their tanks and left in damp air for 15 to 24h, a procedure which has been shown to produce a distinct line in bivalve shell sections (RICHARDSON, 1989). The line clearly dates the shell structure and provides a reference to which all subsequent growth can be related. Several animals were killed before the main experiments began to ascertain the structure of the last few bands and to determine if the microgrowth patterns differed between shell plates or within the same shell plate from different animals.

The first experiment lasted from 28/12/92-29/3/93. The periodicity of band deposition was assessed under different conditions of immersion and emersion. One group of barnacles was maintained in constant immersion while a second group was subjected to a simulated tidal regime of 10h immersion:2h emersion. The tanks, which were essentially similar to those used by Richardson (1989), were supplied with continuously flowing seawater. The level of water in the tank was controlled by a timer which alternately opened a solenoid valve allowing water to flow into the tank to a depth of 10 cm for 10h and then closed the valve and opened another valve for 2h to allow the water to drain from the tank.

Four animals from each group were killed on 12/1/93 and the rest emersed for 15h to mark the shell plates. Two weeks later, further representatives of each group were killed and the rest emersed for 24h to induce a further reference mark in the shell plates. Thereafter, time lapse video recording was employed to determine moulting frequency for later correlation with the outer ridges/shell banding. The water temperature in both tanks was  $16 \pm 0.8 (\pm SD)$  °C. From 28/12/92 until the 26/1/93 the barnacles were subjected to a 12h light/12h dark regime and then to continuous illumination until the end of the experiment. Animals were fed twice a day on a diet of the micro-alga *Tetraselmis chui* and 1-2 day old *Artemia* nauplii, with the occasional supplement of the chain forming diatom *Skeletonema costatum*.

A second experiment was conducted between 6/4-12/5/93 to investigate the factors which could affect the rate of shell deposition, using the shell banding patterns. Using the same tanks as for the first experiment, groups of barnacles were exposed to sets of contrasting conditions. Experience from the first experiment showed that the barnacles settled into a stable pattern of shell deposition after about

one week in the experimental conditions, hence all animals were acclimated for one week before marking the shells through aerial exposure. The water temperature was kept at  $17.3 \pm 1.3 (\pm \text{SD})$  °C, and the air temperature at an average value of  $16 \pm 1.1 (\pm \text{SD})$  °C. Barnacles were continuously illuminated. All the barnacles were subjected to a tidal regime of 10h immersion:to 2h emersion for the first 26 days; during the first 13 days (6/4-18/4/93) they were fed twice a day (as detailed above), then for the following 5 days (19/4-23/4/93) starved to investigate the effect on the width of the bands, and thereafter fed until the end of the experiment. On the 1/5/93 all the animals were submitted to heat stress for 2 hours during low tide (an average temperature of  $31.7 \pm 3.8 (\pm \text{SD})$  °C was attained). Subsequently, all the animals were subjected to continuous immersion (1/5-6/5/93) then to a semidiurnal tidal regime (7/5-11/5/93). At the end of both experiments, 1 and 2, all barnacles were sacrificed.

Shell plates, largely the terga, were dissected from barnacles at the end of each set of observations, cleaned and dried tergal plates were embedded in "Metaset" resin. Hardened resin blocks were then sectioned with a hacksaw through the axis of maximum growth, treated and examined as described previously (see general material and methods). Peels were examined under a light microscope to observe the microgrowth patterns deposited during the experimental periods, and the number of bands deposited counted and the width of the bands measured.

Whole shell surfaces from the animals videoed and kept in tidally emersed conditions from the first experiment (period III) were coated with gold and examined in the S.E.M. (Scanning Electron Microscope), and the number of external ridges counted. The aim was to ascertain if the external ridges corresponded to the internal growth pattern *i.e.* growth bands.

### **1.3 RESULTS**

#### **(1). Shell plate structure**

To establish which shell plates to use routinely for the study of the growth patterns and to determine whether there were major variations in the banding patterns between shell plates and individuals, four large individuals (of similar size) were collected from the field at the same time. One tergum, one scutum and the carina were removed from each individual, embedded and acetate peel replicas of polished

shell sections examined. Essentially the banding pattern was similar in all the plates examined from the barnacles. For each barnacle the average number of bands deposited in the last 4mm of shell at the plate margin was counted and the average band width was estimated. The mean band frequency and width were very similar between shell plates and the data exhibited no significant heterogeneity of variance (see Table 1.1). A two-factor analysis of variance demonstrated no significant difference in either mean banding frequency ( $F=0.91$ ,  $df=2$ ,  $p<0.472$ ) or band width ( $F=0.83$ ,  $df=2$ ,  $p<0.498$ ) either between plates, see Table 1.2 or between animals ( $F=2.67$ ,  $df=4$ ,  $p<0.183$  and  $F=3.95$ ,  $df=4$ ,  $p<0.109$ , respectively; see Table 1.2).

Plate 1.1 illustrates the appearance of an acetate peel replica taken from a *P. pollicipes* scutum plate radial section (Dec. 1992). The peel replicates an outer thin uncalcified layer (c, Plate 1.1), and an inner layer (il, Plate 1.1) running approximately parallel to the inner surface of the shell. The banding appears as thin dark lines with thicker, more transparent incremental zones between. Adopting the terminology of Bourget and Crisp (1975b), the former one is referred to as growth lines (gl), and the transparent regions as growth bands (gb).

A seasonal pattern of growth can be inferred from Plate 1.1. The most recently deposited bands (left of the picture, Plate 1.1) are closely spaced and correspond to the Autumn and Winter months when the barnacle was collected. To the right of these, the bands are much more widely spaced corresponding to periods of more rapid growth, presumably during the Summer.

**Table 1.1**

Mean band frequency and width ( $\pm$  S.D.) in the carina, scutum and tergum shell plates of *P. pollicipes* collected from the field and, results ( $B_c$ ) of Bartlett's test for heterogeneity of variance.

	Carina	Scutum	Tergum
Mean band freq. (bands/mm)	67.5 $\pm$ 2.5	68.3 $\pm$ 13.6	70.7 $\pm$ 11.0
Mean band Width ( $\mu$ m)	14.8 $\pm$ 0.5	15.2 $\pm$ 3.4	14.1 $\pm$ 2.5
N° of animals examined	3	4	3
Bartlett's test:			
Band freq.	$B_c = 3.623$	$\chi^2_{0.05,2} = 5.99$	
Band width	$B_c = 4.051$	$\chi^2_{0.05,2} = 5.99$	



**Table 1.2**

Two-factor analysis of variance comparing mean band frequency (a.) and mean band width (b.) in *P. pollicipes* carina, scuta and terga shell plates for four individuals. Seq=sequential, Adj=adjusted for entry order in the anova model.

**a.**

Two-way analysis of variance for band frequency (bands/mm)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
<b>Plates</b>	2	16.33	122.98	61.5	0.91	0.472
<b>Animals</b>	3	541.0	541.0	180.3	2.67	0.183
<b>Error</b>	4	269.9	269.9	67.47		
<b>Total</b>	9	827.22				

**b.**

Two-factor analysis of variance for band width ( $\mu\text{m}$ )

Source	DF	Seq SS	Adj SS	Adj MS	F	P
<b>Plates</b>	2	1.94	5.04	2.5	0.83	0.498
<b>Animals</b>	3	35.9	35.87	11.96	3.95	0.109
<b>Error</b>	4	12.1	12.11	3.03		
<b>Total</b>	9	49.916				

Plate 1.1 Acetate peel replica of a radial section through a scutum shell plate of a *Pollicipes pollicipes* collected from Castelejo, Portugal, in December 1992. c, thin uncalcified layer; il, inner layer. Scale bar=200 $\mu$ m.



## (2). Experiment I

### (2.1). Periodicity of growth band deposition

Plate 1.2A shows an example of a peel from the plate of a *P. pollicipes* grown during the first experiment. The effect of 15-24h aerial exposure can be seen as a prominent line, the result of the cessation of normal shell deposition (small arrows Plate 1.2A). The deposition of this “stress line” (arrows Plate 1.2A) is usually followed immediately by a very narrow increment, although at the magnification shown in the figure it is difficult to discern. In some cases in association with the line a disruption in the outer layer of the plate could be seen (arrow 2, Plate 1.2A). Such lines (arrows 1 and 2, Plate 1.2A) mark the beginning of experimental periods and further such lines delineate subsequent experimental changes (see Plate 1.2B and C). Barnacles after initial marking were continuously immersed (arrows 1, Plate 1.2A) then after remarking (arrow 2, Plate 1.2A) subjected to 122 semi-diurnal emersions (arrow 1 to plate edge, Plate 1.2A). Gradually throughout the experiment the semi-diurnal lines appeared to become more clearly defined.

Table 1.3 shows the results of the first laboratory experiment. The predicted number of bands (assuming that one band is deposited per tide), and the observed number of bands in the tergal shell plates of *P. pollicipes* when subjected to periods of semi-diurnal emersion and continuous immersion are listed (Table 1.3a). Initially bands are daily, then after a period of entrainment the 12h light/12h dark cycle becomes less stimulating and the tidal emersions and bands coincide tidally. Under constant illumination and no tidal cue, banding patterns become variable (cv=76% as opposed to 11% for the tidal animals) and at an average frequency which neither coincides with the number of days or tides (every 3-4 days).

The individuals under constant immersion exhibited a larger mean band width than those in the tidal regime (see Table 1.3b), a difference which is greatly enhanced in those individuals under constant illumination and immersion. A two factor analysis of variance performed over the two light/dark periods demonstrated no significant difference in mean band width either between illumination periods or immersion conditions ( $F=2.30$ ,  $df=1$ ,  $p<0.137$  and  $F=3.12$ ,  $df=1$ ,  $p<0.085$ , respectively; see Table 1.4b), however, there was a significant difference in the number of bands deposited under different tidal regimes ( $F=11.14$ ,  $df=1$ ,  $p<0.002$ ,

see Table 1.4a). The individuals under tidal conditions during the second period deposited significantly more bands than those continuously immersed (see Table 1.5). The lack of a significant difference during the initial growth period is likely to result from a period of entrainment to the tidal influence.

Fig. 1.1 shows the existence of a significant linear relationship ( $r=0.946$ ,  $df=56$ ,  $p<0.001$ ) between the number of bands deposited and the increment in shell length. These results might be expected if one envisages that shell deposition proceeds in such a way that crystals of equal size are deposited sequentially.

## **(2.2). Shell surface**

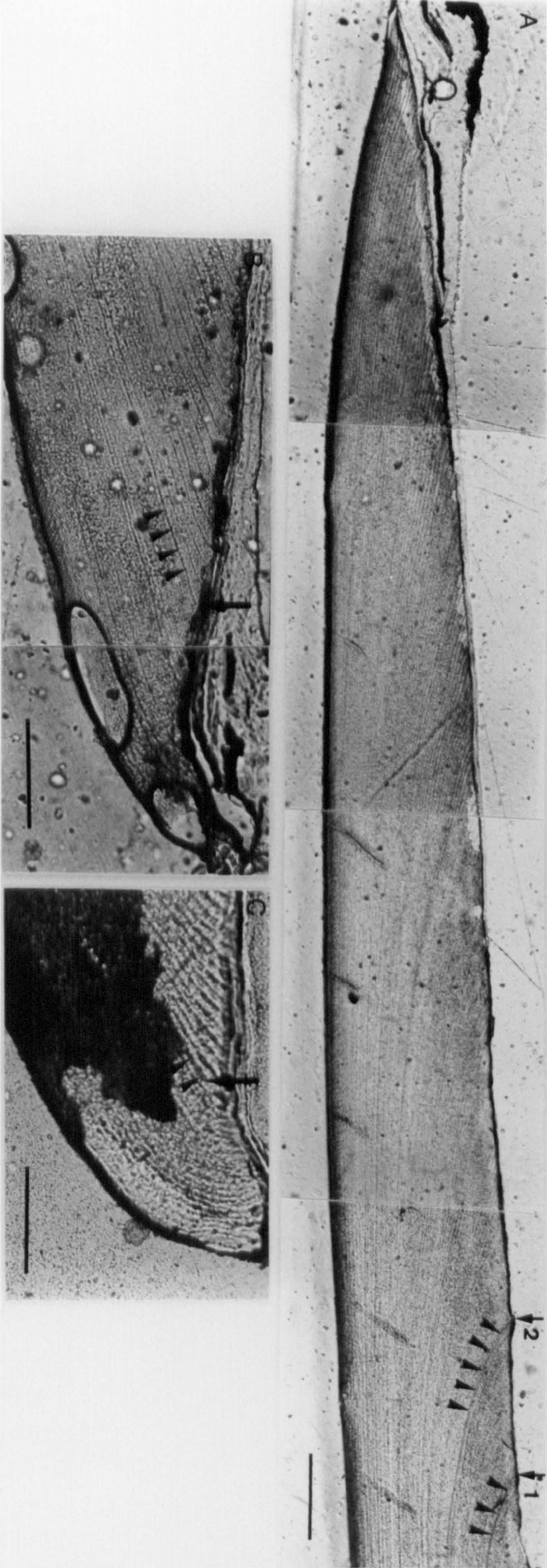
When the outer surfaces of the tergal shell plates were viewed with the S.E.M. they revealed a pattern of thin light ridges (r) with darker increments (i) between as illustrated in Plate 1.3A. At higher magnifications (see Plates 1.3B and C) the details of the ridges clearly show the presence of a series of fine lines (li) perpendicular to the direction of deposition of the ridges (Plate 13C). The average number of ridges observed on the external surface does not directly coincide with the number of tidal periods during the observations (see Table 1.6). However, the average frequency of deposition is closer to a tidal frequency (0.8 bands per tide) than to a daily frequency (1.5 bands per day). There is insufficient data to determine a relationship between the number of bands counted in the shell sections (internal bands) under the light microscope and the number of ridges counted from the external surface (external bands) under the S.E.M. ( $r=0.762$ ,  $df=4$ ,  $p=0.08$ ). On average only 80% ( $\pm 9\%$   $\pm$ SD) of the bands seen inside the shell plates were evident on the shell surface. More data would be required to confirm that there is a relationship between external ridges and internal bands.

Over a 62 day period, each animal moulted on average three times, with a frequency of one moult every 3 to 17 days (mean intermoult period= $13\pm 3.8$  ( $\pm$ SD) days). No hirsute ridges described by Bourget and Crisp (1975b) were observed.

**Plate 1.2 (A-C). Acetate peels of radial sections of tergal plates from *Pollicipes pollicipes*.**

**A) Tergal plate from a barnacle from experiment 1 marked by 15-20h emersion (arrow 1), continuously immersed for 14 days and then remarked again (arrow 2) before being placed in a tidal regime, 10h immersion:2h emersion, for 122 days. Scale bar A=200 $\mu$ m.**

**B and C) Tergal plates from barnacles grown in experiment 2. The arrows mark the point at which the barnacles were emersed for 2h at 30°C prior to transfer into continuously immersed conditions. Scale bar B and C=100 $\mu$ m.**



**Table 1.3**

**a.** Mean numbers of bands counted during the three experimental periods under varying environmental conditions during the first experiment. Pattern—the closest approximation to noticeable rhythm.

	<b>PERIOD I</b> Light/Dark		<b>PERIOD II</b> Light/Dark		<b>PERIOD III</b> Light	
	Tidal	Constant immersion	Tidal	Constant immersion	Tidal	Constant immersion
<b>Exp. days/tides</b>	16/31	16/N.A.	14/26	14/N.A.	62/122	62/N.A.
<b>Mean number of bands</b>	18.5	15.0	23.2	14.3	115.0	21.7
<b>S.D.</b>	7.8	5.6	4.0	6.9	12.6	16.5
<b>95% C.I.</b>	4.3	3.6	2.5	5.3	13.2	17.3
<b>No animals</b>	15	12	12	9	6	6

<b>Pattern</b>	<b>Daily</b>	<b>Daily</b>	<b>Tidal</b>	<b>Daily</b>	<b>Tidal</b>	<b>-----</b>
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**b.** Mean band widths measured in *P. pollicipes* tergal plates during the three experimental periods under varying experimental conditions during the first experiment.

	<b>PERIOD I</b> Light/Dark		<b>PERIOD II</b> Light/Dark		<b>PERIOD III</b> Light	
	Tidal	Constant immersion	Tidal	Constant immersion	Tidal	Constant immersion
<b>Exp. days/tides</b>	16/31	16/N.A.	14/26	14/N.A.	62/122	62/N.A.
<b>Mean band width (<math>\mu\text{m}</math>)</b>	24.6	25.5	24.5	37.9	24.3	72.9
<b>S.D.</b>	15.1	14.5	9.4	13.5	2.0	70.3
<b>95% C.I.</b>	8.4	9.7	6.0	11.3	2.1	73.8
<b>No animals</b>	15	11	12	8	6	6

N.A. not applicable



**Table 1.4**

Two-factor analysis of variance comparing (a.) the mean numbers of bands counted and (b.) the mean band widths measured in *P. pollicipes* terga over two periods of 12h light/ 12h dark under tidal and continuous immersion. Seq=sequential, Adj=adjusted for entry order in the anova model.

**a.**

Two-way analysis of variance for mean numbers of bands

Source	DF	Seq SS	Adj SS	Adj MS	F	P
<b>Tidal/cont.</b>	1	408.17	444.00	444.00	11.14	0.002
<b>Period</b>	1	63.85	45.68	45.68	1.15	0.290
<b>Interaction</b>	1	81.55	81.55	81.55	2.05	0.160
<b>Residual</b>	44	1753.40	1753.40	39.85		
<b>Total</b>	47	2306.98				

**b.**Two-way analysis of variance for band widths ( $\mu\text{m}$ )

Source	DF	Seq SS	Adj SS	Adj MS	F	P
<b>Tidal/cont.</b>	1	426.4	560.0	560.0	3.12	0.085
<b>Period</b>	1	286.1	412.7	412.7	2.30	0.137
<b>Interaction</b>	1	416.6	416.6	416.6	2.32	0.135
<b>Residual</b>	42	7537.6	7537.6	179.5		
<b>Total</b>	45	8666.8				

**Table 1.5**

Tukey-Kramer multiple comparison method comparing mean numbers of bands in *P. pollicipes* over two periods of 12h light/12h dark under tidal and constant immersion.

<b>Tidal2</b>	-4.6		
<b>Constant1</b>	+3.5	+8.2*	
<b>Constant2</b>	+4.2	+8.8*	+0.67
	<b>Tidal1</b>	<b>Tidal2</b>	<b>Constant1</b>

\*significant differences

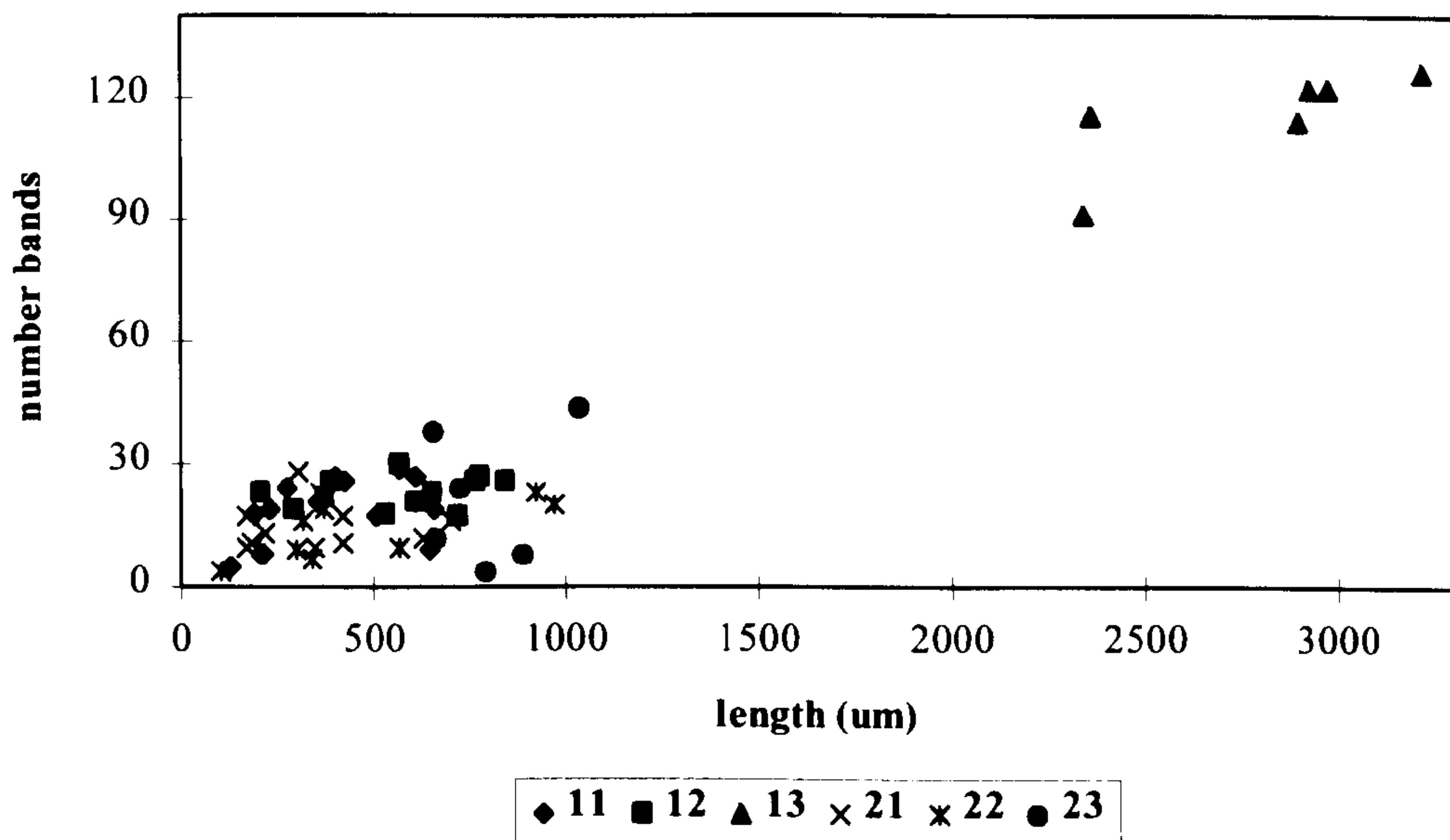


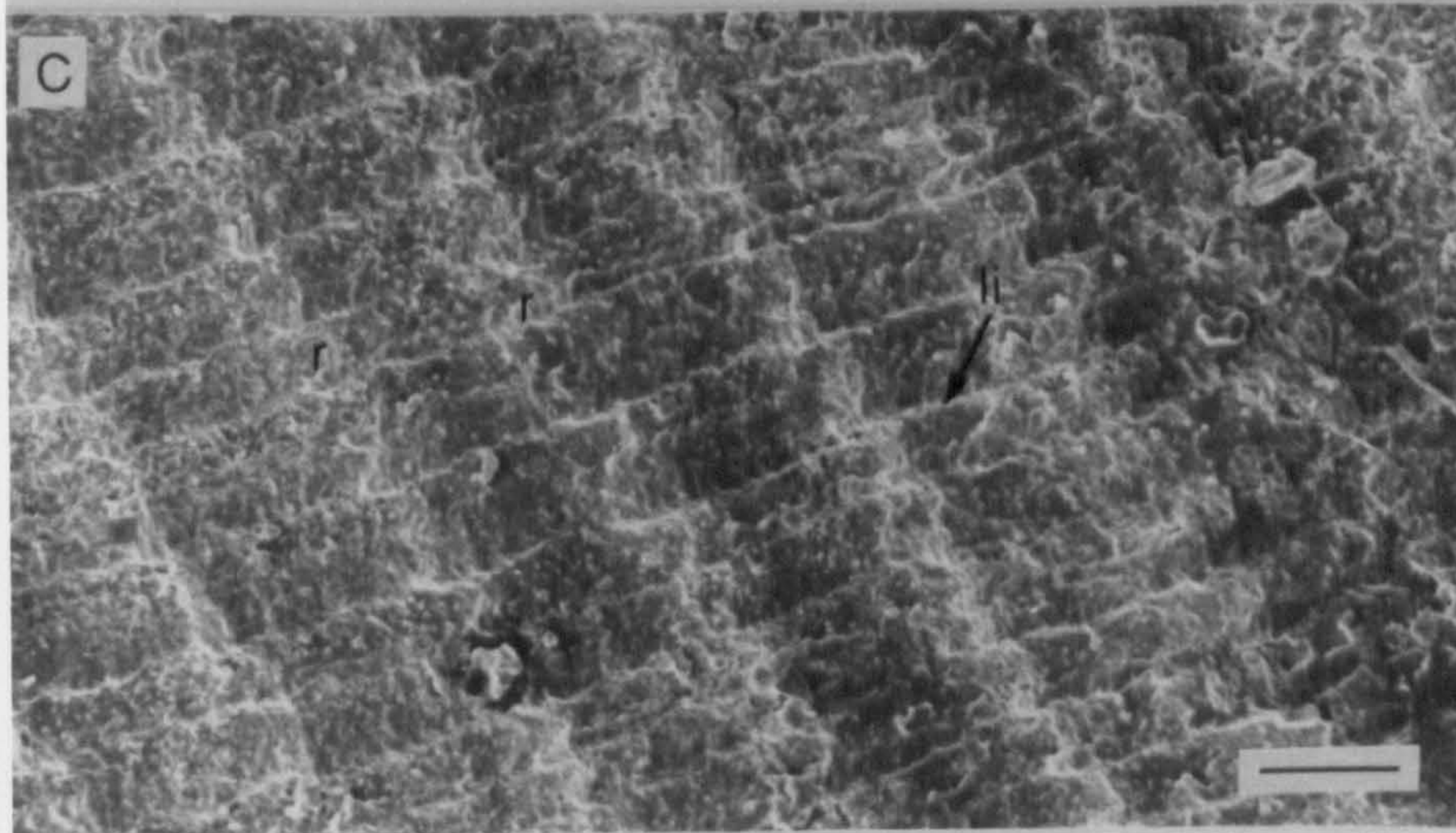
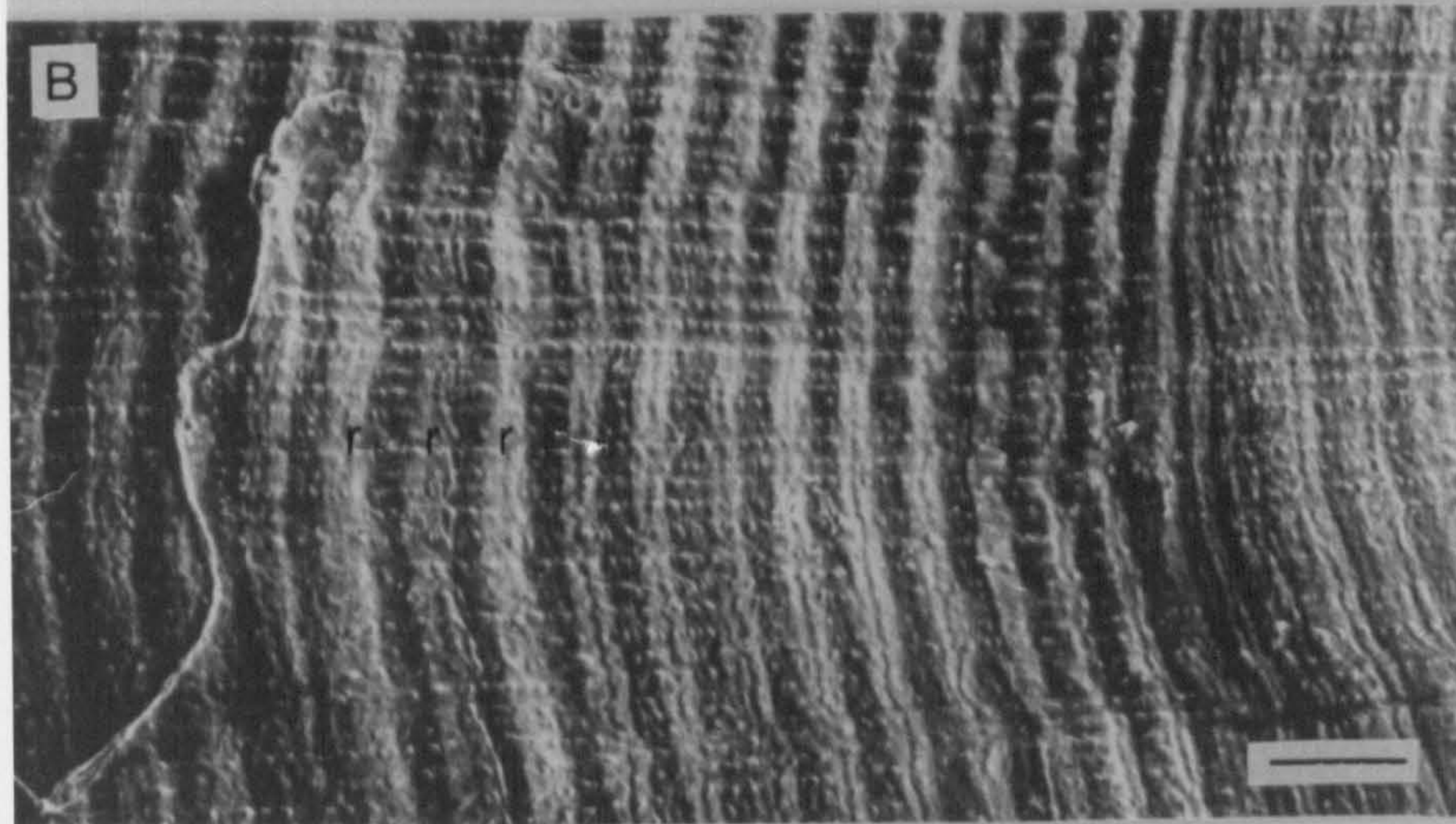
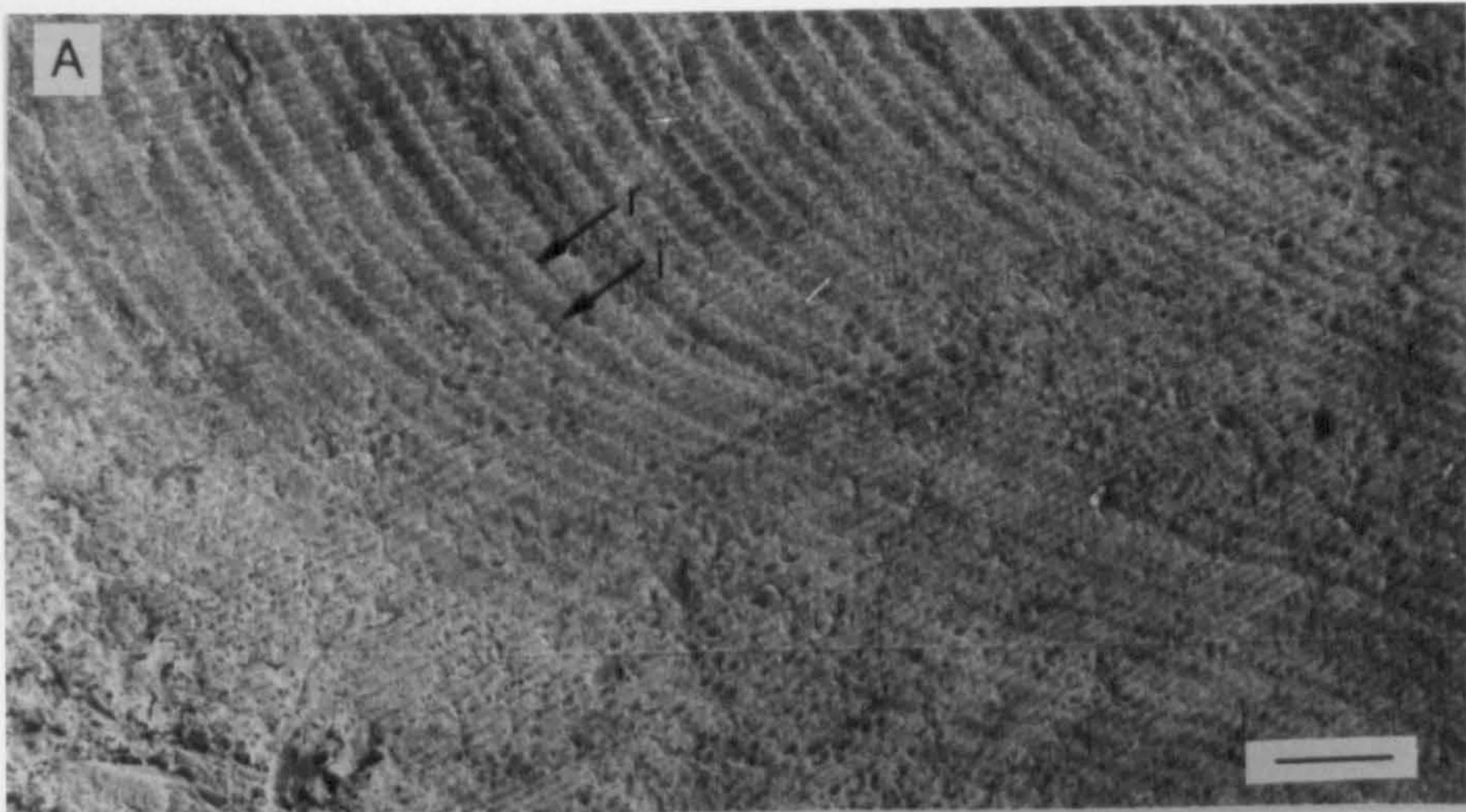
Fig. 1.1 The relationship between the number of bands deposited and the increment in shell length ( $\mu\text{m}$ ). 11, 12, 13, 21, 22, 23 are the different experimental periods and conditions (e.g. 13=data from 3<sup>rd</sup> experimental period from the barnacles subjected to tidal simulation).

**Plate 1.3 (A-C) S.E.M. photomicrographs of the outer surface of the tergal plates from *Pollicipes pollicipes* grown during the first experiment.**

**A) A pattern of thin light coloured ridges with darker wider increments (i) between; scale bar=100µm.**

**B) Higher magnification of the ridges (r); scale bar=50µm.**

**C) Fine lines (li) are deposited perpendicular to the ridges (r); scale bar=20µm.**



**Table 1.6**

The number of ridges observed on the surface of *P. pollicipes* terga during period III of experiment 1 with constant light and a semi-diurnal tidal rhythm of emersion.

<b>PERIOD II</b> Light/Tidal	
<b>Exp. days/tides</b>	62/122
<b>Mean number of ridges</b>	95.0
<b>S.D.</b>	15.9
<b>95% C.I.</b>	16.7
<b>No animals</b>	6
<b>Pattern</b>	<b>Tidal</b>

### (3). Experiment II

In the second experiment the barnacles were fed for a period of 13 days, then starved for 5 days before food was again supplied. The period of starvation had virtually no effect on the rate of growth band formation. For the initial 26 days of this experiment there appeared little difference in the banding rate whether the organisms were fed or not. The banding frequency varied from 0.7 to 1.5 bands per tide (average 1.1 bands per tide over all 3 periods, see Table 1.7a), hence a roughly tidal periodicity. The band widths likewise showed no significant difference (One-way Anova  $F=0.005$ ,  $df=2,6$ ,  $p=0.999$ , see Table 1.8). With such similarity all the data for this initial period were combined for comparison with the second and third periods of the experiment.

At the conclusion of the 26 day period of the experiment, the barnacles were subjected to a short 2h period of emersion at 30°C to simulate as far as possible the temperatures that the barnacle might normally have experienced in their natural rocky habitat, prior to transfer to continuous immersed conditions. The period of stress during low tide did not appear to affect the shell growth of the individuals as

no “stress line” was deposited during emersion (arrow, Plate 1.2 B and C). In most barnacles the “post stress” lines *i.e.* the closest to the plate tip, were narrow and were difficult to count. This decline in band width during continuous immersion, probably reflected the decrease in shell deposition as a consequence of the heat shock.

The second experiment reinforces the results of the first with respect to the pattern of band deposition in the individuals subjected to periods of semi-diurnal emersion (see Table 1.7b). These barnacles deposited approximately one band per tide, with a pattern variability for the first period which was close to the one from the barnacles under similar conditions in the first experiment ( $cv=10\%$ ) but more variable in the experiment period three ( $cv=31\%$ ). The continuously immersed individuals deposited roughly 1.6 bands per day, a frequency which was higher than in the animals from the first experiment subjected to similar conditions, although with a lower pattern variability ( $cv=32\%$ ).

#### **1.4 DISCUSSION**

In order to age an individual using the skeletal patterns, validation of the periodicity of the discontinuities is essential. The production of patterns found in skeletons has been linked with environmental factors (*e.g.* CRISP, 1960; BOURGET and CRISP, 1975a; CRISP and RICHARDSON, 1975) and, in some cases, the frequency of the patterns has been likened to a chronometer of environmental change.

In this Chapter I have for the first time demonstrated the periodicity of the growth patterns and investigated the effects of simulated tides, light, food and heat on the deposition of the shell of *P. pollicipes*. In contrast to Bernard (1988) the tergum was chosen as the plate best suited for studying the deposition of bands since it is the largest, and is more easily sectioned in the right plane and the spacing of the bands is easier to determine than that of the carina. Furthermore, the carinal plate was often seen to be broken at the tip, with the consequent loss of that part of the skeletal record. No significant differences in banding frequency were observed between the three largest shell plates hence for convenience, ease of preparation and readability the tergum was chosen to study and measure the shell plate growth patterns.

**Table 1.7**

**a.** Mean numbers of bands counted during the first experimental period in the second experiment under varying nutritional conditions. Pattern=the closest approximation to noticeable rhythm.

	<b>Fed</b> Light/tidal	<b>Starved</b> Light/tidal	<b>Fed</b> Light/tidal
<b>Exp. days/tides</b>	13/26	5/10	8/16
<b>Mean number of bands</b>	18.3	15.0	16.3
<b>S.D.</b>	5.5	3.6	2.3
<b>95% C.I.</b>	13.69	8.97	5.74
<b>No animals</b>	3	3	11
<b>Pattern</b>	<b>Tidal</b>	<b>Tidal</b>	<b>Tidal</b>

**b.** Mean numbers of bands counted during the three experimental periods in the second experiment under varying experimental conditions.

	<b>Period I</b> Light/tidal	<b>Period II</b> Light/constant immersion	<b>Period III</b> Light/tidal
<b>Exp. days/tides</b>	26/52	5/N.A.	6/12
<b>Mean number of bands</b>	53.1	7.8	10.6
<b>S.D.</b>	5.5	2.5	3.4
<b>95% C.I.</b>	3.07	2.08	2.25
<b>No animals</b>	15	8	11
<b>Pattern</b>	<b>Tidal</b>	-----	<b>Tidal</b>

N.A. not applicable

**Table 1.8**

One factor analysis of variance comparing mean band widths in *P. pollicipes* terga over three periods (feeding/non-feeding/feeding) under a tidal regime and continuously illuminated conditions. Seq=sequential, Adj=adjusted for entry order in the anova model.

One way analysis of variance for band widths ( $\mu\text{m}$ )					
Source	DF	SeqSS	AdjMS	F	P
Fed/n-fed/fed	2	0.01	0.01	0.005	0.999
Error	6	59.71	9.95		
Total	8	59.72			



The results of the present study show that, on average, there is good agreement between the number of growth bands produced and the number of tidal emersions, and band production is generally independent of the light regime, as found for other barnacle species (*e.g.* *Semibalanus balanoides*, BOURGET and CRISP, 1975a); *Elminius modestus*, CRISP and RICHARDSON, 1975). The results of the first experiment show that continuously immersed barnacles, no tidal cue, displayed a pattern which varied with the light regime. Individuals under a 12h light/12h dark regime produced close to a band per day, while those continuously illuminated deposited less than a band a day. However, in the second experiment, continuously illuminated and immersed barnacles deposited more than one band a day. A possible explanation for the difference could be related to the length of the immersion period, which was possibly too short (only 5 days) in the second experiment to negate the influence of the previous tidal entrainment. Daily growth bands are commonly found in the shells of subtidal bivalve species *e.g.* *Pecten diegensis* (CLARK, 1968) and fish otoliths (PANNELLA, 1971). Periodicity of the shell deposition in *Pollicipes* seems to be regulated by tidal immersion and emersion which could be considered the strongest available environmental cue, given a period of entrainment. The increased variability in banding frequency shown by barnacles given a specific cue (*i.e.* continuously illuminated and submerged) points to the absence of an endogenous rhythm as suggested for other species (*e.g.* *S. balanoides*, BOURGET and CRISP, 1975b).

Barnes (1956) reported that continuous immersion lead to an enhanced growth rate of previously maintained intertidal *Chthamalus stellatus*. Here, in this study *P. pollicipes* showed a significantly reduced shell deposition rate when continuously immersed and illuminated. If the mechanism of shell deposition is dependent on the availability of calcium carbonate (BOURGET and CRISP, 1975a) then shell growth should be expected to be enhanced by continuous immersion. However, calcium carbonate deposition is not a simple process of chemical equilibrium. Further, calcium and carbonate ions are seldom, if ever, limiting in the marine environment, and acorn barnacles use carbonic anhydrase to control the pH of shell deposition to ensure an accumulation rather than dissolution of the shell (YULE *et al.*, 1982). The

structure of the shell also requires that a protein matrix is present to support the integrity of the otherwise brittle calcium carbonate structure.

Alternate periods of feeding neither changed the frequency of growth band deposition, nor their width, at least over the short periods employed. Barnacles in general can withstand fairly lengthy periods of food deprivation and, presumably, have more than enough reserves to provide the matrix for shell deposition over the periods of deprivation. The frequency of formation of the striped pattern of the shell in *Sepia esculenta* is reduced in low food concentrations (CHOE, 1963) and the shell deposition rates in *Cerastoderma edule* were found to be proportional to the fraction of time that the animal was immersed and able to feed (RICHARDSON *et al.*, 1980). Yet, Bourget and Crisp (1975b) noted that short interruptions in feeding (of up to 6h) caused no corresponding interruption to shell growth in immersed barnacles.

According to Bourget and Crisp (1975b) heat stress in *S. balanoides* could result in missing bands and the formation of a dark line or “stress” band. The warm summer seawater temperature was also implicated in the suppression of shell growth and appearance of clefts in the shell of the Mediterranean bivalve *Chamalea gallina* (RAMON and RICHARDSON, 1992). Despite the temperature shock experienced by *P. pollicipes* during the current experiments no clefts or dark “stress” lines were ever observed in the shells. Most barnacles close their shell valves in response to elevated temperature and the resultant anaerobiosis and low haemolymph pH has been suggested to cause the formation of shell “stress” lines through dissolution of the inorganic constituents of the shell in bivalves (BOURGET and BROCK, 1990). *P. pollicipes* normally experiences exceptionally high temperatures (> than 35 °C) for short periods during tidal exposure during the summer months in the Algarve, yet no potential “stress” lines are evident in the shells of animals collected from the wild (see Plate 1.1). Nevertheless, barnacle shells do exhibit a seasonal pattern of deposition which seems to reflect the rate of shell deposition being faster in summer than in winter (Plate 1.1).

A mechanism of band formation in barnacles was proposed by Bourget and Crisp (1975b) which suggests that the outer membrane covering the shell plates (epicuticular plus cuticular layers) is responsible for the production of the surface growth rings. Bourget and Crisp (1975b) also concluded that the number of ridges

and their rates of formation do not correlate with the moulting cycle or with tidal immersions and emersions. Their theory also explains the presence of a hirsute ridge associated with body moulting. In *P. pollicipes*, the internal microgrowth patterns seen in the shells roughly correspond to the patterns visible externally. However, no hirsute ridges were seen on the external surface of the shell even though many of the barnacles moulted up to three times during the experiment.

Clearly the deposition of shell plate material in *P. pollicipes* is encouraged when environmental discontinuities are present. The strongest environmental cue appears to dominate hence light/dark provides a stabilising cue for continuously immersed barnacles but a superimposed tidal cycle will become established as the major controlling cue and supersede the presumably weaker cue of a changing light regime.

## **CHAPTER II: RELATIVE GROWTH IN *POLLICIPES POLLICIPES***

## 2.1 INTRODUCTION

The body shape of an organism does not always change uniformly with an absolute increase in the size of the whole animal. For example, the relative proportions of the bivalve shell often change with increasing body size (*i.e.* allometric growth), resultant from differential growth at distinct points around the mantle edge (SEED, 1980).

From the basic geometry of simple slopes, it is a general principle that the surface area of a body will increase roughly as the square of a linear dimension while volume, hence mass, will increase roughly as its cube. Thus, if geometric similarity is maintained throughout growth (*i.e.* isometric growth), the surface area/volume ratio will progressively decline. Because most exchanges between the external environment and the organism occur across body surfaces, however, relatively constant area/volume ratios are generally an adaptive necessity and are only possible through changes in shape, *i.e.* relative body dimensions (SEED, 1980).

Pholo (1964) has shown that the ontogenic changes in the bivalve *Tressus nuttalli* can be related to differences in life style between the actively burrowing juveniles and the more sedentary adults, which live a more protected life deeper in the sediment. Positive allometric growth of the posterior region of the shell is here associated with the increase in space required for the enlarged siphons. Similar environmentally induced ontogenetic shape changes have also been recorded in other bivalves by Kristensen (1957), for *Cardium*, by Johannessen (1973), for *Venerupis* and for *Mercenaria* by Stanley (1970). Crisp and Patel (1961) postulated that when *Elminius modestus* Darwin reaches a certain size, roughly coincident with maturity, growth ceases to be isometric because some structure, vital to food collection or utilisation, such as the cirral net or gut, grows at a rate that is less than proportional to that of the rest of the animal. Another more likely explanation (CRISP and PATEL, 1961) is that growth is dependent on the physical limitations caused by diffusion and the need to maintain a constant surface to volume ratio (SEED, 1980).

The use of bivariate data in allometric studies may be criticised based on the fact that only two variables are compared at any given time. Even if such data do not satisfactorily describe changes in body form they can still provide useful and straight

forward comparisons to be made between populations which may exhibit environmentally or genetically linked variability in morphology (SEED, 1980).

The morphology of a barnacle (particularly the shell plates) has great plasticity, so that its form may be much modified during growth without greatly hindering its vital activities (CRISP and BOURGET, 1985). Although there have been attempts to relate shape to environmental conditions (see CRISP and BOURGET, 1985), only four main factors have been well documented as causing modification in shape: a) crowding (MOORE, 1934; BARNES *et al.*, 1963; BOURGET and CRISP, 1975a; CRISP and BOURGET, 1985); b) the shape of the attachment substratum (BARNES and POWEL, 1950; CRISP and PATEL, 1967; CRISP and BOURGET, 1985); c) salinity (BARNES and HEALY, 1965, 1969; CRISP and BOURGET, 1985; FURMAN, 1990) and, d) pollution (FURMAN, 1990; ROYO-GELABERT and YULE, 1994). Changes in the slopes of the weight relationships have been attributed to the breeding condition of the barnacles (BARNES *et al.*, 1963; CRISP AND PATEL, 1961); they may reflect the abundance of plankton, the level of storage materials (BARNES *et al.*, 1963; BARNES and BARNES, 1968) and differences in the gut contents (HINES, 1978).

The current study concerns the stalked barnacle *P. pollicipes* which is of considerable commercial interest in Portugal and collected in vast quantities from their natural habitats. There is thus a necessity to assess the sustainability of the barnacle fishery and this chapter aims to report on information on the biology of *P. pollicipes* with a view to managing the fishery. It is concerned with the relative morphology and gravimetry of *P. pollicipes* from four sites Castelejo, Zavial, Sagres and Ponta-da-Fisga, and examines comparisons between the capitulum width/capitulum height, wet weight/capitulum height, wet weight/dry weight and ash free dry weight/dry weight for animals from each site throughout the year and between the sites.

## 2.2 MATERIALS AND METHODS

Samples of *P. pollicipes* Gmelin were collected and measurements taken as described in the general materials and methods, from four different sites (Castelejo, Zavial, Sagres and Ponta-da-Fisga) in southwest Portugal.

The relationship between the dimensions of two parts of the body can often be expressed by the power function:  $Y=aX^b$ , where  $Y$  and  $X$  are measures of the dimensions and  $a$  and  $b$  are constants. The exponent,  $b$ , can be considered as a growth coefficient defining the nature of the proportionality between the two dimensions. The constant,  $a$ , represents the value of dimension  $Y$  for unitary dimension  $X$ , and hence is particularly dependent on the units of measurement. If  $b=1$  then  $a$  is the constant of proportionality between the two dimensions. When the dimensions measured are both lengths (*i.e.* both variables have the same dimensions but not necessarily the same units) then an exponent  $b=1$  defines direct proportionality between the dimensions and the relationship is said to be isometric. Biologically we interpret an isometric relationship between dimensions as reflecting a constant ratio of “growth” rates in both dimensions. A significant departure from 1 in the exponent defines lack of proportionality between the dimensions and the relationship is said to be allometric, a unit increase in one dimension is not accompanied by a constant increase in the second. Biologically we interpret an allometric relationship as reflecting unequal growth rates for the two dimensions, hence a general change in shape of the organisms as they grow. Dependent on which function is fitted ( $X=aY^b$  or  $Y=aX^b$ ) the exponent will be greater or less than 1 (so called negative or positive allometry), the difference is trivial since one is always the reciprocal of the other. When the measured variables differ in dimension, *i.e.* length to area or weight to length, then the proportionality discussed above will refer to a different value of  $b$ , *viz.*  $b=2$  for area/length or  $b=3$  for weight/length. Biological inferences regarding lack of proportionality again relate to changes in shape as the organism grows.

Barnacles were measured over as wide a size range as possible and analysis has only been performed on data of comparable dimension ranges to avoid the inclusion of artefacts due to analysis of incomplete bivariate, normal distributions (see RICKER, 1975). As far as possible, the most readily obtained and most precisely determined measure of animal “size”, the capitulum height, has been used as the reference variable throughout. All morphometric work suffers in analysis since very few powerful techniques are available for testing the functional relationships between two randomly distributed variables which are generally approximately

normal. Regression techniques have generally been applied to such data in the past with scant regard to the problems associated with the departures from the assumptions of the analysis shown by the data. Regression techniques are perfectly applicable approximate analyses to use when variability is low and correlation high. When the converse is true then using regression provides much less reliable answers and throws doubt on the interpretation (see RICKER, 1975). Where appropriate, all morphological and gravimetric relationships have been fitted using least squares regression with the adequacy of each fit ascertained from plots of the residuals from the fit. Comparisons between site and collection times were performed using analysis of covariance techniques.

### 2.3 RESULTS

Since samples collected at different tidal heights for each pair of morphological and gravimetric variables revealed no significant effect of tidal height within months the measurements were combined for comparison between sites. As a first approach each bivariate data set was plotted (see Fig. 2.1 to 2.4) using an appropriate scale for expected linearity. Figs 2.1-2.4 show fairly good linearity with the expected biological variability and occasional outlier. To these data, relationships have been fitted using least squares regression with the adequacy of each fit ascertained from plots of the residuals from the fit. The analysis of the residual plots and the high regression correlation coefficients demonstrated no evidence of non-linearity. Tables 2.1 and 2.2 summarise the relationships (data pooled from each sampling period) between the morphometric and gravimetric characteristics of *P. pollicipes* populations sampled from the four sites in the Algarve. Table 2.1 shows the relationships between width and height, wet weight/dry weight and ash free dry weight/dry weight, the constant  $a$  (slope of regression forced through the origin) slope, when  $b$  in the equation  $Y=aX^b$  is equal to one, together with the correlation coefficient and the values of  $s$  of the variability in the  $Y$  values. In Table 2.2 the exponent  $b$ , and  $a$  intercept, are shown together with the correlation coefficient and the significance of a t-test between the fitted exponent and the proportional constant (*i.e.*  $b=3$ ).



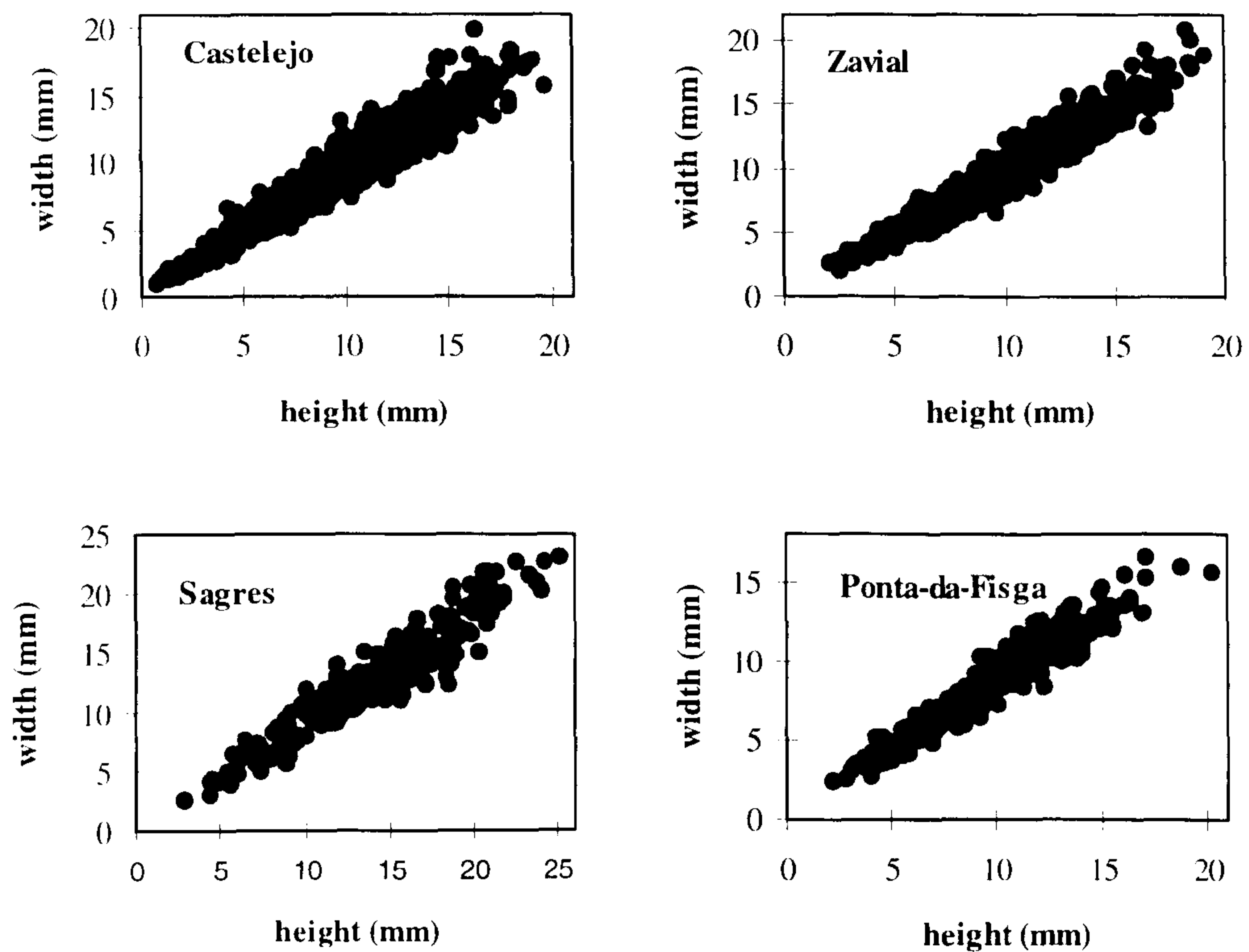


Fig. 2.1 Capitulum width versus capitulum height plots for *P. pollicipes* at four sites Castelejo, Zavial, Sagres and Ponta-da-Fisga.

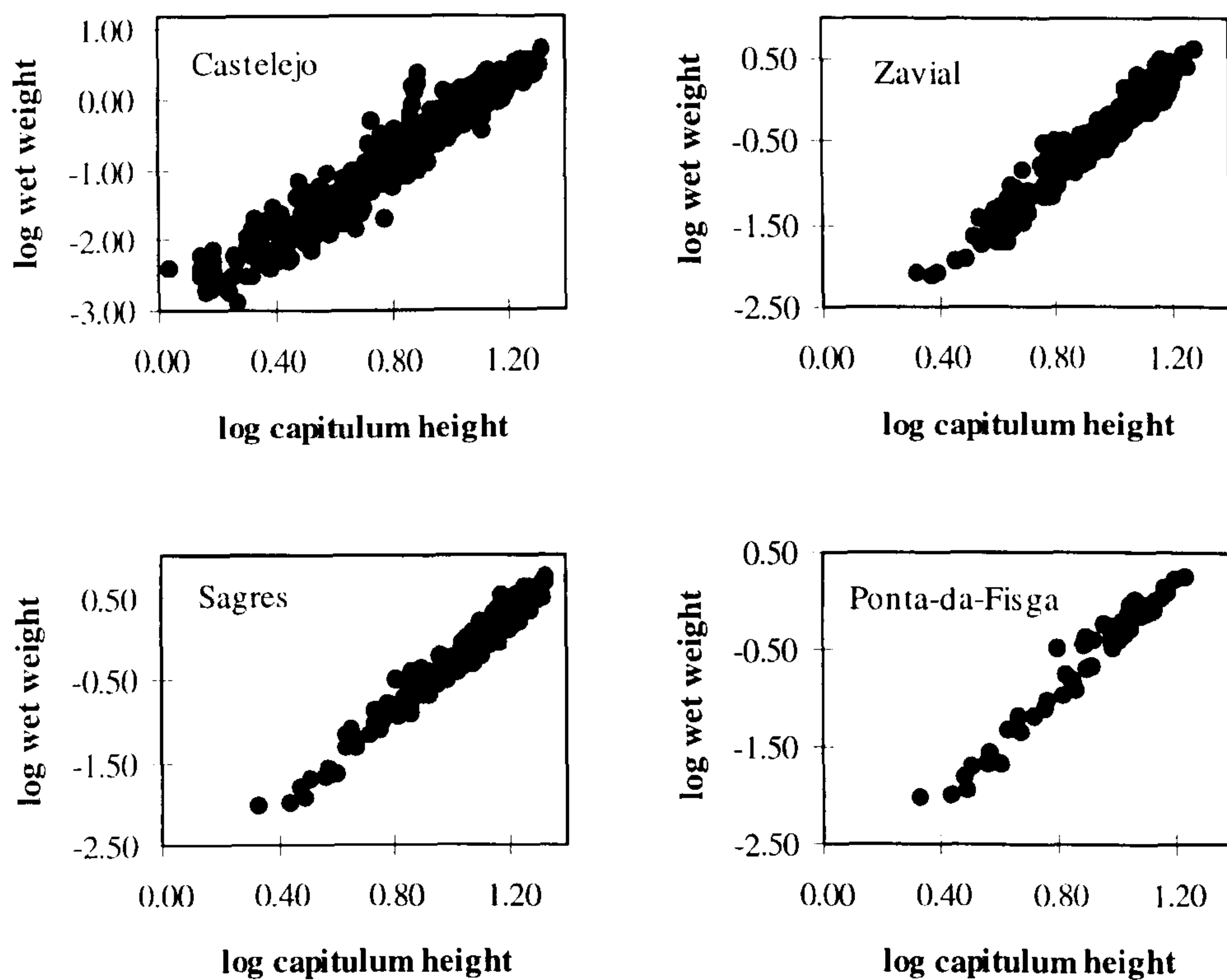


Fig. 2.2 Logarithm wet weight versus logarithm capitulum height plots for *P. pollicipes* at four sites Castelejo, Zavial, Sagres and Ponta-da-Fisga.

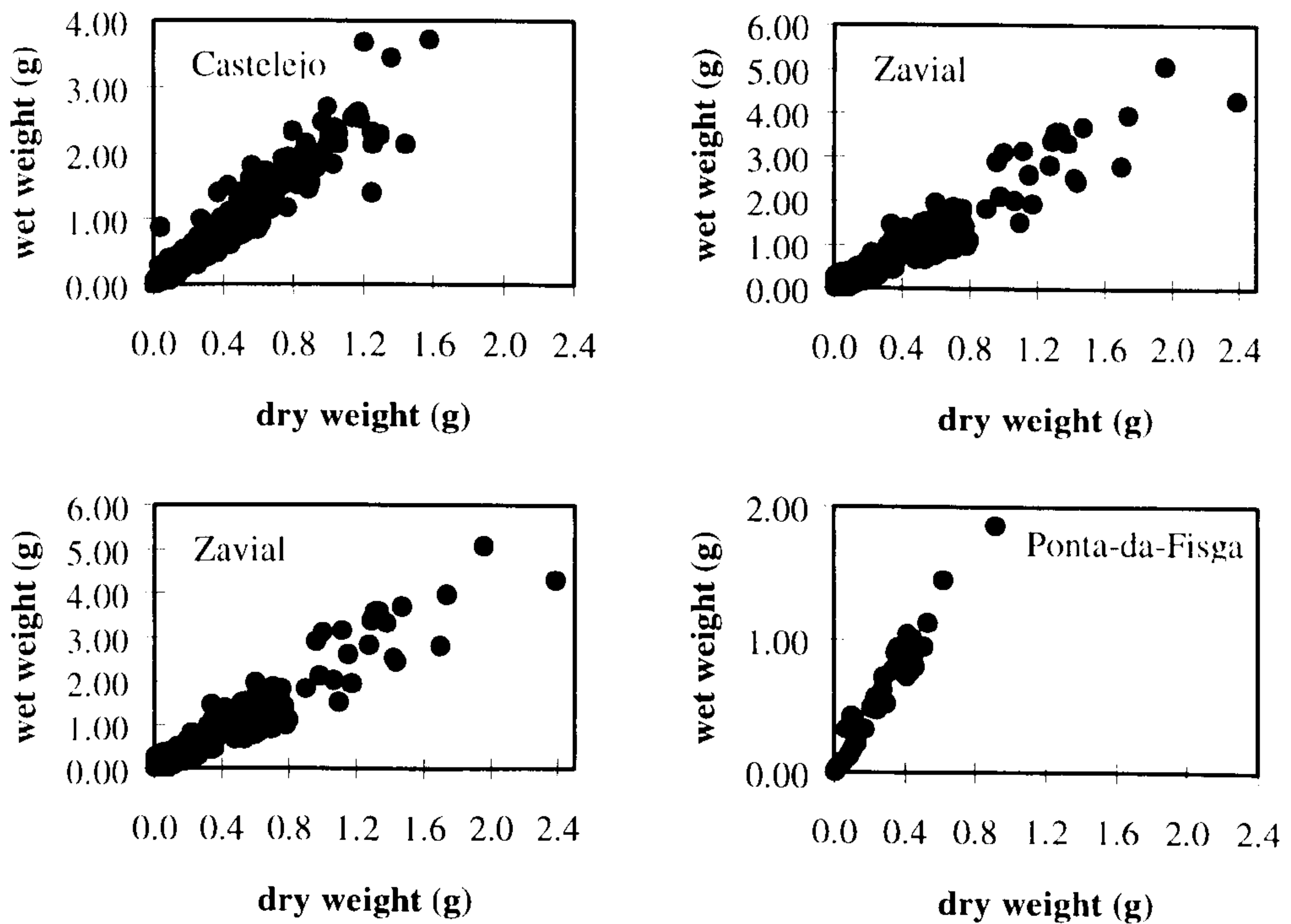


Fig. 2.3 Wet weight versus dry weight plots for *P. pollicipes* at four sites Castelejo, Zavial, Sagres and Ponta-da-Fisga (Fisga).

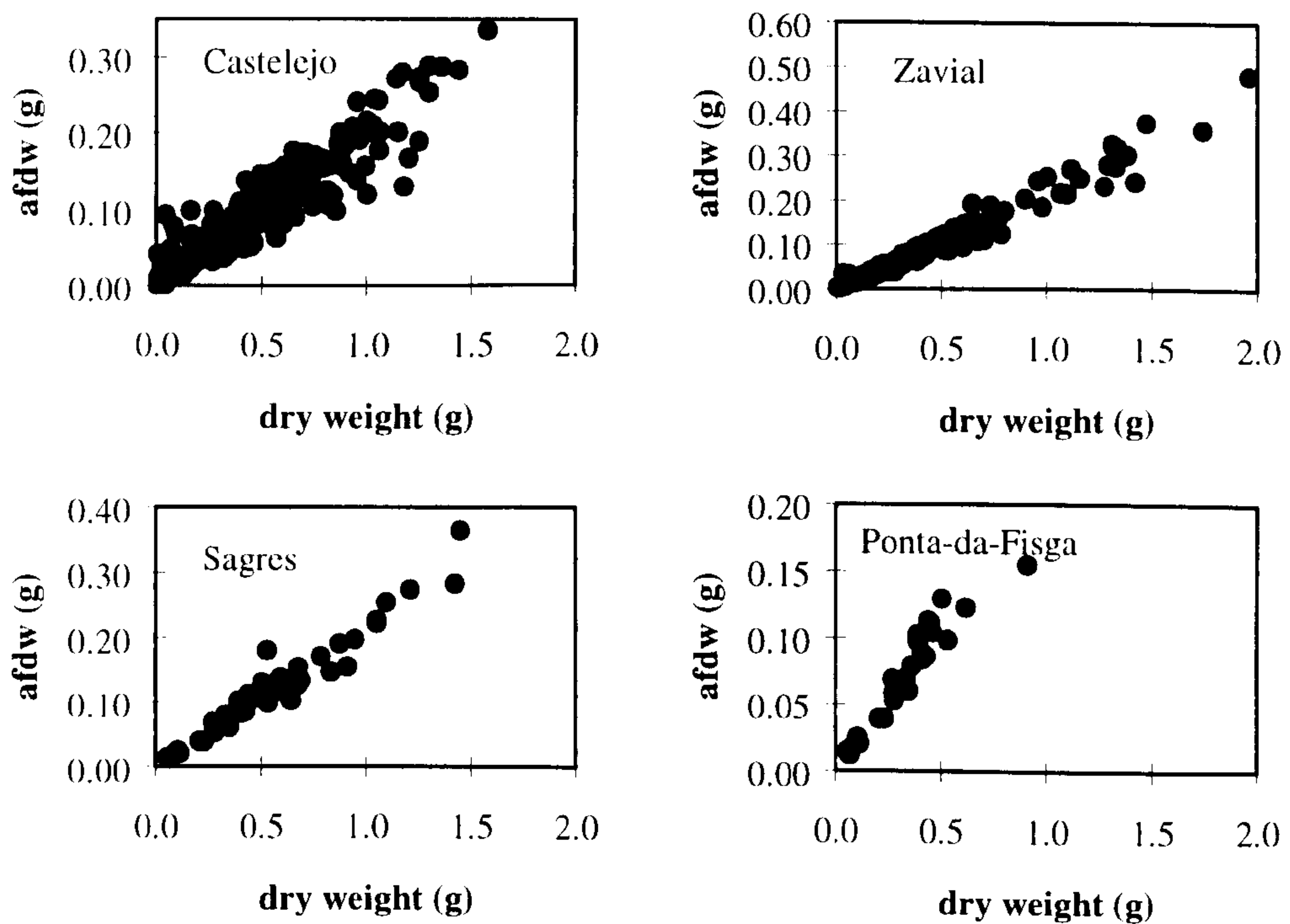


Fig. 2.4 Ash free dry weight (afdwt) versus dry weight plots for *P. pollicipes* at four sites Castelejo, Zavial, Sagres and Ponta-da-Fisga (Fisga).

**Table 2.1**

Morphometric and gravimetric relationships for *P. pollicipes* populations from 4 sites on the Algarve coast. St=site; Ca - Castelejo, Za - Zavial, Sa - Sagres, PF - Ponta-da-Fisga. Parameter a from  $Y=aX^b$  where  $b=1$ . SE=Standard Error. r=correlation coefficient. df=degrees of freedom for r. s=unexplained variability.

<b>St</b>	<b>a</b>	<b>±SE</b>	<b>r</b>	<b>df</b>	<b>s</b>
<b>Capitulum width vs capitulum height (mm)</b>					
Ca	0.946	0.002	0.969	1705	0.858
Za	0.973	0.003	0.966	684	0.898
Sa	0.903	0.006	0.945	129	1.408
PF	0.878	0.004	0.962	342	0.800
<b>Total wet weight vs total dry weight (g)</b>					
Ca	2.08	0.013	0.971	748	0.1396
Za	2.158	0.032	0.947	265	0.269
<b>Sa</b>	<b>6.244</b>	<b>0.217</b>	<b>0.943</b>	<b>101</b>	<b>0.386</b>
PF	2.116	0.049	0.975	39	0.103
<b>Ash free dry weight vs Total dry weight (g)</b>					
Ca	0.201	0.001	0.964	788	0.015
Za	0.214	0.002	0.983	227	0.014
Sa	0.208	0.001	0.971	44	0.016
PF	0.209	0.006	0.953	26	0.012

**Table 2.2**

Wet weight (g) versus capitulum height (mm) for *P. pollicipes* populations from 4 sites on the Algarve coast. St= site; Ca - Castelejo, Za - Zavial, Sa - Sagres, PF - Ponta-da-Fisga. Parameters a and b from  $Y=aX^b$ . SE=Standard Error (g). t=comparison for allometry 3 minus b divided by SE. r=correlation coefficient after Log/Log transformation. n=number of observations.

St	a+SE	a-SE	b	±SE	t	r	n
Ca	0.46	0.50	2.85	0.0007	214.29*	0.94	1039
Za	0.0005	0.0006	2.95	0.0027	18.51*	0.94	284
Sa	0.96	1.32	2.68	0.0043	74.42*	0.90	212
PF	0.59	0.79	2.83	0.0089	19.10*	0.97	60

\* significance

There is noticeably little variation in parameters between sites when the monthly samples are combined as in Tables 2.1 and 2.2. The capitulum widths are between 88% and 97% of the capitulum heights and unexplained variability (s) was similar at all sites, with the variability in Sagres being greater than the rest reflecting the wider size range of organisms measured (variability greatly increased with animal size). The dry weights of the barnacles from Castelejo, Zavial and Ponta-da-Fisga are between 46% and 48% of their wet weights. Strangely, when for all other relationships the relative growth of barnacles from Sagres differs little from the relative growth of animals from other sites, the dry weight of the *Pollicipes* from Sagres was only 15% of their wet weight. The ash-free dry weight is 5% of the dry weight in barnacles from all four sites, with *Pollicipes* from Castelejo with a slightly lower organic weight relative to organic plus inorganic weight than animals from other sites. Wet weight was negatively allometric relative to capitulum height in all four populations (see Table 2.2). These results show that capitulum height for all sites grows faster than the increase in wet weight. Within the size range of organisms measured, the estimated wet weight of a 25mm capitulum height barnacle at Zavial, Sagres or Ponta-da-Fisga ranges between 6.3-7.3 g. Those at Castelejo have the

lowest estimated wet weight, for a 25mm capitulum height 4.6g, despite the second highest exponent (see Table 2.2). The influence of the interaction between constant and exponent in determining estimates from power functions is clear from Table 2.2 where the lowest exponent is associated with the highest constant from the Sagres sample. In despite the fact that the overall sample data corresponded largely to a linear or isometric pattern the samples taken in individual months often showed some tendency to depart from linearity. The monthly samples of the wet weight/dry weight and ash free dry weight/dry weight relationships were thus analysed as power functions (through logarithmic transformation) with curves fitted by least squares regression. The slopes of the lines for the relationship between capitulum width/capitulum height and the curves of the former relationships are plotted as a deviation from the appropriate isometric value of  $b$  for each set of variables (Figs 2.5-2.9). The slopes of the relationship of capitulum width versus capitulum height varied between 1.1 and 0.8, with the largest differences from isometry occurring from June throughout September (see Fig. 2.5). One would not perhaps expect to observe monthly changes in shape for individuals at one site. However, a possible explanation could be that there is a change in capitulum width associated with individual *Pollicipes* carrying egg masses. Animals carrying egg masses or an ovary in an advanced stage of development are often bulky in aspect, with the egg masses making the capitulum increase thickness whilst the width decreases. For such an hypothesis to hold the trend of slope departure from 1 for the relationship between capitulum thickness and capitulum width should show a slight positive difference from isometry during the Summer period. Fig. 2.6, shows no such constant trend, although there seems to be an increase in departure from isometry for animals at Castelejo in July, August and September, and for animals at Zavial in August.

The slopes of the log wet weight versus log capitulum height vary fairly irregularly throughout the year with no constant trend between sites (see Fig. 2.7). Fig. 2.8 shows the difference from isometry for wet weight vs dry weight and a slightly more consistent pattern of increasingly negative departure from isometry is

seen from July to September but the trend is not consistent between sampling years. Fig. 2.9, departures for organic versus dry weight shows a slight trend for the largest data

set (Castelejo) of negative allometry during spring and summer and positive during winter. The scale of departure, however, is very small and samples from other sites do not show the same trends.

The data for the relationship between each pair of dimensions were compared between sample dates among locality, and between localities, pooled data from all sites. There were significant differences on the slopes of all the relationships using analysis of covariance in the general linear model (GLM) (Table 2.3).

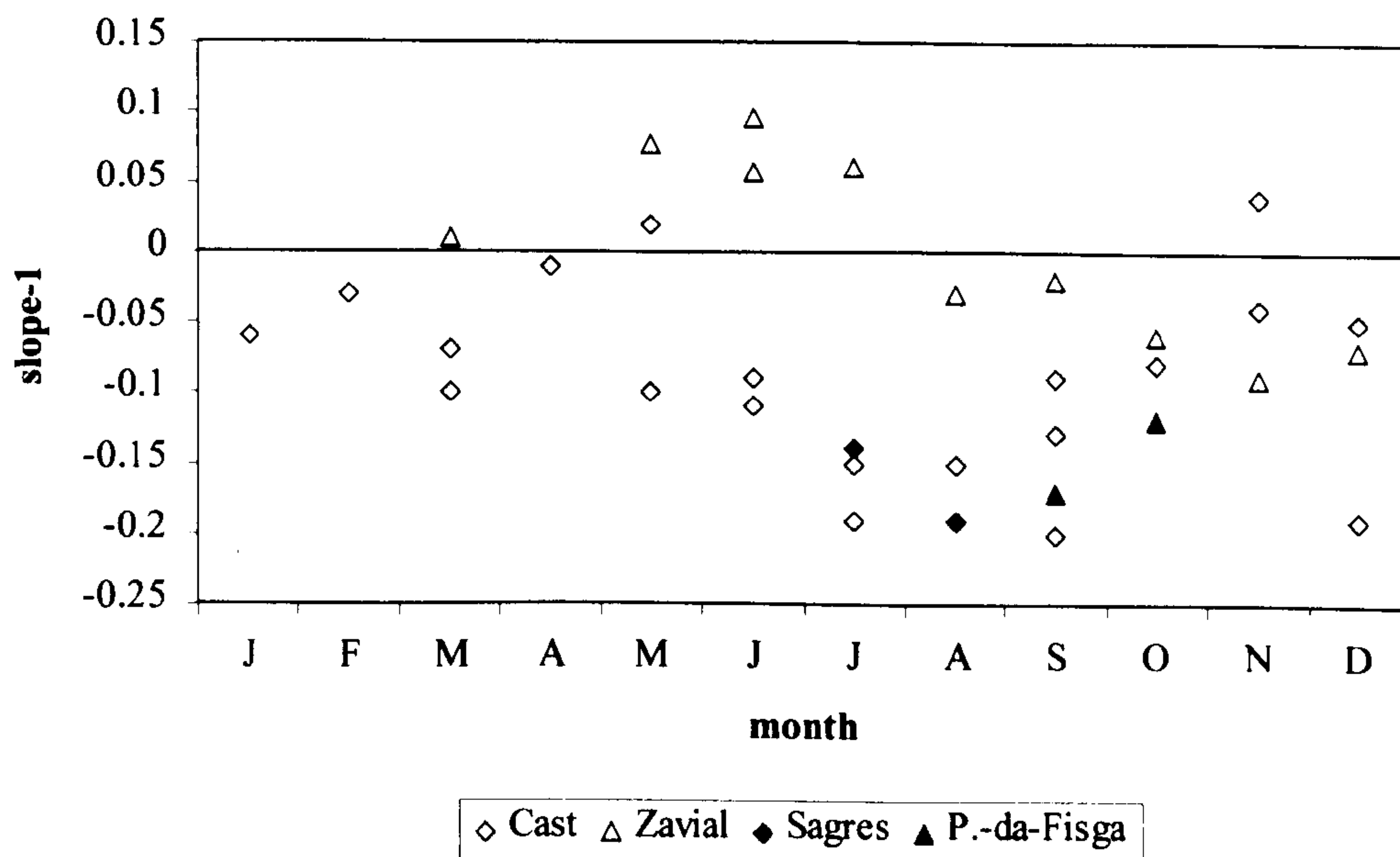


Fig. 2.5 Change in the slope, calculated from the difference from isometry, of the simple linear regression capitulum width/capitulum height for *P. pollicipes* throughout the year at four sites Castelejo, Zavial, Sagres and Ponta-da-Fisga.

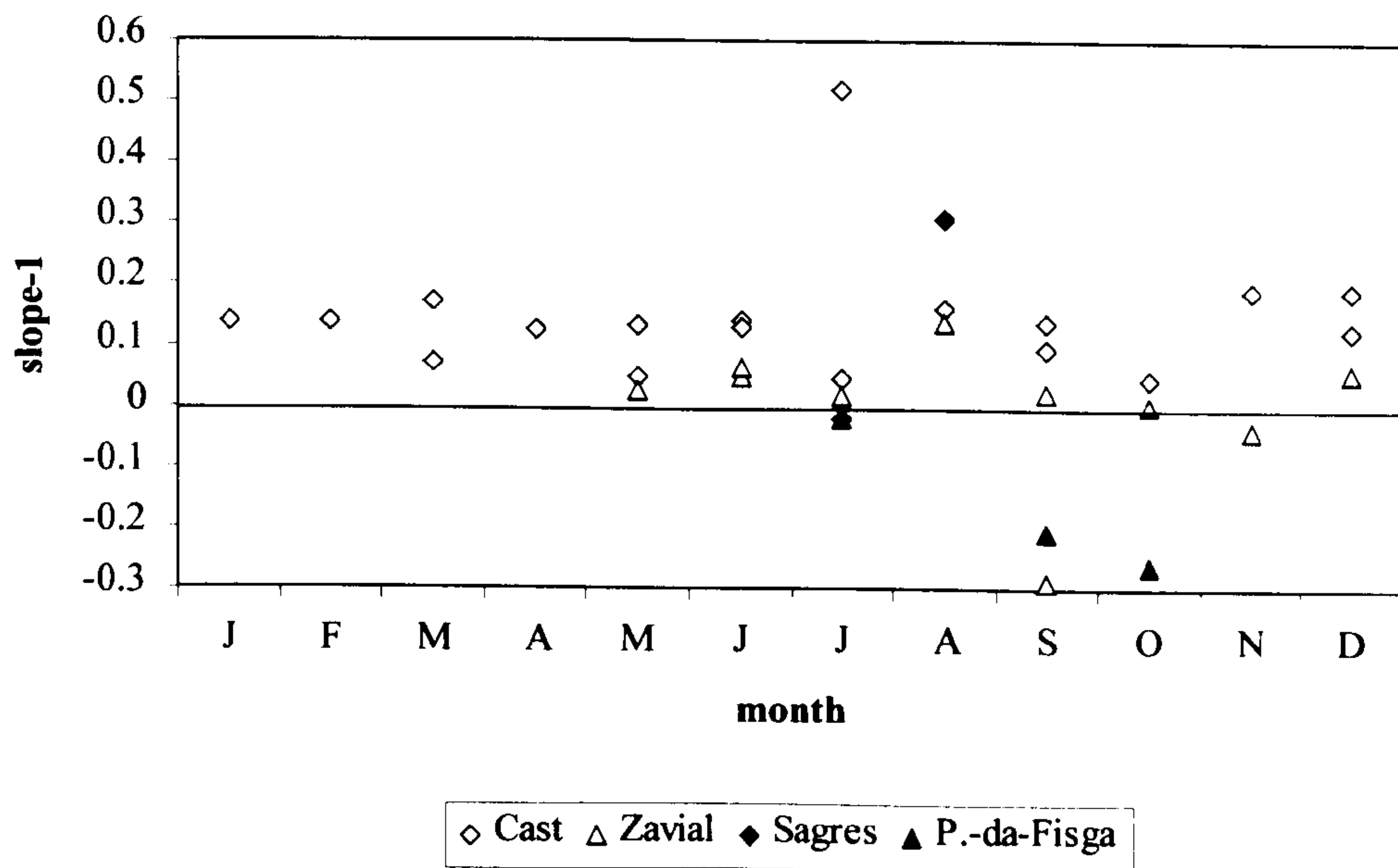


Fig. 2.6 Change in the slope, calculated from the difference from isometry, of the simple linear regression log capitulum thickness/log capitulum width for *P. pollicipes* throughout the year at four sites Castelejo, Zavial, Sagres and Ponta-da-Fisga.

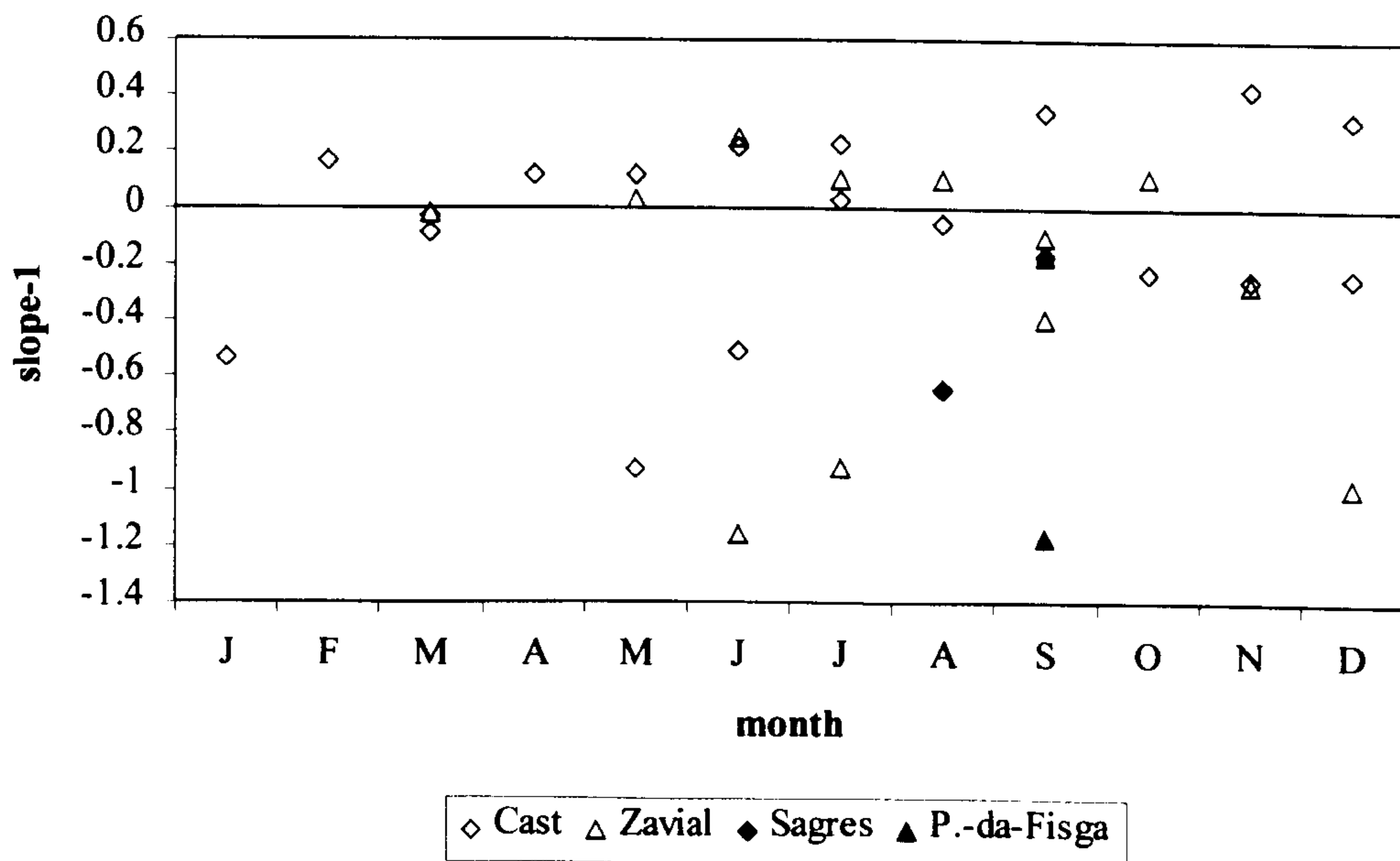


Fig. 2.7 Change in the slope, calculated from the difference from isometry, of the simple linear regression log wet weight/log capitulum height for *P. pollicipes* throughout the year at four sites Castelejo, Zavial, Sagres and Ponta-da-Fisga.

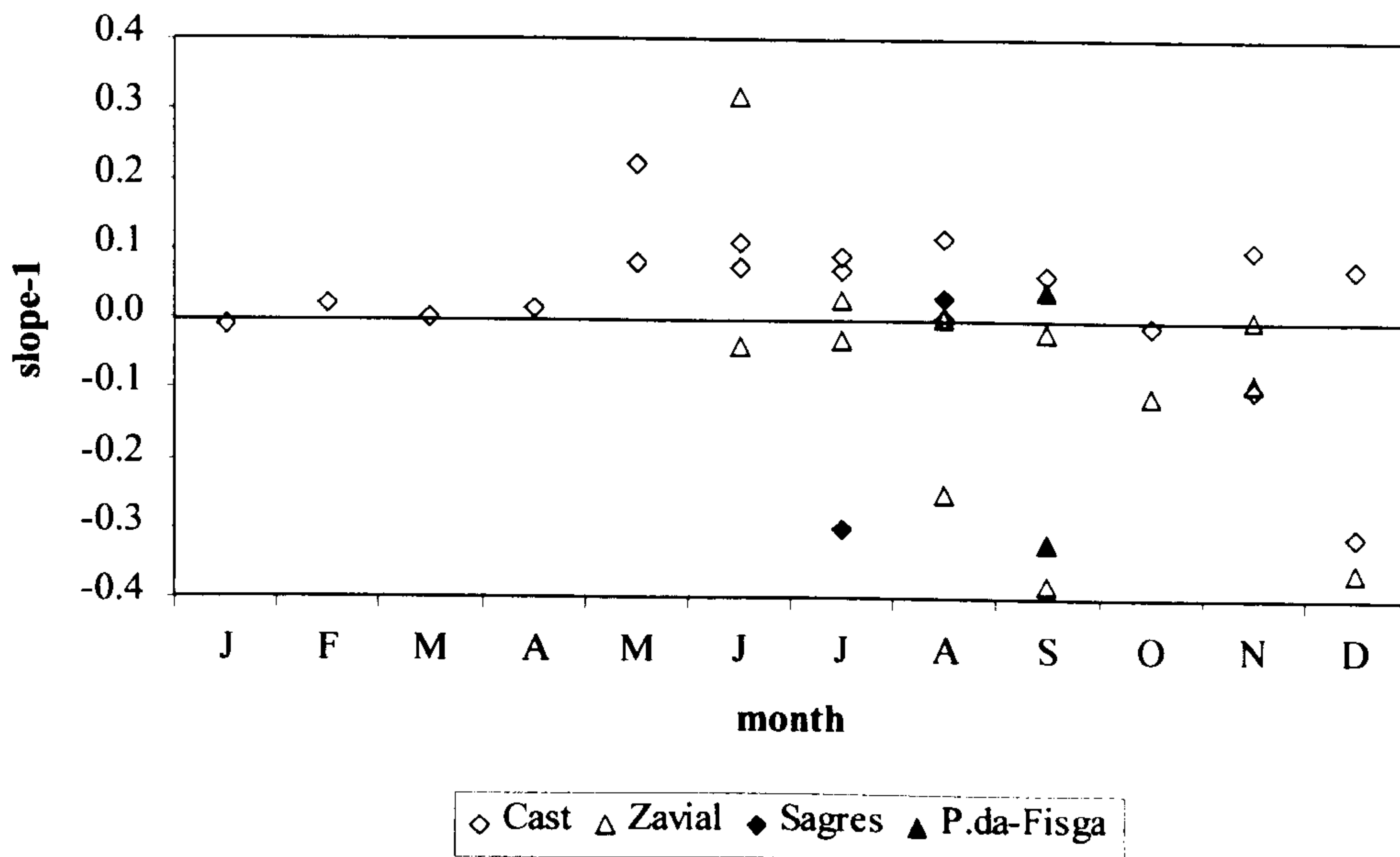


Fig. 2.8 Change in the slope, calculated from the difference from isometry, of the simple linear regression log wet weight/log dry weight for *P. pollicipes* throughout the year at four sites Castelejo, Zavial, Sagres and Ponta-da-Fisga.

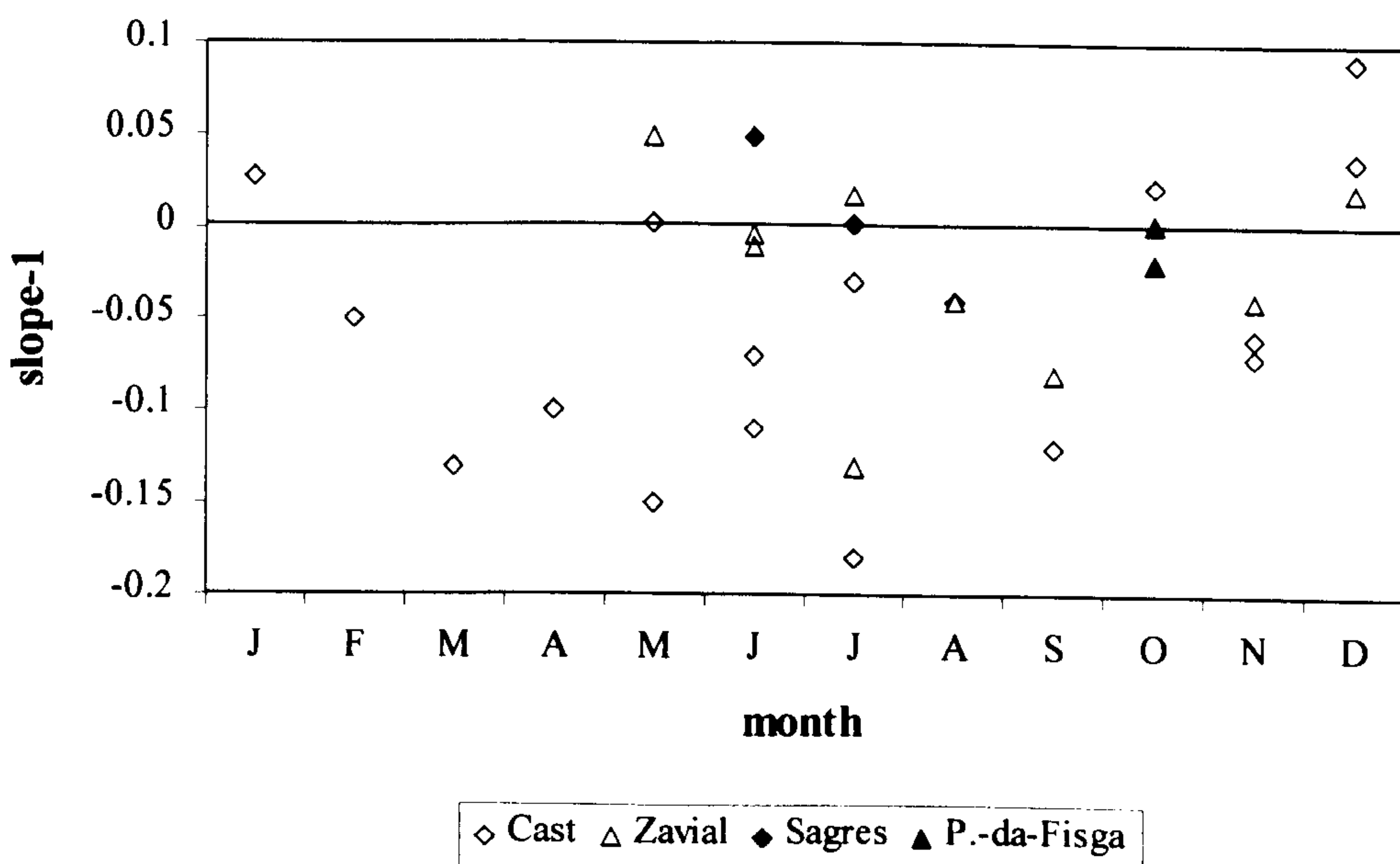


Fig. 2.9 Change in the slope, calculated from the difference from isometry, of the simple linear regression log afdw/log dry weight for *P. pollicipes* throughout the year at four sites Castelejo, Zavial, Sagres and Ponta-da-Fisga.



**Table 2.3**

Anova results on the pooled data from all sites for the several combinations of size parameters in *P. pollicipes*. F=variance ratio of the interaction term and P=its associated probability. df=degrees of freedom.

Independent variable	Dependent variable	Pooled data from sites		
		F	P	df
Height	Width	34.85	<0.001	3,2860
Log Height	Log Wet weight	4.57	< 0.001	3, 1647
Log Dry weight	Log Wet weight	20.97	<0.001	3.1154
Log Dry weight	Log AFDW	6.11	<0.001	3,1081

## 2.4 DISCUSSION

The relative growth of four populations of *P. pollicipes* from Castelejo, Zavial, Sagres and Ponta-da-Fisga was studied through the relationships between two linear parameters (capitulum width and capitulum length) and three weight measurements (wet weight, dry weight and ash free dry weight). The biometric variable that was considered to describe the linear growth in *P. pollicipes* was capitulum height, although Cruz (1993) has proved that the rostro-carinal dimension (capitulum width in this study) best represented the linear growth, and thus was chosen to be related to the other variables.

The sites from where *P. pollicipes* were collected for this study differ in their environmental conditions, with the greatest differences between Castelejo and Zavial (see description of the sites in general materials and methods). Castelejo, located on the west Portuguese coast facing the open expanse of the North Atlantic, is subject to intense wave exposure, while Zavial faces south and is generally more sheltered. The SW coast is influenced by cold, nutrient rich, upwelling water during the summer months (usually between May or June and October). On the west coast the upwelling is almost a permanent feature through the Summer but its effect is much more

variable on the south coast where local wind conditions dominate the general current movements.

Different immersion times, food quantity and light intensity have been suggested as possible reasons for differential growth in *P. pollicipes* from Cabo de Sines and Cabo Sardao (CRUZ, 1993), two sites to the north of the sites from where the barnacles for the current study were collected. The analysis of the morphometric and gravimetric power functions on a spatial and temporal basis, reflect minor differences in shape and weight of *P. pollicipes* with site and season. Yet, these are largely the result of the large sample size which result in significant differences because the standard errors are so small; whether these differences are of any biological significance is questionable. The relationships determined for several combinations of dimensions proved largely isometric, with dimensions growing proportionally. Although, as the relationships between the different variables showed differences between localities no single function provides an adequate estimator of weight from height from the various sites.

The extremely low value of dry weight relative to wet weight for barnacles from Sagres requires some explanation. If one takes into consideration that only 20% of the dry weight of a barnacle is organic weight, then of the weight of barnacles from Sagres only 3% is organic weight and 85% of the body is water. However, the data from Sagres is the only data from 1991, and this slope value either reflects a real biological difference between barnacles here and elsewhere during that year only or in all years or a systematic error caused by an unobserved technical problem with the measurement scale.

The comparison of the monthly samples amongst Castelejo and Zavial for the relationship capitulum height versus capitulum width displayed significant differences, which are difficult to explain, as no differences in shape from season to season within a particular locality seems plausible. Although, there is a slight increase in the difference from isometry of the relationship log capitulum thickness versus log capitulum width which may partially explain such differences. Once again sample sizes are large and minor differences exaggerated.

The differences in slopes of the weight relationships could be explained either in terms of breeding or food availability. Differences in body weight due to breeding

condition have been described by several authors (*e.g.* BARNES *et al.*, 1963; CRISP and PATEL, 1961). On the other hand, the body weight has been found to reflect plankton abundance as storage materials (BARNES *et al.*, 1963; BARNES and BARNES, 1968). Yet, here in this study the body weights fluctuated erratically throughout the year, and maybe, as Hines (1978) has suggested differences in the body weights may reflect differences other than stored nutrients, or breeding activity, for example the quantity of material in the gut.

### **CHAPTER III: ABSOLUTE GROWTH IN *POLLICIPES POLLICIPES***

### 3.1. INTRODUCTION

Growth rate can be determined in one of two ways: 1) either the size of the whole organism is related to age (=absolute growth), or 2) the rate of growth of one parameter is related to that of another (=relative (allometric) growth) (SEED, 1980).

The absolute growth and age of an organism can be estimated both by direct and indirect methods. The direct methods are based on: a) direct observations of growth in captivity (*e.g.* BARNES and POWELL, 1953; BARNES, 1956; CRISP, 1960; GOLDBERG, 1984); b) mark recapture experiments in the wild (*e.g.* BRETOS, 1978; SCHIEL and BREEN, 1991; SCHNUTE, 1992) and, c) examination of growth recorded in hard structures (*e.g.* BRETOS, 1980; BERNARD, 1988; ANWAR *et al.*, 1990; RICHARDSON and WALKER, 1991; RAMON and RICHARDSON, 1992; RICHARDSON *et al.*, 1993).

The former two methods involve the measurement at known intervals of either free-living marked animals or caged animals. In sessile animals both methods are equivalent. Direct measuring methods are also used to determine growth rates of animals under experimental conditions (*e.g.* BARNES and POWELL, 1953). The third method is based in the use of growth “checks” recorded in the animal's skeleton (*e.g.* SEED, 1969; RICHARDSON *et al.*, 1980). A wide range of different sized individuals can be categorised into age groups according to the number of clear growth checks. Assuming that the origin/periodicity of the “checks” is known weight or age curves can thus readily be derived. Alternatively, back calculations from the patterns in the skeletal structure completed with adequate length/weight relationships can provide adequate growth estimates (references are as above).

The indirect methods are based on the analysis of sequential length frequency distributions for the population. Size frequency histograms have been widely used to estimate population growth rates (*e.g.* HOFFMAN, 1988; FRANCE *et al.*, 1991), despite several serious deficiencies in the method. The method assumes that obvious modes in a length frequency diagram approximates to the mean size of year class. The progression of the modes along the size axis over successive time intervals represents growth of the year class. The average growth rate can then be determined from a plot of modal size against time.

The technique can work well when recruitment to the population occurs as single, perhaps annual, event leaving an identifiable large 0 age class to follow in successive time periods. The intervention of mortality with time often leads to imprecision in assigning year classes to the older age groups when identifiable modes are not existent. When recruitment is sporadic or virtually continuous throughout the year the method becomes difficult to employ. Length frequency analysis should not be used in the complete absence of information on growth and recruitment patterns, or with very small data sets (CASTRO and ERZINI, 1988). However, it can be a useful tool in conjunction with other methods.

A limited number of studies reporting on the growth and the effects of various factors on growth are available for barnacle species (*e.g.* BARNES, 1956; CRISP, 1960; BARNES, 1962; BOURGET and CRISP, 1975a) b); LEWIS and CHIA, 1981; GOLDBERG, 1984; PAGE, 1984, 1986; BERNARD, 1988; BERTNESS *et al.*, 1991; CRUZ, 1993). Ecological interest, fishery potential and the nuisance caused to structures by these fouling organisms have been the driving force behind investigations. Age determination and growth in the genus *Pollicipes* have largely been assessed by length-frequency analysis and through marking the shells of individuals (*e.g.* LEWIS and CHIA, 1981; GOLDBERG, 1984; BERNARD, 1988; CRUZ, 1993). Bernard (1988) claims to have determined the age of *P. polymerus* individuals using the patterns observed in the carinal shell plate utilising methodology developed for fish otoliths.

*Pollicipes pollicipes* is subject to an intense fishery in the southwest coast of Portugal and yet very little is known about its growth. In Galicia, northern Spain, Goldberg (1984) compared the growth rates of *P. cornucopia* (= *P. pollicipes*) in their natural habitats with those continuously immersed, suspended from rafts. Cruz (1993) studied the relative and absolute growth of *P. pollicipes* from Cabo Sardao and Cabo de Sines. However, if regulation is to be imposed to the fishery of this species a better knowledge of its growth is needed.

Local environmental conditions and phenotypic plasticity influence the population and life history characteristics of barnacles. A considerable variation in maximum size and population structure was reported by Page (1986) for two

populations of *P. polymerus* spatially separated but in close proximity which differ in their exposure conditions. The objectives of the study reported in this chapter were: 1) to ascertain and compare the population age and size structure for four sites in the southwest Algarvian coast (Portugal), and 2) to develop a reliable ageing technique for fisheries monitoring and management.

### 3.2 MATERIALS AND METHODS

In order to investigate the growth history of *P. pollicipes* populations in the southwest coast of Portugal large samples of the barnacle were collected from the shores of four sites in the Algarve, Castelejo, Zavial, Sagres and Ponta-da-Fisga in 1991/92/93 (see General Materials and Methods). The capitulum height (see General Materials and Methods) of all the barnacles was measured to the nearest 0.01mm with vernier calipers. Juveniles were measured under a dissecting microscope using a calibrated eye-piece graticule. The biometric dimension that was considered best to describe the linear growth in *P. pollicipes* is the capitulum height (see Chapter2) and thus is used in this study of absolute growth.

#### 3.2.1 Length frequency analysis

The capitulum height data were grouped into 1mm class intervals and the percentage frequency of each size class calculated and plotted for each sample at Castelejo, Zavial, Sagres and Ponta-da-Fisga. In an attempt to estimate modal sizes in the size frequency distributions an analysis was carried out using the computer programme - LFDA - which was developed under the fish management science programme of the Overseas Development Administration (N.R.A.G. LTD, U.K.).

Because of resource-limiting environmental conditions, a habitat usually imposes a maximum size beyond which further growth proceeds slowly, if at all. In fast growing individuals this asymptotic limit is approached relatively quick, whilst in areas of slow growth this limit may only be approached in much older individuals. Quantitative expressions of growth include the Von Bertalanffy and Gompertz equations. In the Von Bertalanffy equation:

$$L_t = L_\infty [1 - e^{-k(t-t_0)}]$$

where  $L_t$  represents the length of an animal at time  $t$ ;  $L_\infty$  is the maximum asymptotic length;  $k$  is the growth constant reflecting the rate at which maximum size is approached and  $t_0$  is a constant representing the theoretical time when  $L_t=0$ .

Three different methods, under the LFDA package, were applied to the analysis of the *P. pollicipes* capitulum height frequency distributions, these are the Shepherds method, the PROJMAT method and the ELEFAN method. Common to all methods is to consider that growth is described by the Von Bertalanffy growth equation.

The Shepherd's method (SLCA) compares each observed length frequency distribution with a length frequency distribution that would be expected for given values of the Von Bertalanffy growth parameters  $L_\infty$  and  $k$ . A goodness of fit score is then calculated using a certain test function (SHEPHERD, 1987). Large positive values of the goodness of fit score function indicate that the expected length frequency distribution matches well with the observed data. The best estimates of  $k$  and  $L_\infty$  are those that correspond with a maximum value of the score function. The basic idea behind the PROJMAT (Projection Matrix Method) method is to successively project one observed length frequency distribution forward in time, based on the assumed set of Von Bertalanffy growth curve parameters, to obtain a prediction of what that length frequency distribution should have looked like at the time the second observed length frequency distribution was collected. The goodness of fit of the observed and predicted distribution is then compared and the best estimates of the growth parameters are those that had the best fit between the two distributions. The ELEFAN method is based on the method devised by Pauly (1987). It works by 1st restructuring the length frequency data in a way that emphasises the peaks and troughs in the data, and then calculates a score function of the proportion of available peaks and troughs that can be explained by a Von Bertalanffy growth curve with specified parameters.

### **3.2.2 Shell Growth**

The examination of acetate peel replicas of polished and etched radial sections of the tergal shell plate of *P. pollicipes* from experiments (see Chapter 1, Plate 1.2) reveal a



series of distinct growth bands which were ascertained to be deposited tidally. The patterns in the shell were then used to age individual barnacles.

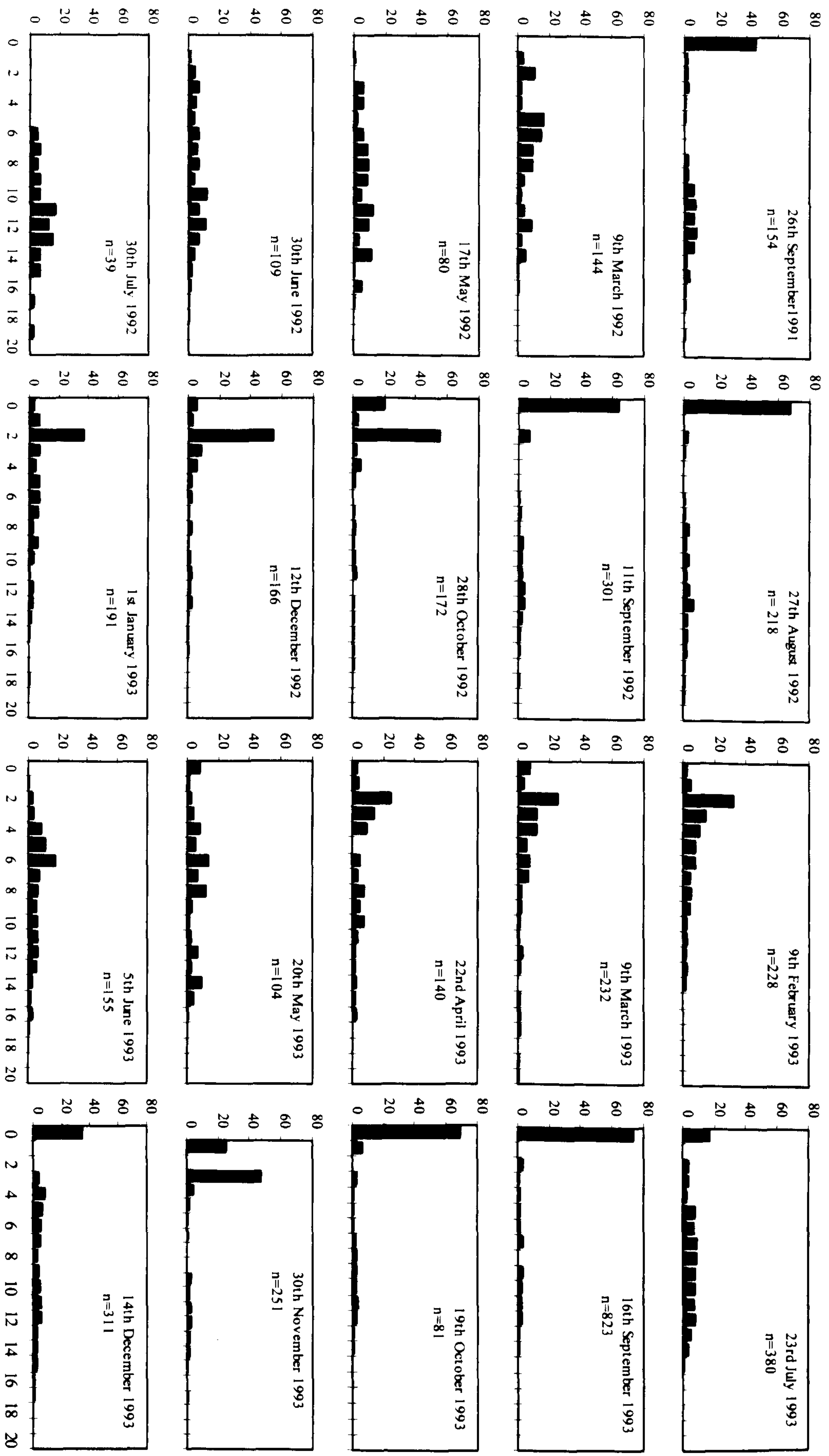
Sub-samples of barnacles belonging to different capitulum height groups and covering small to large individuals were taken from each month sample of the barnacles from Castelejo and Zavial. One of the tergal plates was removed and cleaned, and its height (from base of insertion in the capitulum tegument to the plate tip) recorded. Acetate peel replicas of sectioned, ground, polished and etched surfaces were then prepared (see General Materials and Methods). Acetate peels were examined under a low power objective (x10) in a transmitted light microscope. In order to quantify whether there were any seasonal variation in band separation, the number of bands in the most recently deposited 0.5mm of shell from the tip of the plate was counted. An analysis of the entire shell was then performed to search for any regular growth “checks” along the length of the tergal plate.

### **3.3 RESULTS**

#### **3.3.1 Analysis of size-frequency distributions**

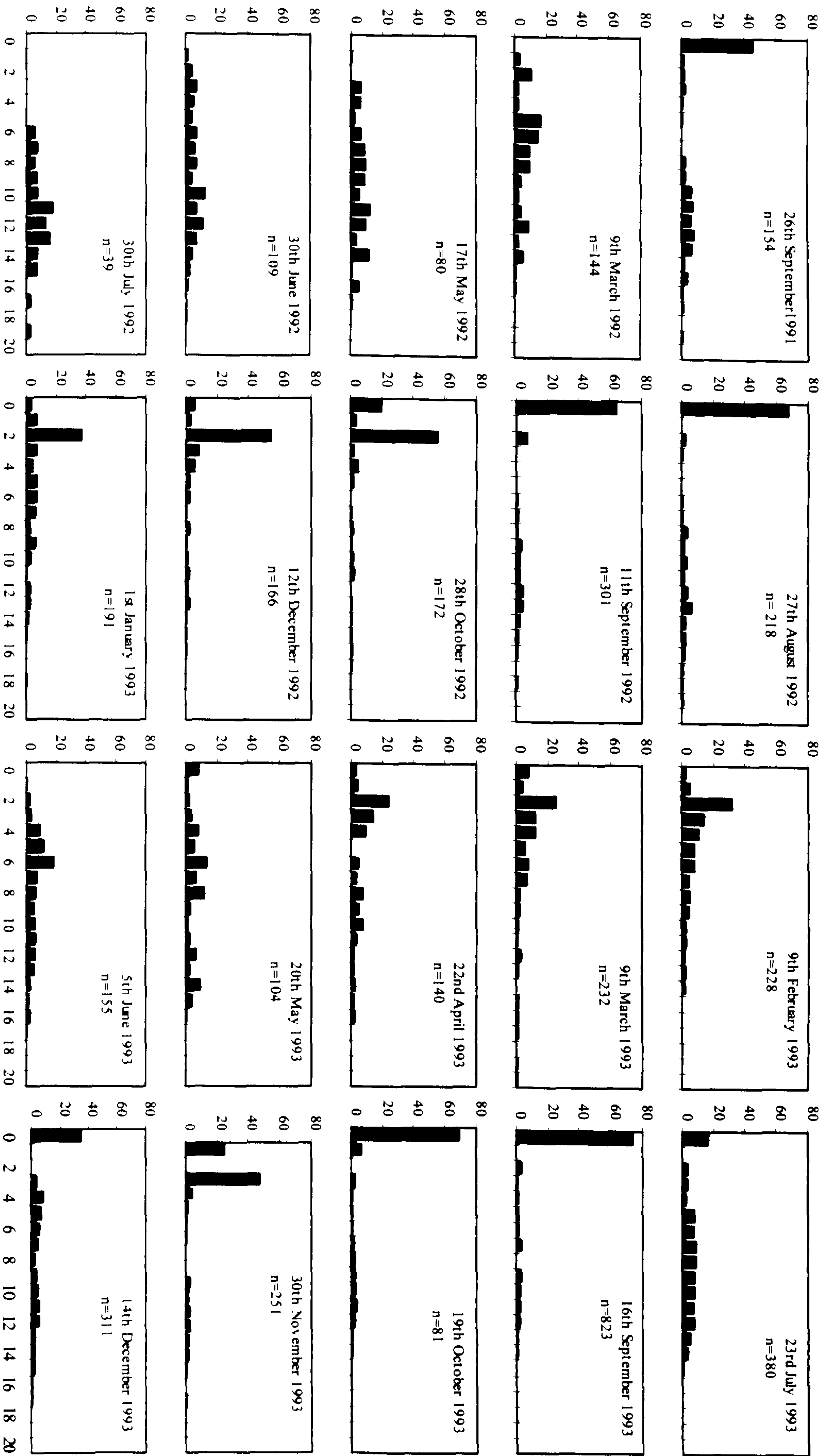
Capitulum height frequency histograms of *P. pollicipes* from the four studied populations are shown in Figs 3.1 to 3.4. and no obvious modes can be observed within each population. The most striking feature in the population structure of *P. pollicipes* from all sites is the positive skewness of the histograms for most monthly samples due to an almost year round recruitment. When there was no new recruits to the populations there seemed to be no differences in the size structure from month to month samples. The maximum size attained for barnacles from Castelejo and Zavial was 20mm capitulum height, with few barnacles larger than 16mm capitulum height. The barnacles from Ponta-da-Fisga attained a maximum size of 16mm capitulum height, whereas the barnacles from Sagres reached the maximum size of 24mm capitulum height and the capitulum height frequency histogram of the population in August shows a slightly negative skewness (Fig 3.4). *P. pollicipes* population from Sagres in August seemed to have slightly larger barnacles than during July.

The three LFDA methods used to determine the growth parameters from the Von Bertalanffy equation and to age the populations were totally unable to



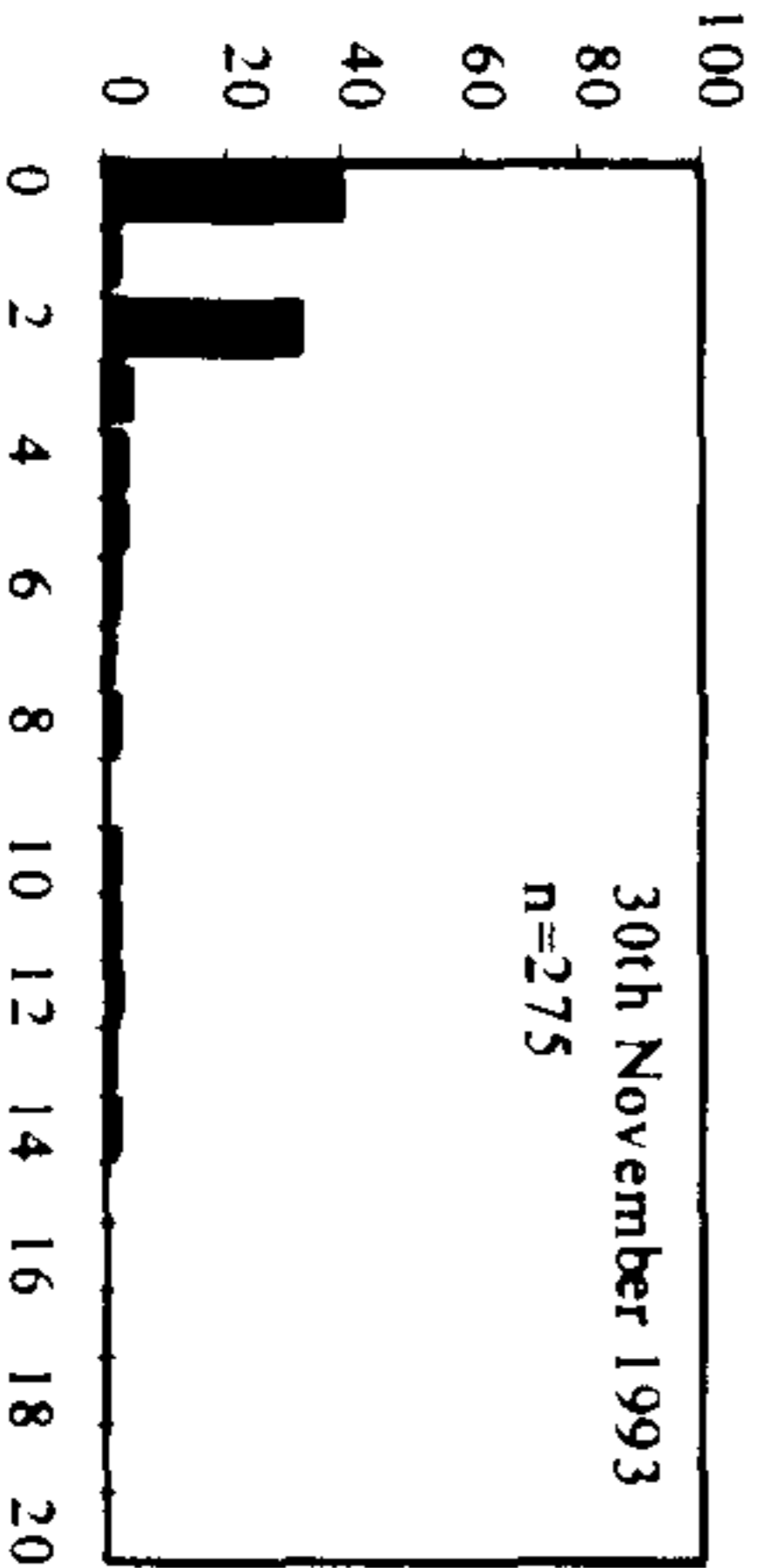
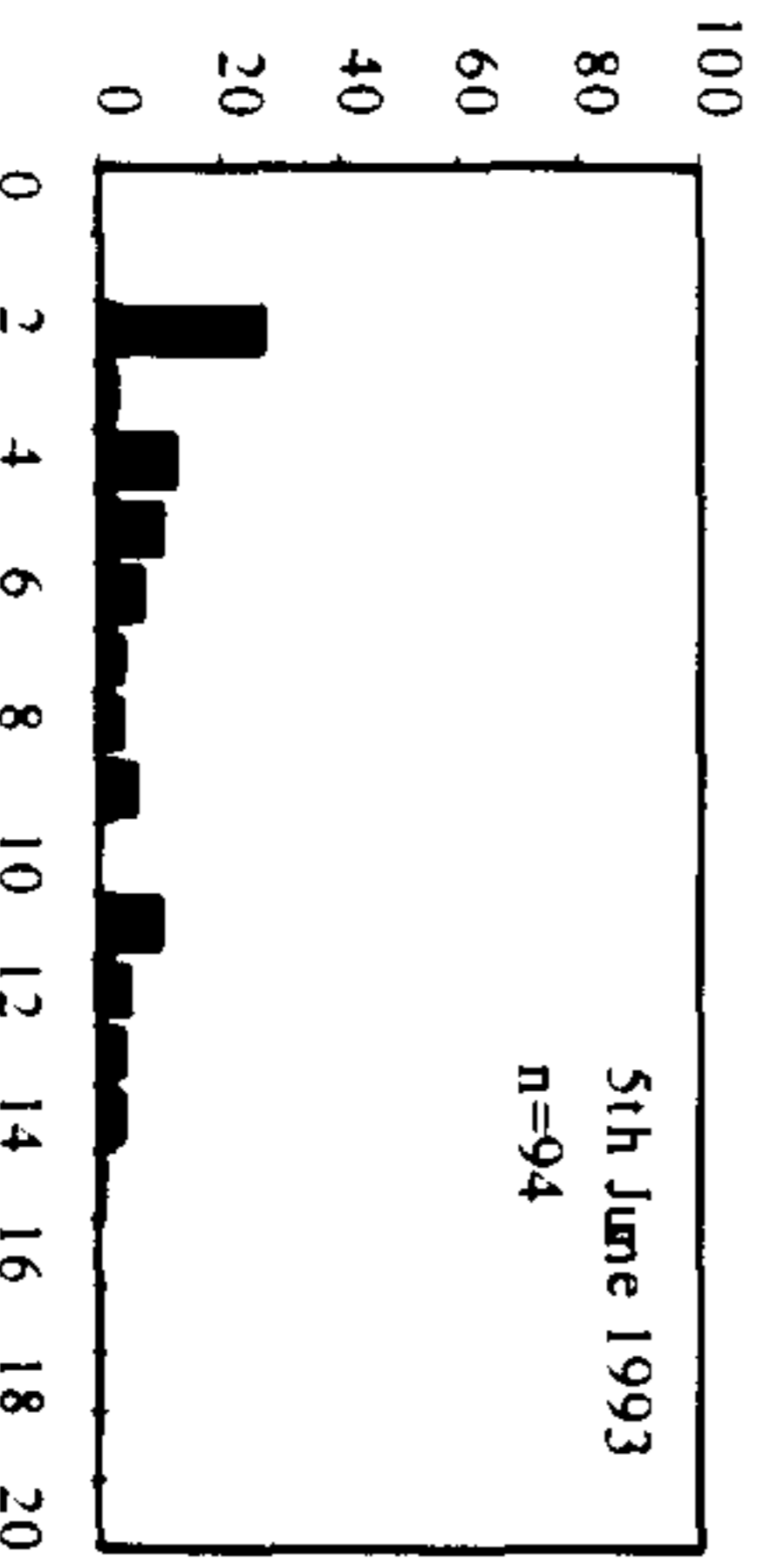
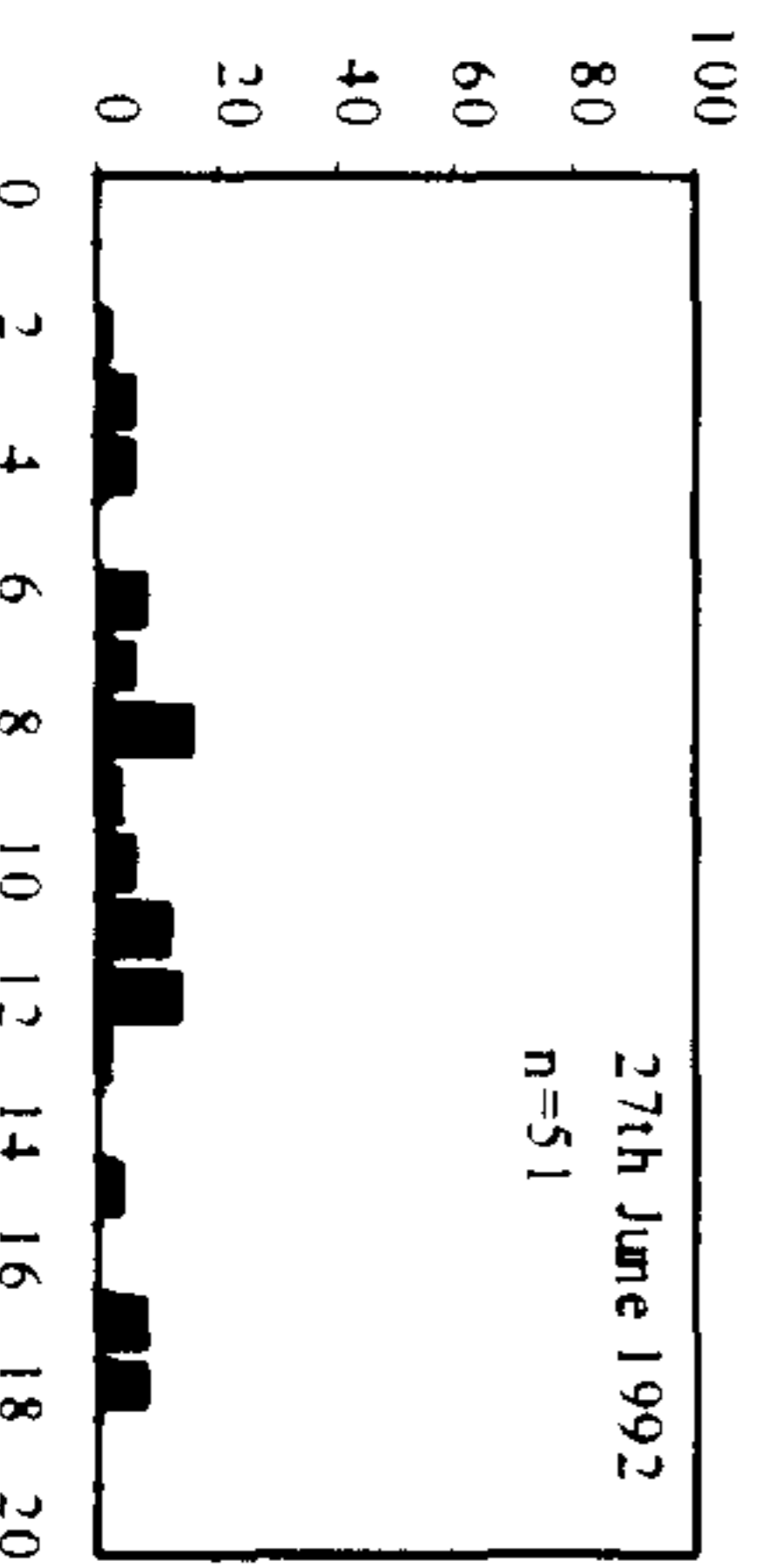
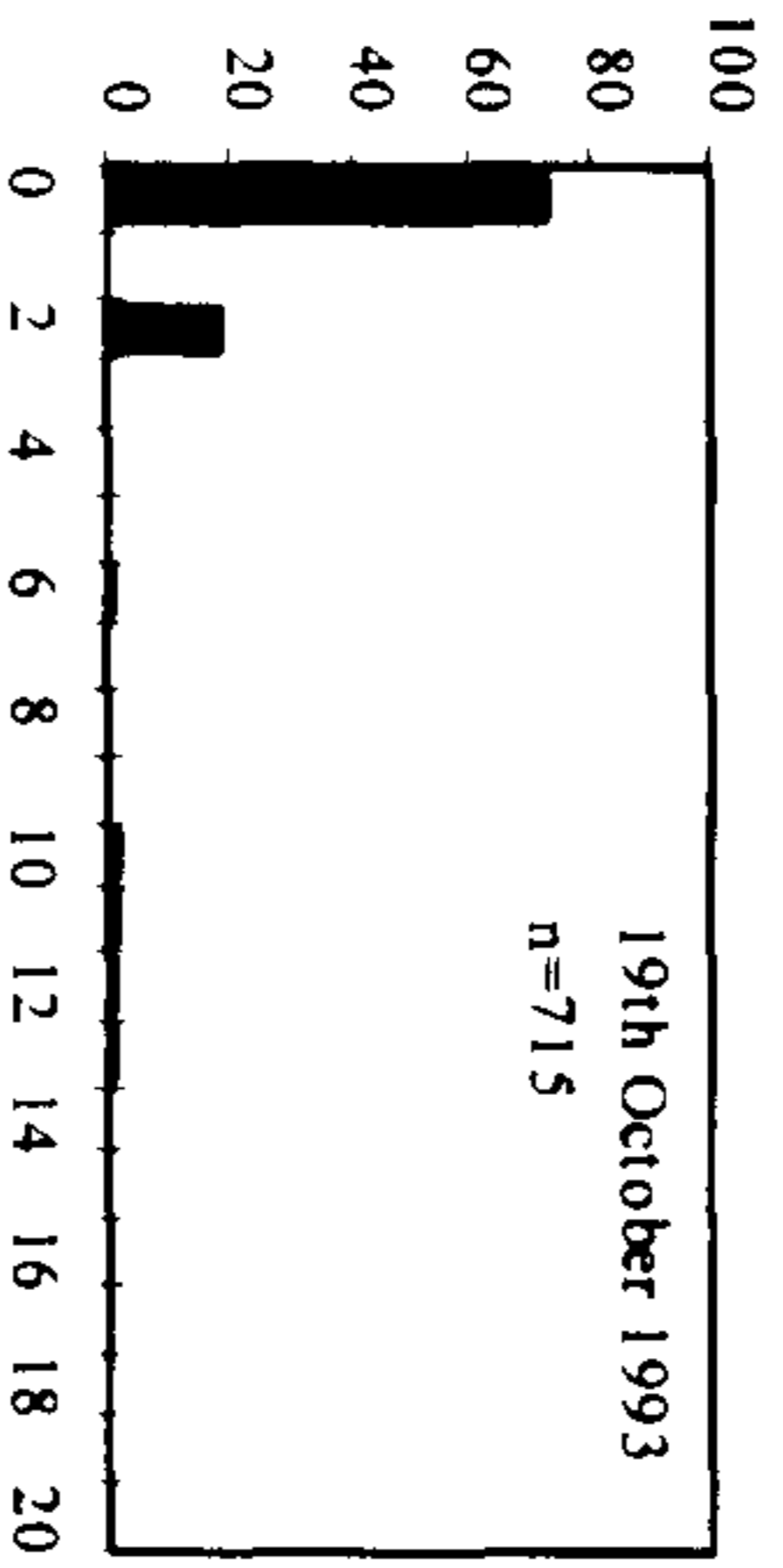
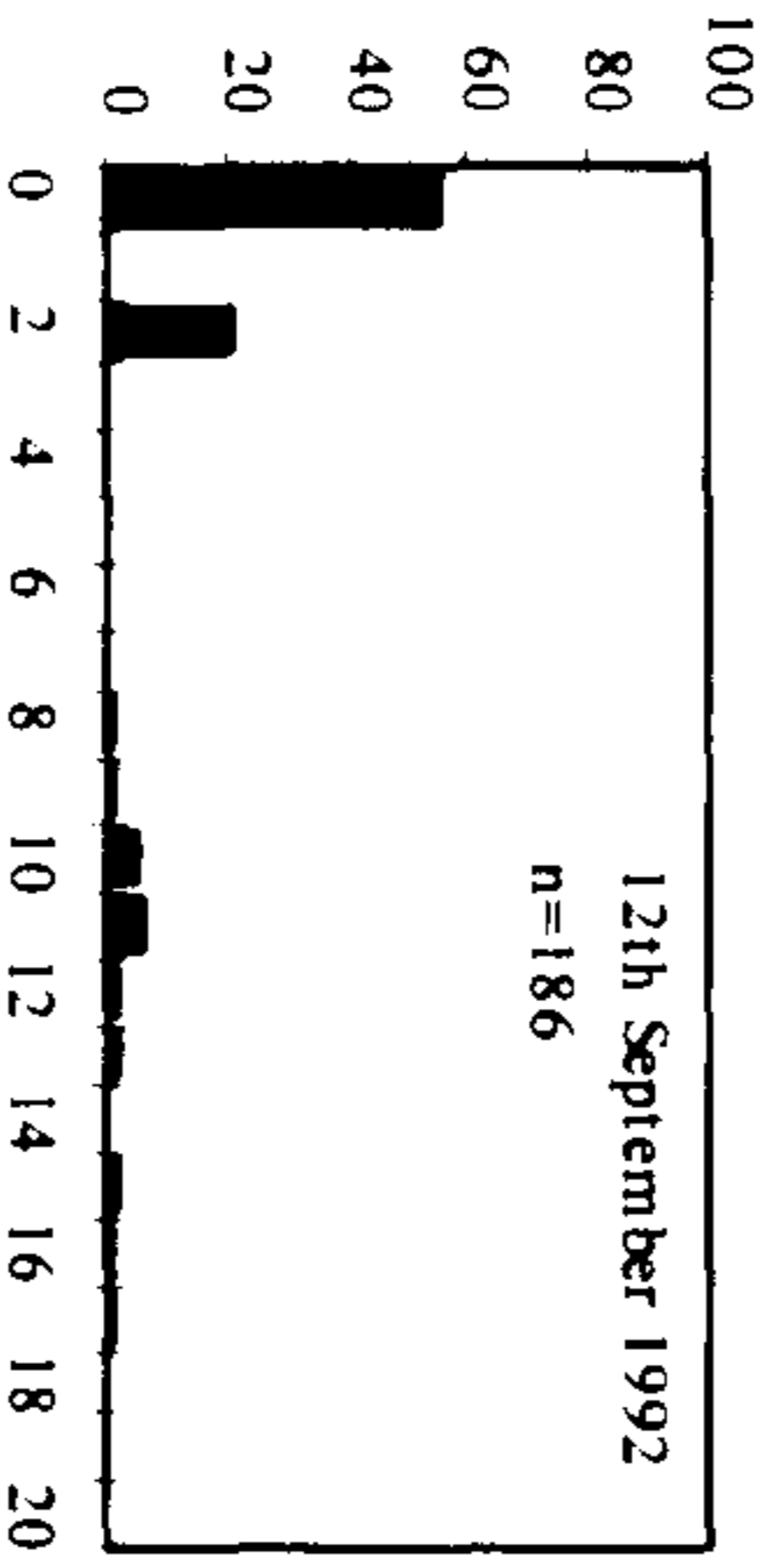
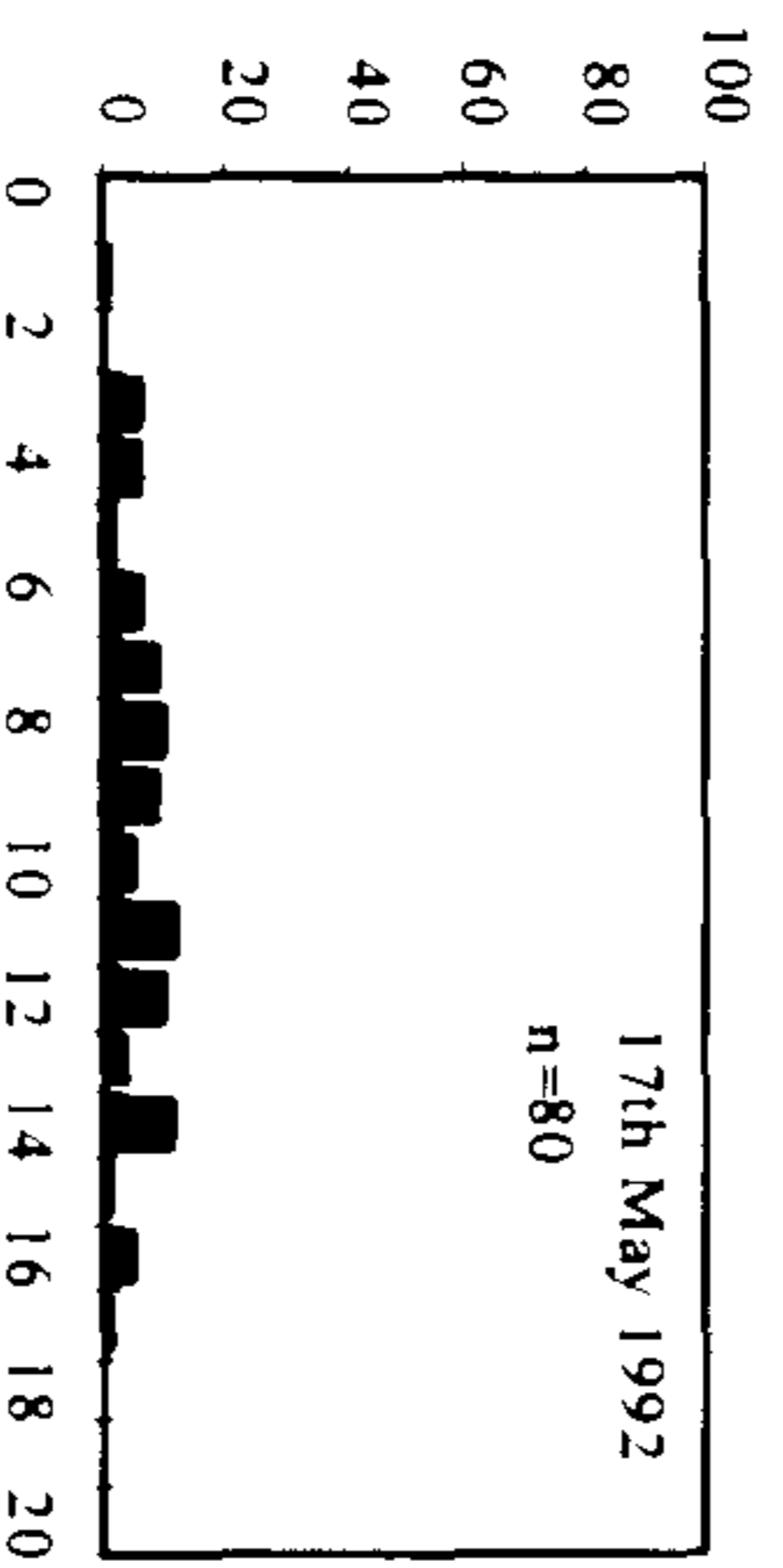
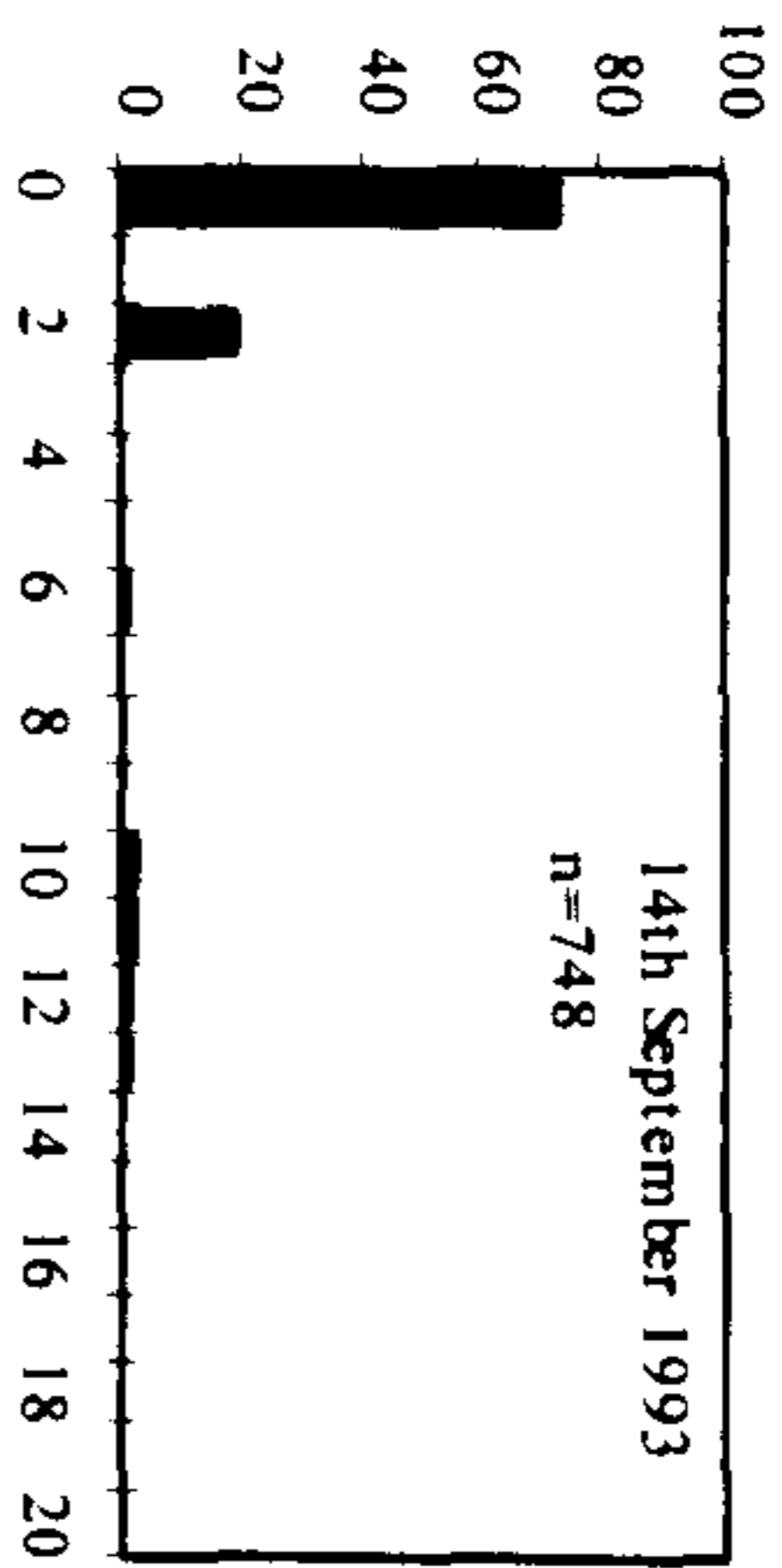
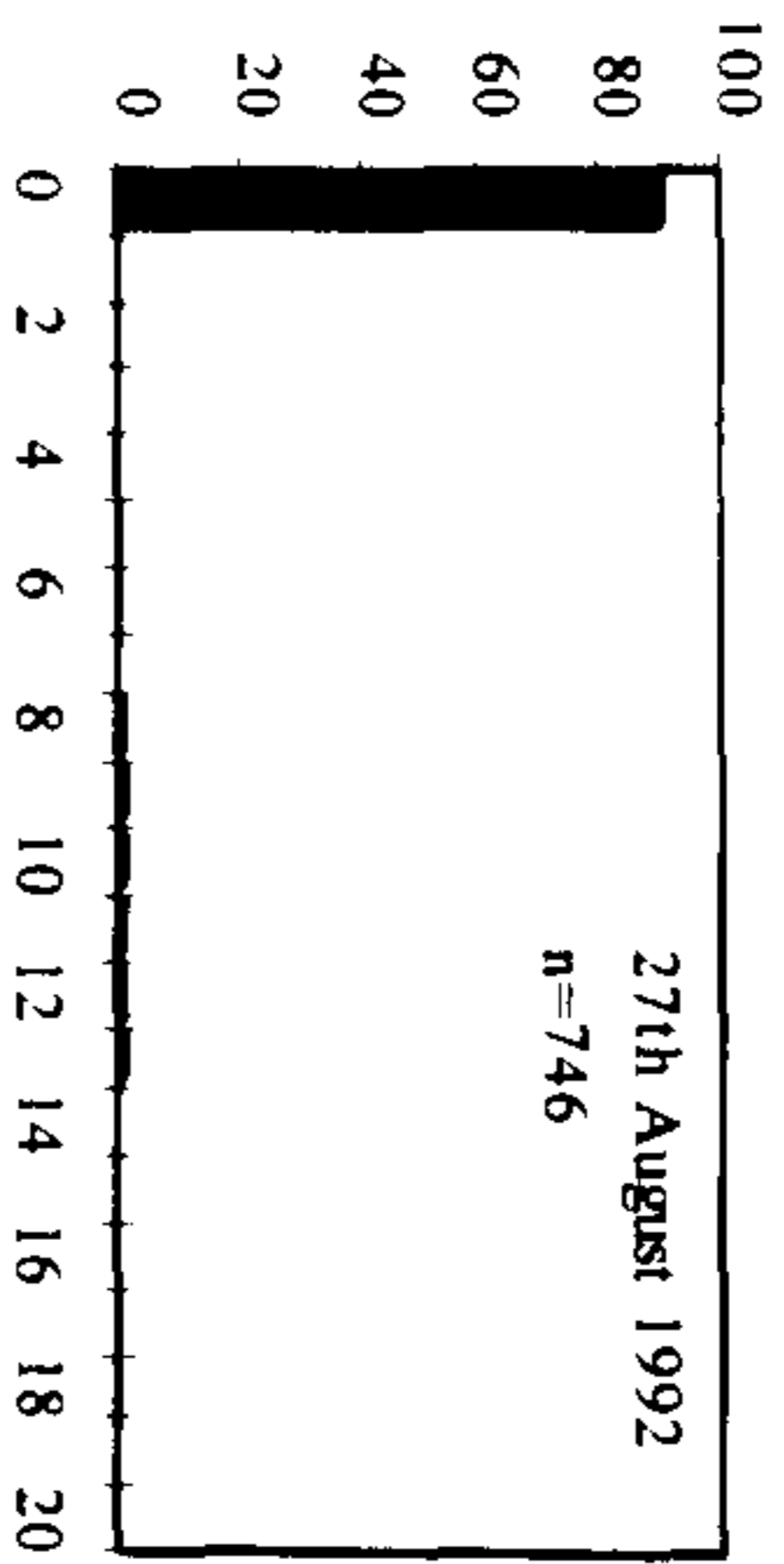
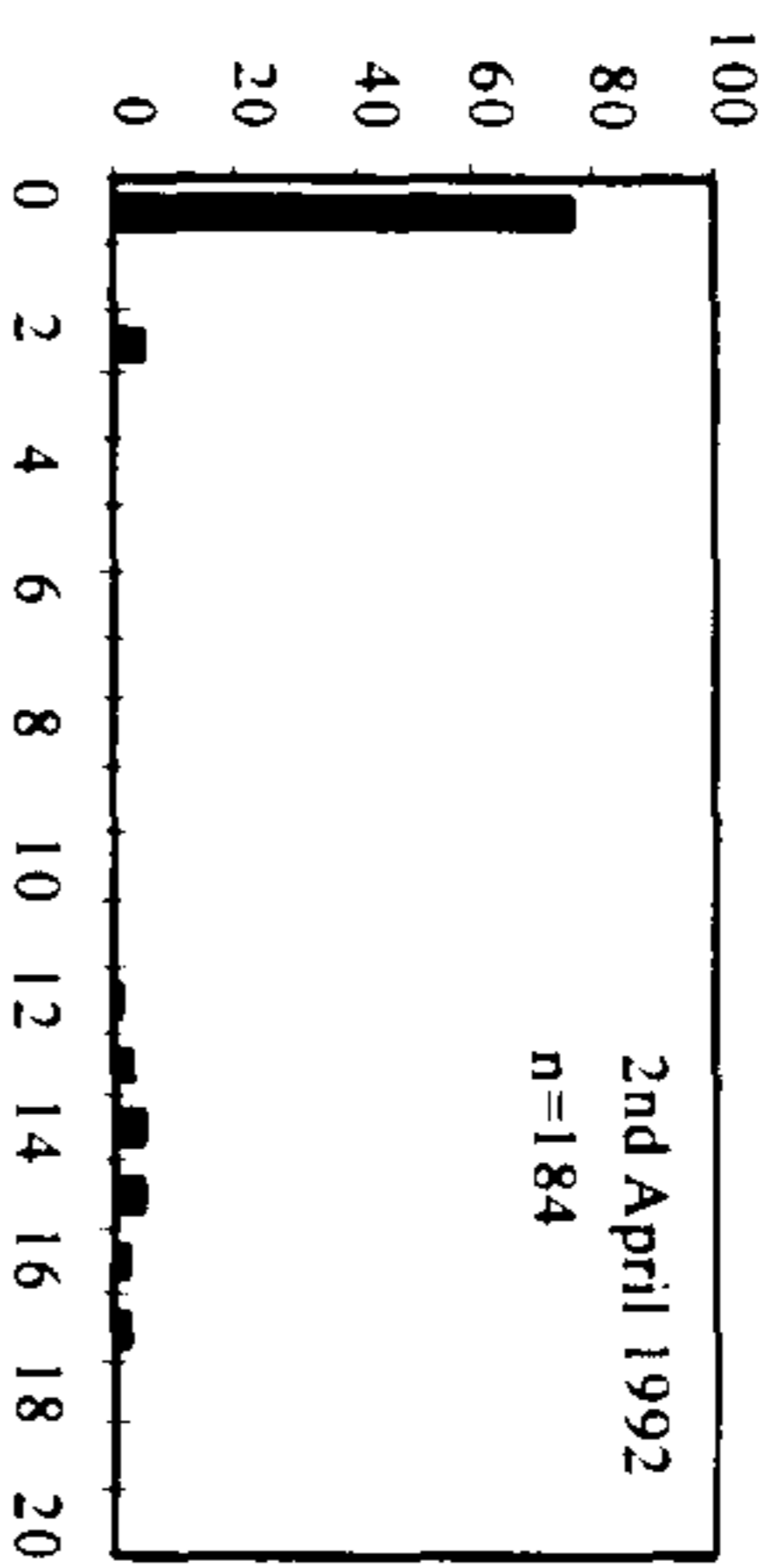
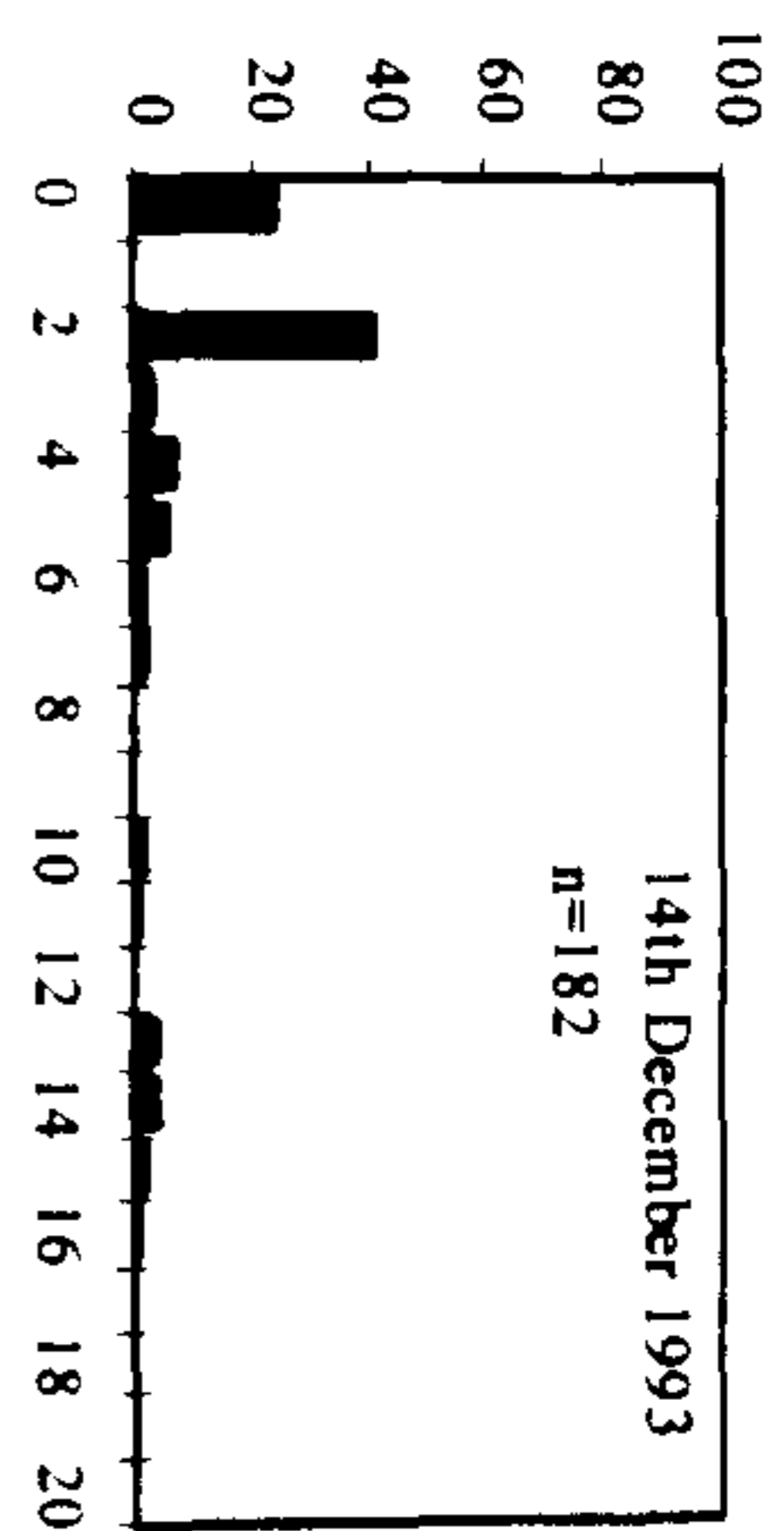
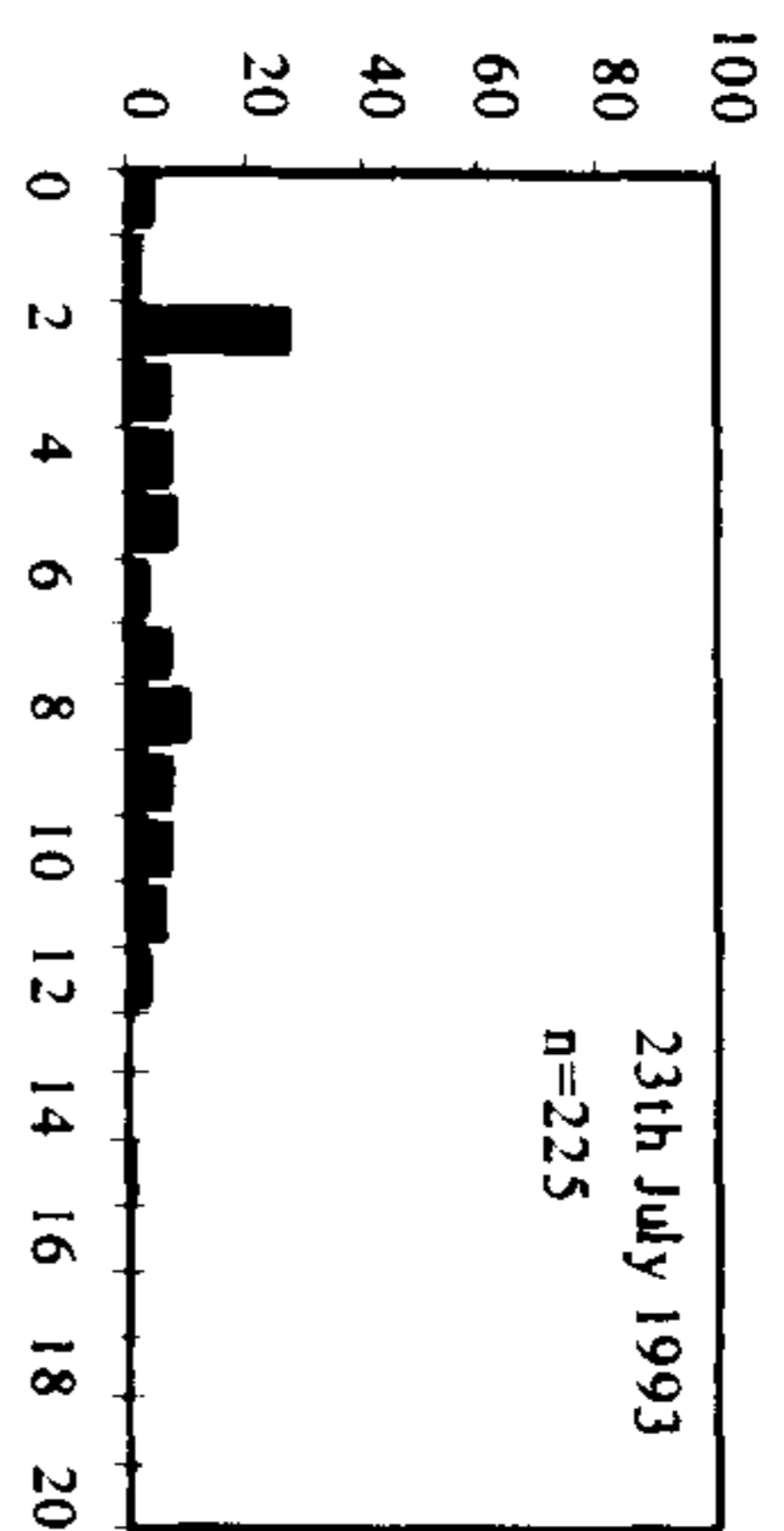
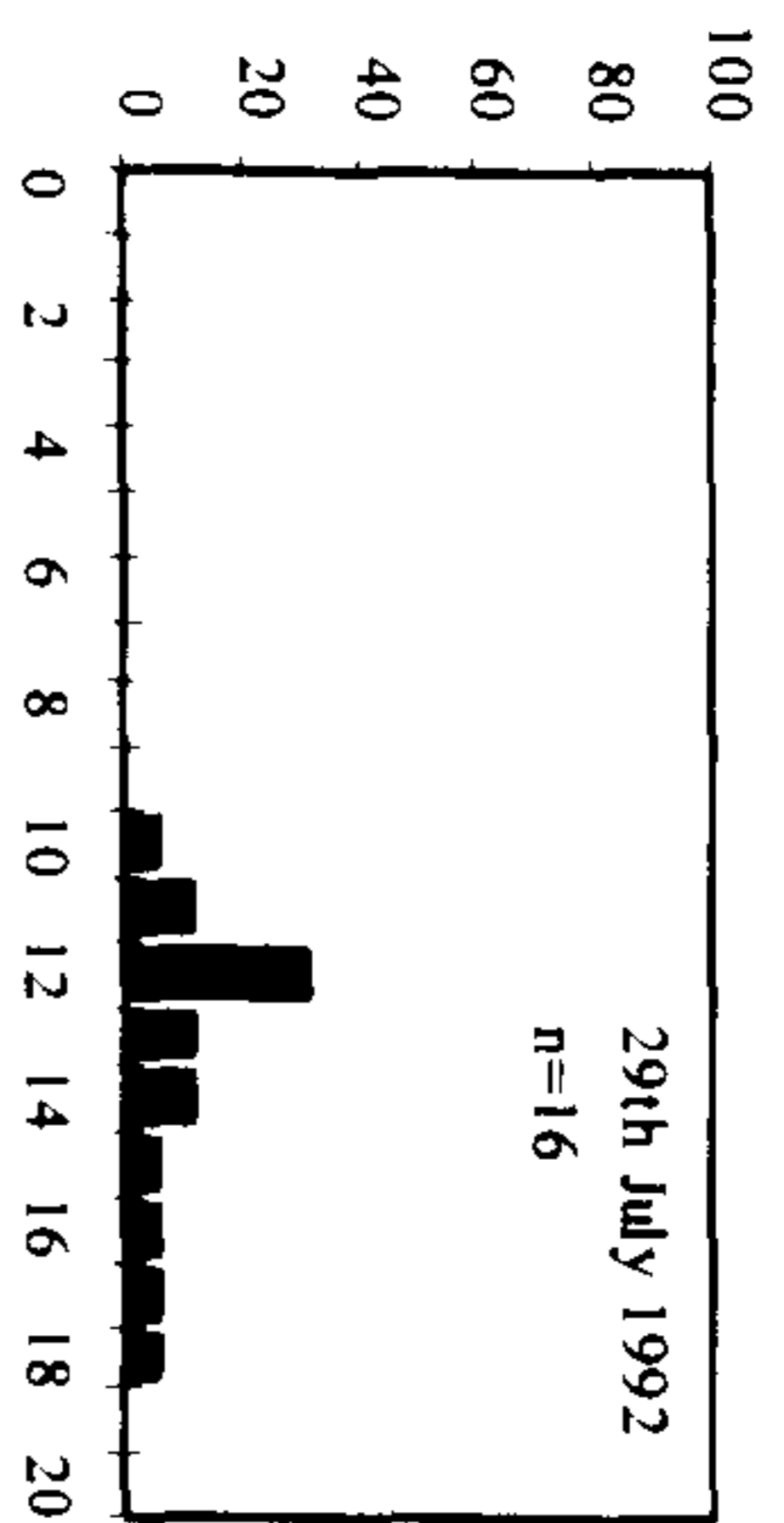
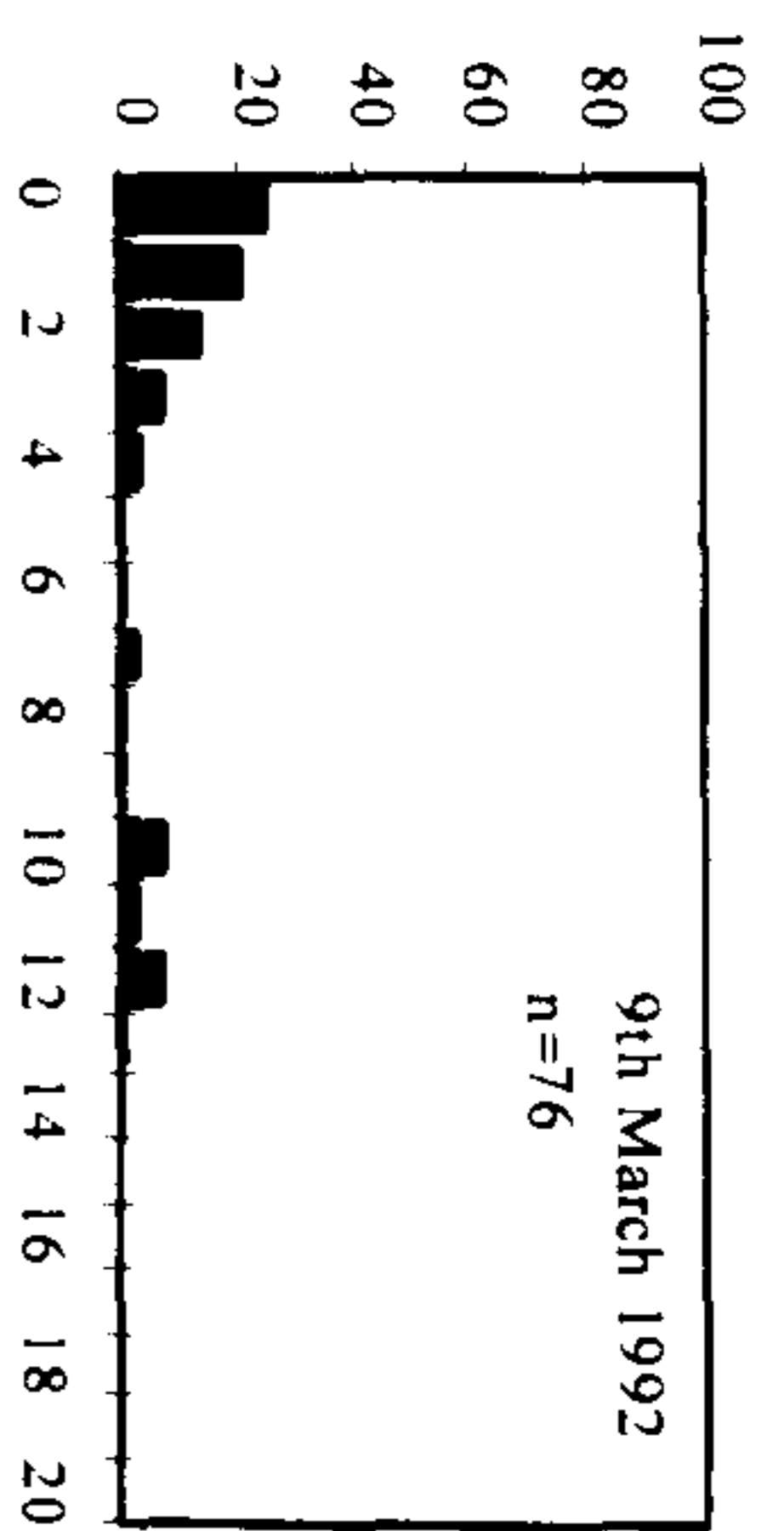
height (mm)

**Fig. 3.1 Capitulum height relative frequency distributions of *P. pollicipes* from Castelejo.**



height (mm)

**Fig 3.2 Capitulum height relative frequency distributions of *P. pollicipes* from Zavial.**



height (mm)

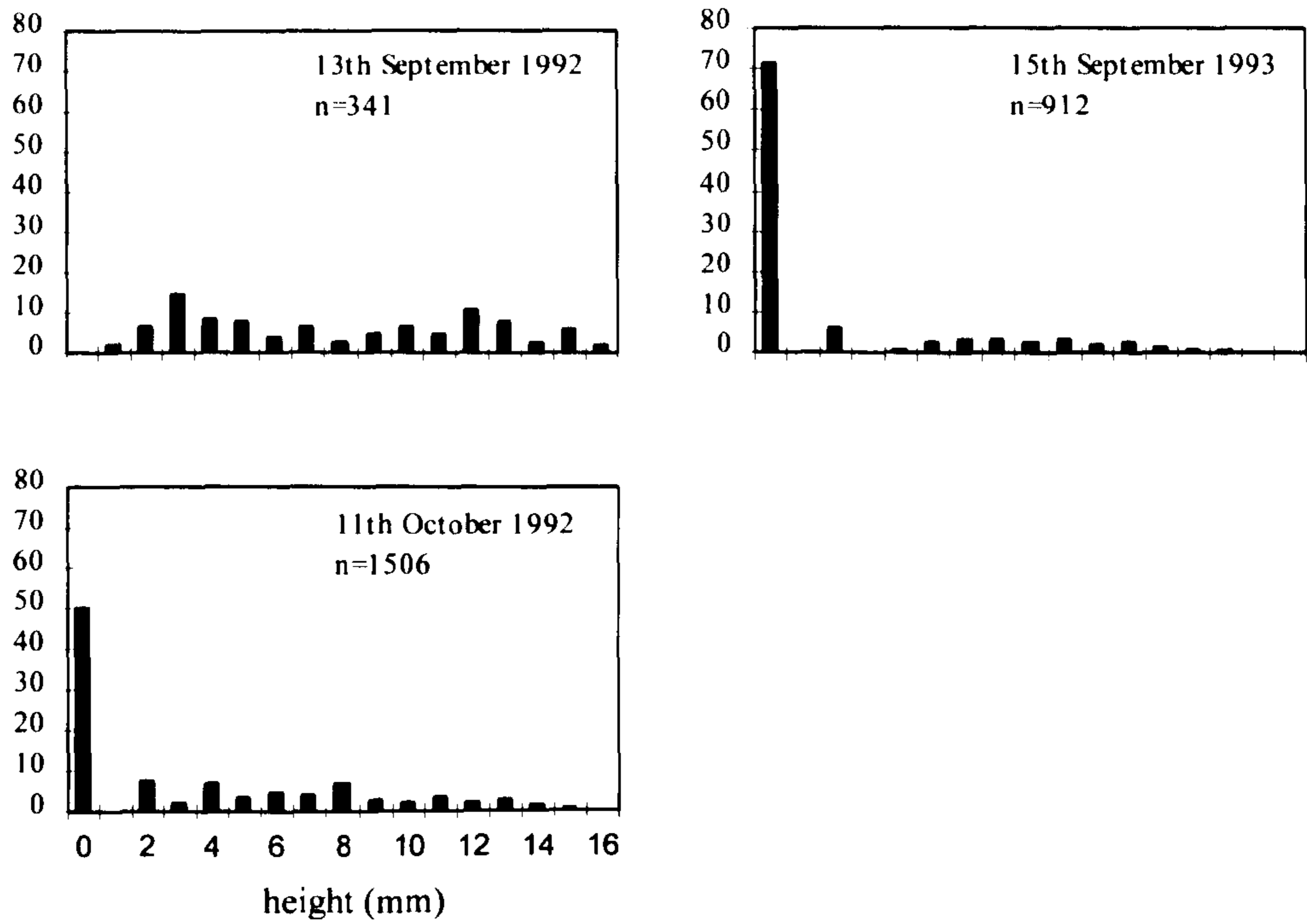


Fig. 3.3 Capitulum height relative frequency distributions of *P. pollicipes* from Ponta-da-Fisga.

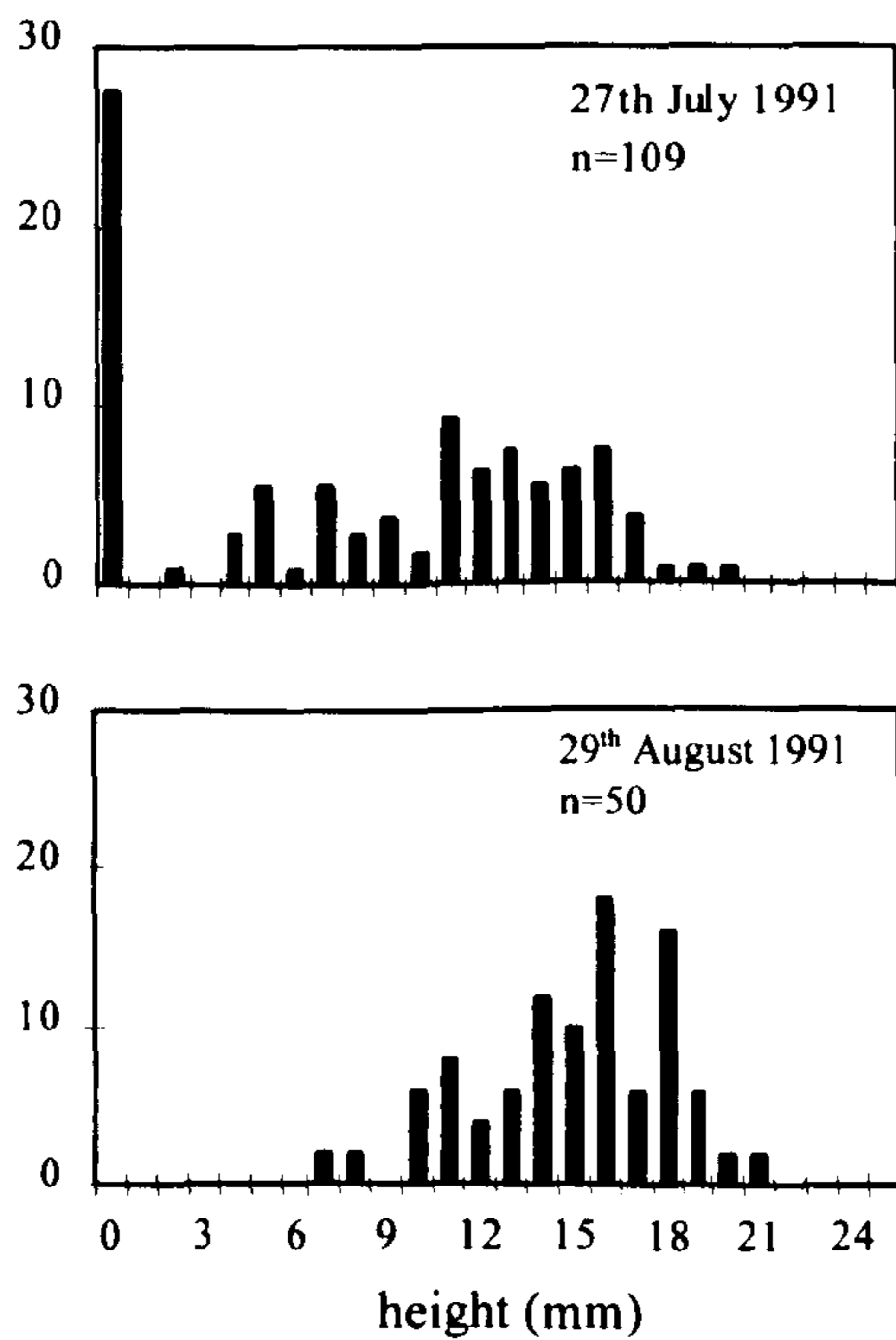


Fig 3.4 Capitulum height relative frequency distributions of *P. pollicipes* from Sagres.

discriminate any successive cohorts from the populations capitulum height frequency distributions.

### **3.2.2. Shell microgrowth patterns**

A description of the shell of *P. pollicipes* is given in Chapter one (Plate 1.1). Plate 3.1A) illustrates the typical microgrowth patterns in a tergal plate of a *P. pollicipes* along its entire length from a barnacle collected from Castelejo in September 1993. The observation of the patterns all along the length of the peel show a well defined relatively even spaced banding pattern.

In the current study it was not possible to obtain data from field mark and recapture experiments as would be desirable since attempts to mark animals in the field were thwarted (see General Materials and Methods). Instead an alternative method was employed to investigate whether there was any seasonal pattern to the formation of growth bands. Counts of the number of bands in the most recently deposited 0.5mm of shell from monthly samples of barnacles from Castelejo and Zavial were made. Mean capitulum heights for the barnacle samples used to evaluate deposition patterns are plotted in Fig. 3.5 with their associated standard deviations, and it shows only small differences in size of animals used. Yet, and to standardise the results for size, the numbers of bands counted were divided by the animals capitulum height, and these values are plotted in Fig. 3.6 for each month for Castelejo and Zavial. A high number of bands per unit area indicates a slow rate of shell deposition, and conversely a small number of bands per unit area indicates a high rate of growth. The pattern displayed is irregular throughout the year.

The relationship between tergum plate length and the capitulum height was calculated for animals from Zavial and Castelejo and no significant difference in slope was found ( $F=0.30$ ,  $p=0.584$ ;  $df=1,161$ ). Consequently, the values of the two variables for the two sites were grouped and showed a significant correlation ( $r=0.938$ ) with the relationship expressed by the equation:

$$Y = 0.509 + 0.747X \text{ (Fig. 3.7).}$$



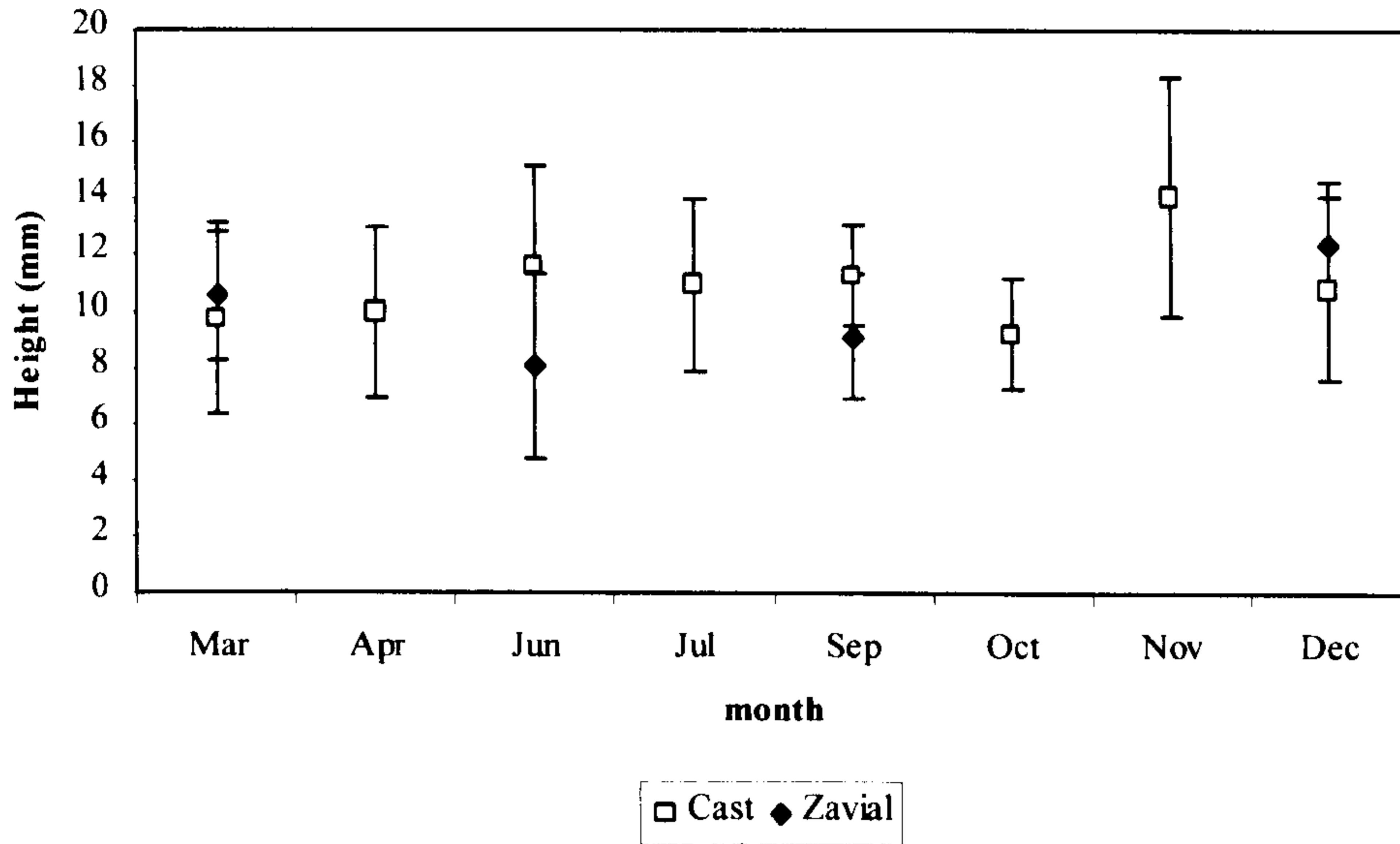


Fig. 3.5 Mean capitulum heights and associated standard deviations of *P. pollicipes* samples used for the analysis of the shell deposition patterns.

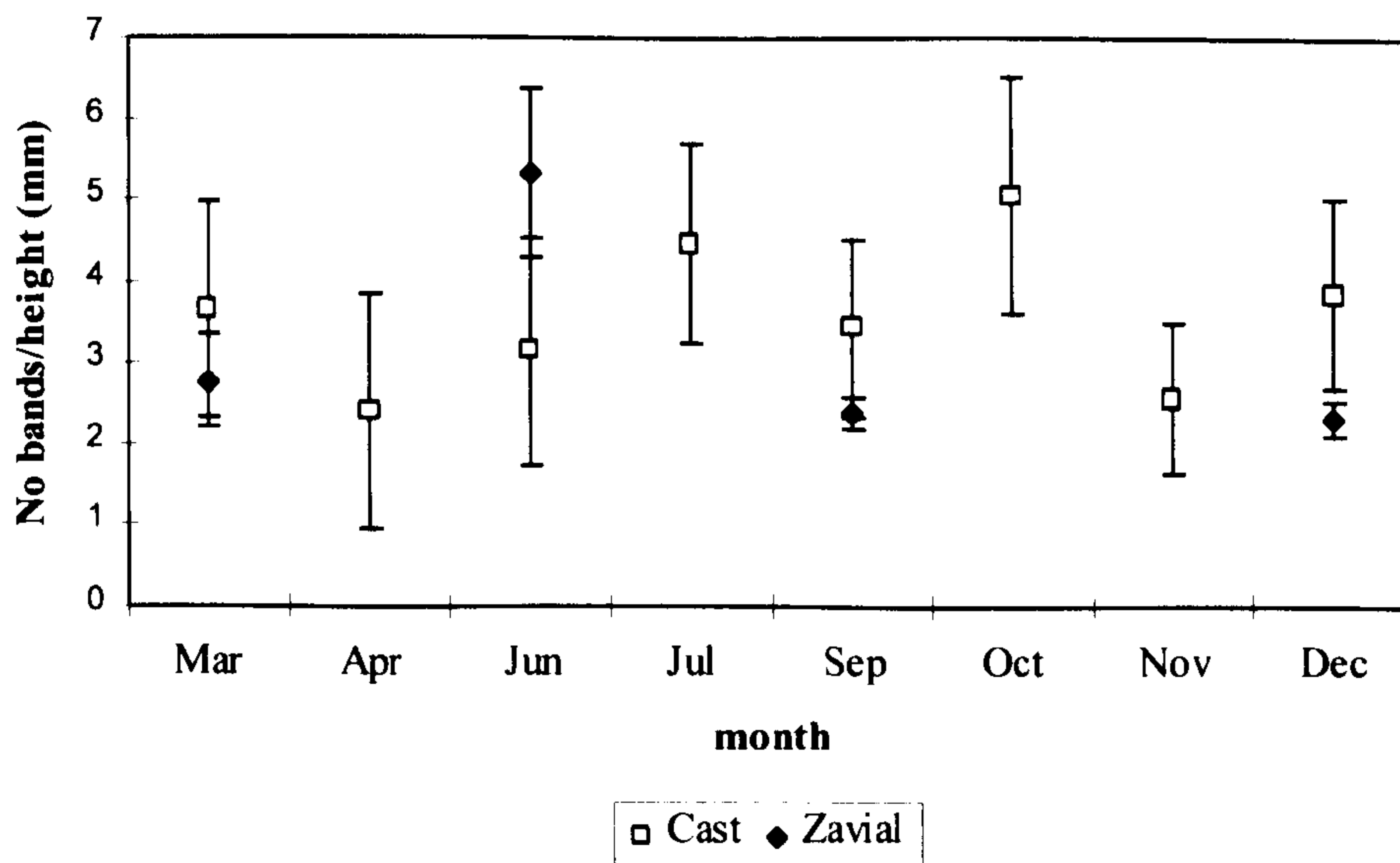


Fig. 3.6 Seasonal variation in the number of bands deposited in the last 0.5mm of the tergal shell plate in *P. pollicipes*, values were standardised for animal size.

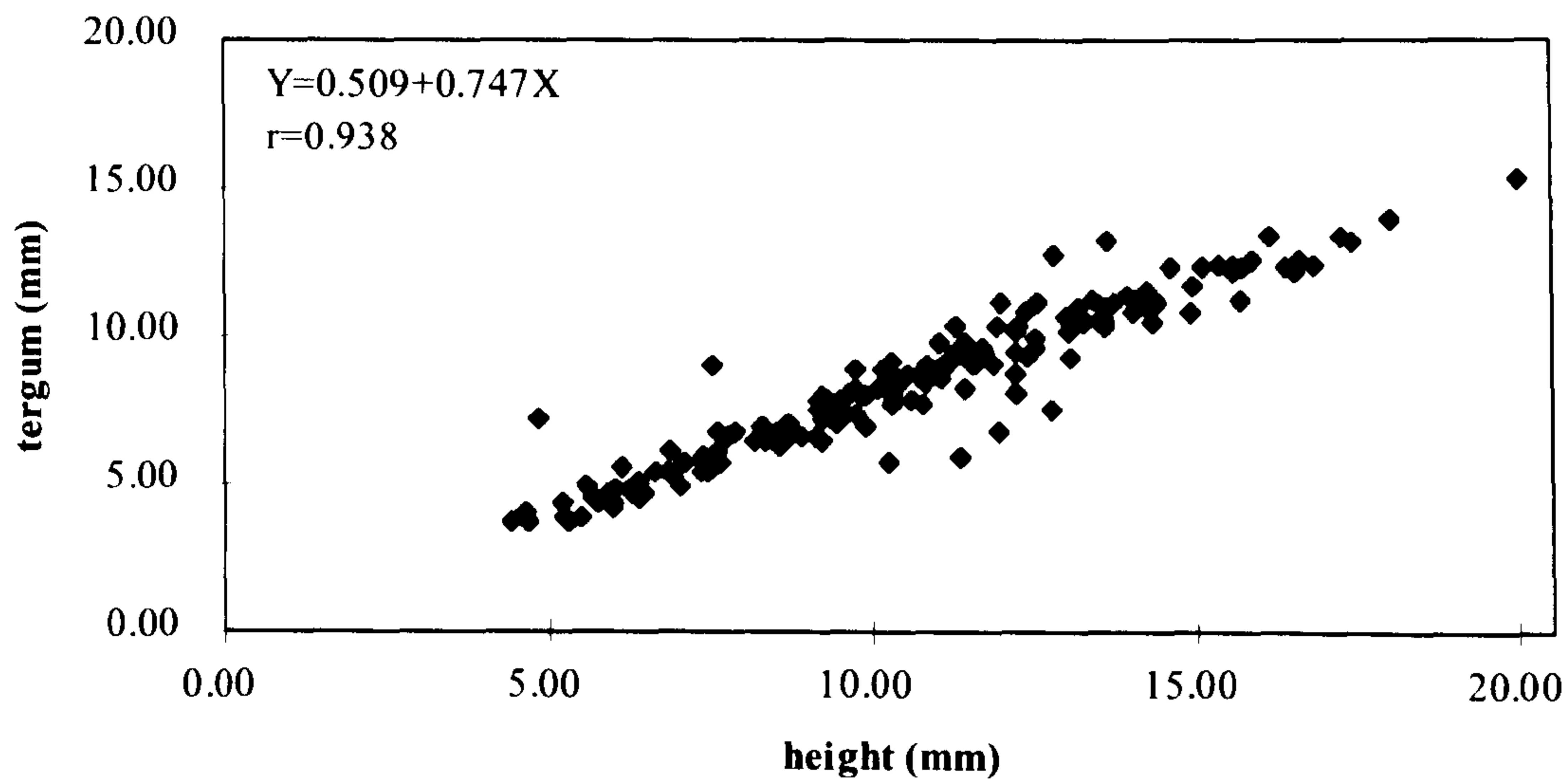


Fig. 3.7 Relationship between the tergum length (mm) and the capitulum height of barnacles from Castelejo and Zavial.

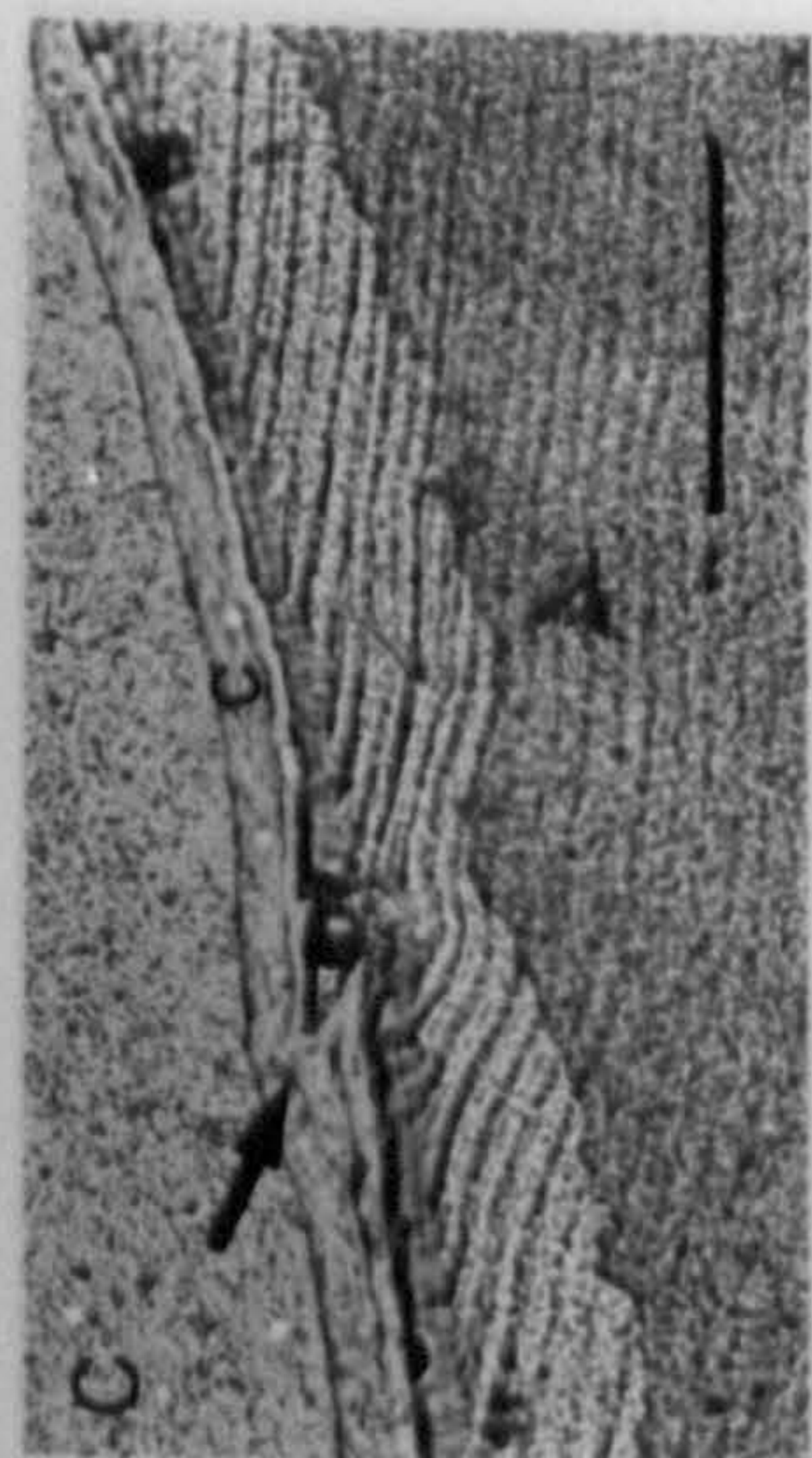
The existence of other but seasonal checks or clefts along the radial section of the shell plate was also noted (Plate 3.1 A. and B.), but these were generally random and were not consistently present.

### 3.4 DISCUSSION

The absolute growth of the *Pollicipes* in the current study remained largely undetermined. The maximum size attained by animals from Castelejo, Zavial and Ponta-da-Fisga was 20mm capitulum height, while barnacles from Sagres reached a maximum size of 24mm. Page (1986) described two different population structures of *P. polymerus* one from a rocky headland and the other from an offshore platform, in California. Barnacles at the rocky headland attained a maximum individual size of 26mm and the population was positively skewed, while barnacles from the offshore platform attained a maximum size of 36mm and the structure was bimodal (PAGE, 1986). The differences in size of these two populations were attributed to a much faster growth of barnacles from the offshore platform, and differences in population structure were attributed to a poor long term survival of barnacles on the

**Plate 3.1 Photomicrographs of acetate peels of tergal plates from *Pollicipes pollicipes*.**

- A) Microgrowth patterns along the entire length of a plate taken from a barnacle collected from Castelejo in September 1993. For ease of presentation the photo montage has been split into two parts. Scale bar=200 $\mu$ m.**
- B) Two disturbances to shell deposition recorded in the outer surface and in the microgrowth lines (arrows) in a barnacle collected from Zavial in June 1993. Scale bar=200 $\mu$ m.**
- C) The detailed appearance of the lines either side of the disturbance check (arrow); c, uncalcified layer. Scale bar=100 $\mu$ m.**



headland, in part because of the soft shale rocky substratum there. The bimodal distribution at the offshore platform resulted from stability of the substratum and intraspecific competition. In the studied populations, particularly for Castelejo and Zavial the two more extensively sampled sites, recruitment takes place throughout most of the year with barnacles carrying egg masses from April through September (see CARDOSO and YULE, 1995), and the barnacle populations are subjected to an intense fishery. These two factors explain the positive skewness of the capitulum height population structures and the relative small maximum size attained.

The populations are probably largely composed of very young individuals of 1 to 2 years. Cruz (1993) estimated the growth rates of juvenile *P. pollicipes* from Portugal (Cabo Sardo and Cabo de Sines) to be between 0.17 to 0.66 mm rostro-carinal increment per month. If barnacles grow at similar rates at the sites studied here an individual of 1 year is expected to have a rostro-carinal width of 2.0 to 7.9 mm (=2.3 to 8.8 mm capitulum height using the equations relating capitulum width to capitulum height in Chapter 2). An animal of 2 years could thus be expected to have a capitulum length of 4.5 to 17.6mm. In cases like the present where natural populations have an almost constant inflow of new individuals to the population and a constant loss of large individuals by fishing growth curves constructed by connecting modal or mean length values for successive time intervals are unlikely to reflect real growth for each or any generation. Indeed, intra-year generations may even be interpreted as year classes. In fact the software utilised to follow cohorts within the population was totally unable to discriminate between any modes. Thus, length frequency data cannot be used to define growth rates for such populations.

In many bivalves (e.g. *Mercenaria mercenaria*, RICHARDSON and WALKER, 1991; *Chamalea gallina*, RAMON and RICHARDSON, 1992) patterns in the shell deposition have been demonstrated to correspond to annual patterns of shell growth. Narrow growth increments are deposited during the winter when conditions for growth are less favourable. During the summer when conditions for growth are optimum the growth increments are considerable wider. In *P. pollicipes* from this study the seasonal patterns if present are not immediately obvious. Some of the barnacles examined exhibited clefts in the shell plates and these have in other

**CHAPTER IV: ASPECTS OF THE REPRODUCTIVE BIOLOGY OF  
*POLLICIPES POLLICIPES* (CIRRIPIEDIA; LEPADOMORPHA)  
FROM THE SOUTHWEST COAST OF PORTUGAL**

The results in this chapter were presented in a poster at the symposium “ECSA 24 Northern and southern European estuaries and coastal areas” at the University of Aveiro, Portugal, in 1994. Later these results were published by Cardoso, A.C. and A.B., Yule, 1995. *Neth. J. Aquatic Ecol.*, 29:391-396.

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percentage of barnacles with full seminal vesicles was comparable between sites. The evidence thus suggests that *Pollicipes* cannot self-fertilise.

#### 5.4 DISCUSSION

The results of the laboratory experiment confirm the suggestion from the field data that *Pollicipes* is an obligate cross-fertiliser. There is currently no literature on other scalpellids which relates to their ability to self-fertilise. *P. pollicipes* thus joins the list of hermaphroditic barnacles which tend to settle in high densities and maintain a high genetic diversity through obligate cross-fertilisation (CRISP, 1954; BARNES and CRISP, 1956; BARNES and BARNES, 1958; BARNES, 1992).

Apart from the Chthamalids, self-fertilisation (facultative) seems more common among inhabitants of marginal habitats or those barnacles which are less gregarious in habit (FURMAN and YULE, 1990). Pioneering new habitats or recolonising old ones after population extinction is possibly easier for facultative self-fertilisers which settle in low densities at irregular intervals, provided some means of larval retention (*e.g.* De WOLF, 1973; SHANKS and WRIGHT, 1987; FARREL *et al.*, 1991) in the vicinity is operating. Thus the estuarine dwelling *B. improvisus* is not only a facultative self-fertiliser but probably also possesses larvae with behavioural responses to retain them in the estuary (FURMAN and YULE, 1990). *P. pollicipes* occurs in large aggregations on the shore under fairly stable ecological conditions and the need for self-fertilisation is less apparent.

The isolated individuals found on the Portuguese coasts are probably the result of fishing activity leaving one or two small individuals behind once an area of rock has been cleared. The capacity for self-fertilisation would clearly have been an aid to the species in maintaining recruitment levels in the face of the fishery. However, the new isolated individuals might well be a focus for new clumps by the next wave of discriminant settlers. Since the species is unable to self-fertilise there is an even closer imperative to assess the impact of the fishery on natural populations and to develop management policies for the fishery in the future.

## GENERAL DISCUSSION

The objective of this thesis was to study the population biology of the goose-necked barnacle *Pollicipes pollicipes* (Gmelin, 1970) in the Algarve, southwest Portugal. After 3 years data collection in the field (sites at Castelejo, Zavial, Sagres and Ponta-da-Fisga) and in the laboratory, several of the initial questions have been answered yet many others raised during the study remain to be tackled.

The current study has provided definitive information on the annual reproductive cycle of *P.pollicipes* that is essential for any attempt at fisheries management. It has shown conclusively that shell-banding techniques are easily applicable to age and growth studies on these lepadomorph barnacles and has provided initial information on the growth rates and factors influencing shell deposition in experimental situations. An exhaustive data set provides a complete set of morphometric tools for the conversion of length measurements into dry and ash-free weights and demonstrates little differences between the shapes of *P.pollicipes* on the different shores sampled. The demonstration that *P.pollicipes* is an obligate cross-fertilising barnacle has subtle implications in managing the fishery since it is imperative that any adopted policy incorporates aggregates of the barnacle as a unit necessary to ensure continuing recruitment.

Concerns over the future of the species and of those who fish them prompted the instigation of the current project. Natural populations of *P.pollicipes* are harvested throughout their geographic range. Population numbers appear to be in decline and the ecological structure of the rocky shores they inhabit is undoubtedly affected by the intense fishery since the clumps of barnacles collected by the fishermen contain many other types of organism reliant on the established barnacle clumps. Dura (1988) reported the disappearance of *P.pollicipes* from Northern Spain at a time when the market for the species seemed to be expanding as shown by the increasing quantity collected from the Algarvian coast (see Table 1). It was doubtless the realisation that European shores could not withstand the expected increase in demand which prompted Bernard (1988) to investigate the feasibility of exporting *Pollicipes polymerus* from British Columbia to Europe. The magnitude of Portuguese *P.pollicipes* stocks has never been quantified and now is the time to ask the questions regarding conservation and management before Portugal suffers the same loss as Northern Spain.

Successful fisheries management is founded on the detailed knowledge of a species' biology and the population dynamics in given localities. Such detailed information is currently lacking for *P.pollicipes* given the paucity of currently available literature. The current thesis goes some way to providing the information needed to, at least, consider the options available for management.

The project was ambitious from the outset and has failed to deliver sufficient information for a number of readily identifiable reasons. The chosen sampling sites were expected to provide populations that would differ in their relative responses to the differing environmental conditions each was subject to. The most influential difference expected was that due to the upwelling experienced along the west coast (Castelejo) possibly providing more food but lower temperatures to those barnacles in comparison to those on the south coast. Such differences were not evident and food limited populations were not found.

Determination of the population size and age structure is a traditional tool in fisheries research. Attempts to use random monthly samples of population size structure from the sites in the Algarve to determine growth parameters failed largely because of the constant settlement of larvae throughout the summer months and the difficulties of sampling the area during the winter months. Traditional methods of using size frequency distributions to determine age and growth parameters were not readily applicable to the data which consisted of fairly small samples containing large numbers of small juveniles in virtually every month. Following cohorts from month to month was not possible without considerable imagination. The use of regular bands in calcareous skeletal materials has been successfully used for fish and bivalves with evidence of repeatable periodicity being shown in certain cirripedes (BOURGET and CRISP 1975b; CRISP and RICHARDSON, 1975). The current project demonstrated the efficacy of using shell bands in *P.pollicipes* and indicated several environmental influences on banding periodicity, the strongest being tidal periodicity. However, the application of the technique to field barnacles was less encouraging since band widths were very similar throughout the shell length with no regular "check" marks which could provide age estimation. Growth and age determination of field populations seems possible only by mark and re-capture experiments of the type attempted at Zavial that failed to provide any information due to the disappearance of the marked animals.

Given the successes and limitations of the current data it is possible to indicate what forms of fisheries management are likely to be applicable to Portuguese *Pollicipes* populations. Traditional fisheries are managed by enforcing one or more of the following measures:

1. Licensing and limiting the number of fishermen/ boats etc.
2. Enforcement of a closed season usually to protect reproducing individuals.
3. Enforcement of a minimum size at capture to limit the impact on young individuals.
4. Enforcement of catch quotas.
5. Enforcement of total exclusion from fishing in protected areas.

The choice of one or more of these protective measures depends on the type of population fished, its reproductive dynamics and the social mores of the fishing community.

Limiting the number of fishermen would have a protective influence on the *Pollicipes* population on the Portuguese shores but would be extremely difficult to enforce since most of the fishermen are part-time enthusiasts and tradition plays a major role in determining the number of current fishermen. The reproductive season for *Pollicipes* on the Southwest coast of Portugal lasts from April through to November with settlement occurring virtually continuously through that period. Enforcing a close season for the reproductive season would ensure protection of *Pollicipes* but it would also finish the fishery given the difficulty of fishing during the winter months. A closure during 3 of the calmest months of the year would clearly aid protection but again would be very difficult to enforce given the traditions in the *Pollicipes* fishery and the extent of the coastline. Furthermore, the settlement pattern of *Pollicipes pollicipes* appears highly gregarious with extant adults seen as the focus for larval settlement. Even if the reproducing population was protected and more larvae become available for settlement, recruitment of reproducing adults to the population may be limited by the availability of adults on the shore to act as settlement cues for the larvae.

Enforcement of a minimum size for capture is not possible in the management of *Pollicipes* collection. Populations are composed of dense aggregates of widely different sized animals that are not easily separated. Whole clumps are prised off the rock with no possibility of controlling the size of individuals collected. Vast

numbers of small individuals are always discarded at collection. Quotas are also difficult to enforce given the high discard rate of small individuals and the fact that traditional marketing of the barnacles takes place outside the normal fish market system.

Enforced reserve areas may prove a practical solution for *Pollicipes* management. Protected populations could serve as stocks for recruitment to other areas. A series of protected areas around the coastline would need to be established in areas that could be easily monitored and, if needed, physically protected. It might also be feasible to rotate protected areas every 3-4 years resulting in a better quality and increased quantity of *P. pollicipes* entering fishery.

Measures for protecting a fishery require considerably quantitative information before an appropriate ruling can be recommended. Data on the minimum number of adults to maintain a population, the population age structure, the population size together with estimates longevity, natural mortality and better estimates of fishing mortality are required to develop adequate predictive models and provide a quantitative basis on which to fix quotas, assign a length to a close season or determine the number of and extent of reserves etc. Although the current work provides valuable data, more, quantitative data are needed to estimate the possible effect of one or more proposed measure on the species stocks. There is still an urgent need to identify the stock size throughout Portugal before nation-wide recommendations can be considered.

The study of *P. Pollicipes* is still in its infancy and several aspects of its biology need further investigation:

- Implementation of a reliable ageing technique
- Study of the oogenesis
- Understanding of settling behaviour
- Factors determining recruitment
- Determination of adult, juvenile and larvae mortalities.
- Understanding of the detailed ecology of *Pollicipes* dominated shores.

Such information will allow the detailed formulation of management proposals with a level of confidence determined by the quality of the data. A successful management scheme will only be developed with the full support of the fishermen engaged in this traditional and dangerous fishery. The warriors of the sea (Guerreiros

do Mar as Cruz and Hawkins (1998) describes them) will require convincing that their prey is in danger and their way of life threatened before they can be persuaded to become involved.

The rich, upwelling waters of the Algarve support varied rocky shore communities and are rapidly becoming used for aquaculture ventures with bivalves. It is not inconceivable that *Pollicipes* could form the basis of yet another aquaculture industry given the ease with which the species can be cultured in the laboratory (KUGELE and YULE, 1996). The *Pollicipes* populations of the Algarve may be conserved through the intervention of aquaculture but the price may be the passing of the traditional percebes fisherman.

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