Population Ecology and Genetics of European Species of Intertidal Barnacles

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by

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Population Ecology and Genetics of European Species of Intertidal Barnacles

Federica Gabriella Pannacciulli

ABSTRACT

Barnacles are one of the dominant components of littoral communities. Because their abundance and accessibility on rocky shores they have been the object of a large number of ecological studies. Their importance as fouling organisms and more recently as bioindicators of pollution and long-term climatic changes have also contributed to increased interest. The aim of this work was to investigate in greater depth two important aspects of the biology of European barnacles: their population ecology and genetics.

On the south-west coast of England four species of barnacles (*Chthamalus montagui*, *Chthamalus stellatus*, *Semibalanus balanoides* and *Elminius modestus*) occur together on the shore. Their species composition has been shown to fluctuate with environmental temperatures. Warmer weather favours warm temperate species, such as *Chthamalus* and *Elminius*, while colder conditions favour the boreal *Semibalanus*. These observations were the result of a forty-five year survey carried out on the barnacle populations of South Devon and Cornwall. Barnacles from a shore near Plymouth, Cellar Beach, showed the most extensive variation in species abundance over the long-term study. The present study investigated in greater detail the changes in the barnacle populations of Cellar Beach over a three year period. The beginning of the survey (September 1991) coincided with a decline of *S. balanoides*. The reduction of this species generated a large amount of bare rock which only in part became colonised by the other species. Due to the continuous availability of vacant surface, it was suggested that competition for space was not the determining factor in the decline of the *Semibalanus* population. A combination of rising temperature and reduced larval supply were instead thought to be responsible for the observed pattern.

Data on breeding, settlement and recruitment, growth and mortality rates (including differential post-settlement mortality of different settling cohorts) at different stages of the life history were also obtained for all four species of barnacles present at Cellar Beach. These results allowed speculations on the way population dynamics affect the availability of bare rock and on the adaptive strategies of the various species for coping with such a complex environment.

The population genetic structure of the barnacles *C. stellatus* and *C. montagui* was also investigated. Protein electrophoresis on starch gels was used to compare specimens of the two species collected from the NE Atlantic and Mediterranean coasts. The existence of "Atlantic" and "Mediterranean" forms of *C. montagui* had been suggested in the past but no division of this kind had been noted for *C. stellatus*. With my investigations I attempted to enlarge the previous work on the subject by analysing samples from the species' entire geographical range and also by extending previous analysis to *C. stellatus*.

Both *Chthamalus* species were found to have marked differentiation of allele frequencies between Atlantic and Mediterranean localities at a single locus. Within the Mediterranean basin, allele frequencies indicated a greater degree of differentiation in *C. montagui* than in *C. stellatus*. In both species, mean heterozygosity per locus was higher in the Mediterranean than in the Atlantic; Mediterranean populations also possessed a greater range of alleles. Possible explanations for the differentiation between the Atlantic and the Mediterranean, and for the greater genetic diversity in the Mediterranean, are discussed. These centre on climatic and attendant sea-level fluctuations during recent geological history, and possible differences in the selective regimes imposed by the contrasting environments of the Mediterranean and the Atlantic.

GENERAL INTRODUCTION

Barnacles are important features of littoral communities world-wide. Although they tend to be most abundant on exposed shores, their ability to live under most physical conditions suggests that their occurrence in quantity is controlled less by their own requirements and more by competition with other animals and plants (mainly mussels and fucoid algae). As generally these competitors favour mostly very exposed and sheltered areas, barnacles tend to colonise the areas left available, which are those of intermediate exposure (Stephenson and Stephenson, 1972).

The objects of this study were the European species of intertidal barnacles belonging to the class Cirripedia, superorder Thoracica (Darwin, 1851; 1854). The study concerned symmetrical sessile barnacles belonging to the order Sessilia, suborder Balanomorpha (classification proposed by Newman, 1987), and did not include any investigation on stalked barnacles, such as *Pollicipes pollicipes*, which are also common in the intertidal region of some European coasts.

In the intertidal zone of European rocky shores, five species of sessile barnacles are generally present. On the Atlantic coasts these are: *Semibalanus balanoides*, *Chthamalus montagui*, *Chthamalus stellatus* and *Elminius modestus*. In the Mediterranean area the two *Chthamalus* species, together with *Euraphia depressa*, constitute the whole of the intertidal barnacle population.

The ecological section deals with the barnacle populations of the South-West of Britain and is therefore limited to the four Atlantic species. The genetic study concentrated on *C. montagui* and *C. stellatus* which are the only two species of intertidal barnacles that are common to both the Mediterranean and the Atlantic.

A brief outline of the taxonomy and biogeography of the species studied follows here.

The boreal species *Semibalanus balanoides* (Linnaeus) belongs to the genus *Semibalanus* Pilsbry, subfamily Semibalaninae, family Archaeobalanidae (Newman and Ross, 1976). Until 1976 this species was known under the name of *Balanus balanoides*; after that time, following a major revision of the balanomorph barnacles (Newman and Ross, 1976) its name was changed to *Semibalanus balanoides*. *S. balanoides* is common in the intertidal zone of Western Europe and Atlantic North America (Stubbings, 1975). On the north east coast of the Atlantic Ocean, it reaches its southern limits in the cold Galician rias of north-western Spain (Fischer-Piette and Prenant, 1956; 1957), while its northern distribution stretches over all the European coasts to Spitzbergen (Fisher, 1943).

Charles Darwin (1854) was troubled by the genus *Chthamalus* during his studies on barnacles; his problem was to decide whether similar but recognisable morphological types should be regarded as species or varieties (Southward, 1983). Influenced by his developing ideas on evolution and the search for variation within a species as evidence for the basis of the origin of the species, Darwin grouped five varieties of intertidal *Chthamalus* into the world-wide species *Chthamalus stellatus* (Poli). Darwin's species is now considered to comprise two genera, *Chthamalus* (subfamily Chthamalinae, family Chthamalidae) and *Euraphia* (Newman and Ross, 1976) within which there are five valid species from the original material described (Southward, 1983). In the North East Atlantic *C. stellatus* remained unchallenged until 1976 when Southward reconsidered the status of this species (Southward, 1976). Morphological characters were used to separate two species of British barnacles: *Chthamalus montagui* Southward from the "true" *Chthamalus stellatus* (Poli) (Southward, 1976). Examination of the biochemical genetics of the two species, using enzyme electrophoresis, confirmed the specific rank of the two species (Dando *et al.*, 1979). A detailed investigation of the geographical distribution of the two *Chthamalus* species was later carried out by Crisp *et al.* (1981). This study showed that *C. montagui* and *C. stellatus* are temperate species which overlap extensively in geographical range. They occur together along the Atlantic coasts of Britain, Ireland, France, Spain, Portugal and North Africa, and both also occur in the Mediterranean

Sea. They have almost identical north-eastern limits to their distribution in the English Channel and North Scotland, but *C. stellatus* may extend farther south along the West African coast (for a map of the geographical distribution of the two species, see genetic section).

Elminius modestus Darwin belongs to the genus *Elminius*, subfamily Archaeobalaninae, family Archaeobalanidae (Newman and Ross, 1976). This species was found until quite recently only in New Zealand and southern Australia but it is now well established also in Europe. This species is responsible for one of the most striking invasions of rocky shores. The establishment of this species on British shores from ship-borne populations probably occurred in the early years of the second world war, but went unnoticed until 1945 when it was discovered in Chichester harbour (near Southampton) (Bishop, 1947). The original colonisation is thought to have started from ships immobilised there at the outbreak of war. From here *Elminius* is thought to have spread to the British coasts and to north France either by further ship-borne invasions or natural drift of larvae along the coast. Studies carried out by Crisp document the gradual invasion of *E. modestus* on British shores (Crisp, 1948; 1958; 1960; Crisp and Southward, 1959) and estimate a speed of invasion of 20-30 km per year (Crisp, 1958). Thirty years after its arrival in south of England *Elminius* was already recorded as far north as the Shetland Islands (Hiscock *et al.*, 1978), the German Waddensea (Michaelis, 1978) and the Danish coasts (Theisen, 1980).

Barnacles are good model organisms for ecological work because:

- they are sessile;
- are relatively easy to identify, count and monitor;
- are suitable for various types of experimental manipulation;
- the intertidal species are very accessible.

For all these reasons considerable work has been done on their ecology as reviewed in the introduction to the ecological section.

Less attention has been given to the population genetics of barnacles. In more recent years, the advent of starch gel electrophoresis has stimulated studies of both taxonomy and population differentiation of barnacles on a broad geographical scale, as reviewed in the introduction to the genetics section.

In this study both the population ecology and genetics of the common barnacles of the North Atlantic region were investigated. The overall aim of the work was to understand temporal changes on both decadal (ecological studies) and evolutionary (genetic studies) time scales.

The ecological work focused on the investigation of short term changes in barnacle species composition. This study was aimed to provide useful information to help interpret the long-term changes observed in the barnacle populations of the south-west of England (Southward, 1991). The project also investigated further details of the ecology of *S. balanoides*, *C. montagui*, *C. stellatus* and *E. modestus.*

The work was then extended to a study of changes which took place in evolutionary time. The intraspecific genetic differentiation of populations of *C. stellatus* and *C. montagui* from the Atlantic and Mediterranean basins was investigated by enzyme electrophoresis.

SECTION I

POPULATION DYNAMICS OF FOUR COMPETING SPECIES OF INTERTIDAL BARNACLES ON THE SOUTH-WEST COAST OF BRITAIN

I 1. INTRODUCTION

Barnacles are one of the dominant components of littoral communities (see Stephenson and Stephenson, 1972). Their importance as fouling organisms and more recently as bio-indicators of pollution and climatic change, has demanded basic knowledge about their biology and ecology. Because of their abundance and accessibility on the shore, their ecology has been the subject of study over a hundred years. In his definitive studies of the group, Darwin (1851; 1854) fully realised that many species of barnacles had strong intertidal preferences. The work by Hatton and Fischer-Piette (1932) and Moore and Kitching (1939), started the modern era of studies on barnacle ecology. During the last forty years, Barnes (for studies on reproduction, growth, etc. e.g. Barnes, 1955; 1956a; 1962; Barnes and Crisp, 1956; Barnes and Barnes, 1968), Connell (for studies on competition, distribution etc. e.g. 1959; 1961a; 1961b; 1983), Crisp and coworkers (for studies on breeding, hatching, settlement, growth, distribution, invasion by *E. modestus*, etc. Crisp, 1948; 1950; 1956; 1961; 1964b; Crisp *et al.*, 1981; 1991; Crisp and Bourget, 1985) and Southward (for studies on taxonomy, distribution, cirral activity, long-term changes, etc. e.g. Southward, 1955; 1976; 1991; Southward and Crisp, 1952; Southward *et al.*, 1975; 1995) are among those who produced classic work on this subject (see Southward, 1987 for a complete review). A great deal of literature has also been published on the role of barnacles in the rocky shore community (e.g. Paine (1966; 1969; 1981; 1984), Dayton (1971), Menge (Menge, 1976; Menge and Sutherland, 1976; 1987; Menge and Lubchenco, 1981), Underwood (Underwood, 1978; 1986; 1991; Underwood *et al.*, 1983; Underwood and Denley, 1984; Underwood and Fairweather, 1986) and Hawkins (Hawkins, 1981a; 1983; Hawkins and Hartnoll, 1982; 1983; Hawkins *et al.*, 1992)).

The following is a brief account of some aspects of the biology of the four species (*Semibalanus balanoides*, *Chthamalus montagui*, *Chthamalus stellatus* and

Elminius modestus) which are the object of this study. This account is not intended to review all the literature available on the subject, but simply to introduce some selected information considered useful for a better understanding of population biology.

The four species mentioned above, are generally found on open rock in the eulittoral zone of North Atlantic shores. A fifth species, *Balanus perforatus,* can occasionally be found together with the above species. As *B. perforatus* occurs mainly low on the shore and in crevices, and as only a few specimens were seen during the work, it was omitted from the study.

I 1.1 BARNACLE DISTRIBUTION

I 1.1.1 Horizontal distribution

Important studies on barnacle distribution along the wave exposure gradient were carried out by Lewis (1964). According to this author, barnacles are almost ubiquitous and are the dominant and most characteristic organisms of the eulittoral zone throughout the world (see also Stephenson and Stephenson, 1972). He regarded barnacles as being the main colonisers on moderately exposed shores, as fucoids are on sheltered and mussels and red algae on very exposed ones. In his opinion barnacles are generally restricted to this type of environment, by competition with the other organisms rather than unsuitable physical conditions. This assumption is confirmed by the fact that barnacles can occasionally be found in areas where fucoids and mussels are normally dominant. In these areas barnacle settlement occurs for instance on steep slopes or vertical rocks where fucoids and mussels are generally less abundant (Stephenson and Stephenson, 1972). Barnacles can also be found on flat surfaces of very exposed shores. This type of environment may be too harsh for colonisation by fucoids (Vadas *et al.*, 1990) and desiccation may limit mussels to the lower eulittoral zone (Suchanek, 1978; 1985; Tsuchiya, 1983). Biotic factors can also be responsible for the presence of barnacles on shores which are normally dominated by other organisms. Intensive grazing by limpets for instance, can inhibit algal growth and indirectly favour barnacle settlement (Jones, 1948; Southward and Southward, 1978; Hawkins, 1981a; 1981b; 1983; Hawkins *et al.*, 1992). On heterogeneous coasts, it is often possible to observe all three types of communities within a few metres from each other.

I 1.1.2 Vertical zonation and species distribution

Vertical zonation of barnacles has received much attention. Barnacle species are generally limited by physical factors towards the upper limit and by biological ones at the lower (Connell, 1961a; 1961b; 1972). Brief exposure to sea-water and consequent problems with desiccation and poor food supply, are limiting on the high shore. On the other hand, there are some advantages of living on the high shore: competition for space and predation may be less in this environment. Mid and low shore barnacles instead, are constantly competing for space (Connell, 1961a; 1961b), are more subject to predation and to the destructive force of wave action; turn-over is therefore much more pronounced at these shore levels. The presence of encrusting algae can constraint the expansion of barnacles lower on the shore (Hawkins and Hartnoll, 1982). The very lowest limit of the barnacle distribution is considered to be set by the upper limit of the laminarians below (Lewis, 1964).

The intertidal barnacle community of shores in the south-west of England, the area where this study was made is formed by four different but co-occurring species. These species are: *Chthamalus montagui*, *Chthamalus stellatus*, *Semibalanus balanoides* and *Elminius modestus*.

The two *Chthamalus* species can occasionally be separated by habitat, with *C. stellatus* predominant on exposed shores and *C. montagui* on moderately sheltered ones, but in this particular case their distribution overlaps. Where they overlap, *C. montagui* is found more commonly in the upper barnacle zone (MHWS and MHWN), while *C. stellatus* is more dominant lower down (MTL and below). Occasionally, *C. stellatus* can occur higher up on the shore in wave-washed, wet or shaded places (Southward, 1976; Crisp *et al.*, 1981). According to Burrows (1988), processes of larval dispersal, larval development and settlement appear to be the most important factors producing the different adult distributions in the two *Chthamalus* species. Juvenile mortality also accounts for this difference. *C. stellatus* in fact, is found to be more susceptible to desiccation during the early stages of life than *C. montagui.* Morphological differences in the shape of the opercular plates may account for this (Foster, 1971a). *C. montagui* juveniles may require desiccation for consolidating their shell plates (Burrows, 1988).

S. balanoides is generally speaking distributed between high water of neap tides (MHWN) and low water of neap tides (MLWN) (Stubbings, 1975). Cyprids are capable of settling on the high shore owing to the short period required to attach to the rock surface, but desiccation tends to kill most of them before the next tidal immersion (Foster, 1971a). For this reason, the upper zonal boundary of adult *Semibalanus* is below the limit of larval settlement (Foster, 1971b). When *S. balanoides* is found in conjunction with *Chthamalus*, *Semibalanus* tends to colonise shaded positions lower on the shore. Because of this, it is much more in competition for space with *C. stellatus* than with *C. montagui* which favours sunny situations in the upper shore (Southward, 1976; Crisp *et al.*, 1981). Where barnacles are very abundant, a white and regular "barnacle line" can be observed. This is generally produced by dense aggregations of *S. balanoides,* but in situations towards high shore where it becomes darker and more ragged, then the *C. montagui* component is prevalent. *Chthamalus* species have the ability to survive at very low densities, their capacity to self-fertilise (Barnes and Crisp, 1956; Barnes and Barnes, 1958) can be considered an adaptation to this particular situation. *Semibalanus* and *Elminius* instead are cross fertilizing hermaphrodites and need therefore to be in the vicinity of conspecifics in order to mate (Barnes and Crisp, 1956; Barnes and Barnes, 1958; Walley *et al.*, 1971).

The fourth common inhabitant of the south-west shores, is *E. modestus,* an australasian immigrant which arrived in south England in the forties (Bishop, 1947; Crisp, 1948; 1958; 1960; Crisp and Southward, 1959) and was recorded for the first time at the study site (Cellar Beach, South Devon) in 1953 (Southward, 1991). *Elminius* is usually common at mid and low shore, and in general below MHWN. At these shore levels, competition for space with *S. balanoides* can be very intense. In comparison to the other three species, *E. modestus* is a better coloniser of the subtidal and can tolerate quite low salinities. Of the four in fact, *Elminius* is often the most abundant in estuaries.

I 1.2 BARNACLES as BIO-INDICATORS of LONG-TERM CLIMATE CHANGES

During the last few decades, global warming as a possible consequence of human action has been at the centre of the interest of the scientific community (Gregory, 1988). There have been some contentions about whether the observed warming was due to short term natural fluctuations or to the beginning of a warmer era. The real problem in fact, was to separate the normal variation from a superimposed trend. Increased computing power allowed more precise calculations of global mean temperatures, whilst improved instrumentation provided more accurate measurements of atmospheric constituents. Thanks to these technological improvements, very recently it became possible to demonstrate the existence of a trend of rising temperature and link it to increasing concentrations in the atmosphere, of carbon dioxide, methane and chlorofluorocarbons produced by human activities (Bolin *et al.*, 1986). The link can be explained through the so-called "greenhouse effect". The gases mentioned above are not transparent to all infra-red radiation, and therefore, an increase in their concentration in the atmosphere would lead to greater trapping of the longer wavelengths of solar radiation that would otherwise be re-radiated back into space. Since the end of the past century, air temperatures have increased by between 0.3 and 0.6°C with most of the increase taking place during the past forty years. Predictions of air temperature change, that might result from the greenhouse effect, have estimated an increase in average temperature of between 1 and 3°C in the next fifty years (Southward and Boalch, 1994).

Changes in sea water temperature have been observed in association with changes in air temperature during the last seventy years (Southward, 1960; Russell *et al.*, 1971; Southward *et al.*, 1975; Southward *et al.*, 1995). Records of temperature from various stations in the English Channel, were collected since the end of the nineteenth century. Cyclic fluctuations in sea temperature showed signs of a secular trend superimposed on short term cycles. For the first half of the twentieth century, an increase in sea temperature was observed. This was particularly noticeable for the period between 1928 and the fifties, when the temperature rose by 0.46°C (Southward, 1960). A much cooler period followed these years and lasted until the 1970s. This period corresponded to a secular decline in sea temperature, already expected from the analysis of long-term fluctuations over the century. Since 1979, the trend reversed (Southward, 1980; Southward *et al.*, 1995).

When the noise was removed, the short term fluctuations of sea temperature were found to have a 10-11 year cycle. This cycle was discovered to be perfectly phased with some changes in the solar radiation and in particular with curves of annual mean sunspot numbers (Southward *et al.*, 1975). Marine biological records showed signs of both secular and 10-11 year cycles. Since 1975, however, the biological data diverged from the solar cycle and in the case of intertidal organisms, it started to show more fit with the mean inshore sea temperatures (Southward, 1980). Changes in weather pattern and a global climate shift are believed to be involved (Southward, 1991). Incidentally, it was in the seventies that an increase in the atmospheric concentration of CO_2 was first noticed.

The western end of the English Channel is close to a boundary between species of fish and invertebrates that flourish best in colder waters and others that do better in warmer waters. Among biological records, changes in the distribution of certain marine species in relation to changes in sea temperature, were noticed here during the last century (see Southward, 1980; Southward and Boalch, 1994; Southward *et al.*, 1995 for review). Fluctuations in the herring and pilchard fisheries of Devon and Cornwall were an example (Southward *et al.*, 1988). Another case was the change in the relative proportions of species of intertidal barnacles (Southward, 1967; 1980; 1991).

On the South Devon coast, the two warm-water species, *Chthamalus montagui* and *Chthamalus stellatus*, and the boreo-arctic *Semibalanus balanoides*, are found together in direct competition. In this area, all three species are close to the extremes of their distribution range, with *Chthamalus* reaching its northern limit in the Shetland Isles and *Semibalanus* its southern limit in northern Spain/Portugal. Towards the limits of the geographical range of an animal, both its abundance and exact boundaries may fluctuate from time to time. These fluctuations can sometimes be correlated with environmental changes, and thus may help to assess the relative importance of the factors that control distribution (Southward and Crisp, 1956).

Already in the fifties, Southward and Crisp (1952; 1954) noticed an increase in the density of *Chthamalus* and a decrease in *Semibalanus* in the south-west. They related these changes to an increase in sea temperature that occurred in the previous decades. According to them, warmer temperatures would promote the fecundity and survival of the *Chthamalus* populations. Low winter temperatures would instead favour *Semibalanus* by delaying embryonic development to a point where larvae may benefit more from the spring phytoplankton (Southward and Crisp, 1956). After 1961 the temperature trend reversed. Colder weather characterised most of the following fourteen years and an increase in *Semibalanus* associated with a decrease in *Chthamalus* was detected (Southward, 1967; 1980; 1991). From 1975 up to present day, temperatures rose again and once more *Chthamalus* populations benefited from these favourable conditions. Since its arrival on the south-Devon coasts in the fifties, fluctuations in the density of *E. modestus* were also monitored. Among the four species, this was the one which showed the highest variation, probably in relation to its dependence on "good" years for reproduction and survival. No particular trend was observed, with the exception of the fact that warmer years seemed to favour *Elminius*.

Maximum fluctuations of all four species were found to take place on the low shore. Here the high turn-over, presumably due to predation and competition with algae and mussels, would allow a quicker replacement of species. It is important to note, that in spite of the oscillations in species abundance, the total density of barnacles has always remained fairly constant. Reductions of one species have been balanced by increases in another. In addition, bare rock has always been present, indicating that some factor other than substratum availability was limiting the stockholding capacity of the habitat (Southward, 1991).

In relation to changes in sea-temperature, changes in the barnacle species composition of South Devon, were found to have cyclic fluctuations of 10-11 years. These cycles, however, had a 2-year phase lag on the temperature ones. As well as sea temperatures since 1975 the amplitude of the fluctuations in the proportion of the barnacle species started to decrease and to show an upward trend in the direction of an increase of the *Chthamalus* species (Southward, 1991). Large temperature changes operate on the survival of barnacles of the various species, for instance by altering the frequency of the cirral beat and consequently food intake (Southward, 1955). Temperature also operates on the maturation and reproduction of adult barnacles which are designated to produce larvae for the next settlement season. A good larval supply for the following year though, is not the only requisite to successful recruitment. A certain amount of bare rock in fact, is needed in order to allow cyprids to settle. High mortality of one species due to adverse climatic conditions, will produce the bare rock necessary to allow larvae of the other species to settle successfully. The time required for these processes to take place, can partly justify the observed 2-year phase lag between cycles of temperature and cycles of changes in barnacle species composition (Southward, 1991).

Darwin (1872) cautioned against attributing the distribution of an organism directly to temperature differences in the environment. He pointed out that competition between species would probably be the most exacting factor, but that this would be influenced by conditions of temperature or climate, which favoured one or the other competitor. Southward believed that temperature, like other environmental factors, influences the relative number of *Chthamalus* and *Semibalanus*, by its effect on competition between them for space and food (Southward, 1967) (see also Connell, 1961a; 1961b). He suggested that in the south-west of England, on the border of their distributions, the two competing genera are in a state of dynamic equilibrium and that even quite minor changes in the physical environment, will favour one or the other species. Before concluding, it is worth mentioning that the role of the predator *Nucella lapillus* as a structuring factor on the investigated barnacle zone, has been minimal since the decline of this species in the seventies due to TBT pollution (Bryan *et al.*, 1986; Gibbs and Bryan, 1986). Its decline coincided with the beginning of the irregularities in the fluctuations of the barnacle species composition. It is therefore difficult, to separate the effects of the decrease in predation from those of global warming (Southward, 1991).

According to Southward, continuance of the barnacle observations, could help to distinguish the other biotic and abiotic factors involved in controlling the relative proportions of the species in relation to changes in temperature and climate and provide an early-warning system for the biological effects of global change in climate (Southward, 1991).

I 1.3 MATING SYSTEM

Most sessile aquatic organisms release eggs and sperm into the water for external fertilisation, or transmit sperm between mates through the water. Barnacles are different, they are hermaphroditic organisms. The general prevalence of hermaphroditism among sedentary and sessile animals has been attributed to difficulties in gaining access to a mate (Tomlinson, 1966; Ghiselin, 1974). Such problems will be particularly acute in early colonising individuals, and in populations habitually at low effective density. There are two possible advantages of simultaneous hermaphroditism where opportunities to mate are limited. Firstly, any two mature individuals will be potential mates for purposes of cross-fertilisation (Tomlinson, 1966). Secondly, a completely isolated self-compatible individual will still be able to produce sexual progeny by self-fertilisation (Altenburg, 1934; Baker, 1955). Barnacles perform internal fertilisation by copulation (Klepal, 1990), which imposes extremely restricted mating distances limited by the length of the penis (in *S. balanoides* about two to three times the shell length (Stubbings, 1975)). Gregarious settlement of cyprids (Knight-Jones, 1953) makes cross-fertilisation possible by producing dense aggregations of potential mates.

Chthamalus stellatus may self-fertilise in reproductive isolation, that is when the nearest neighbour is too far away for copulation to occur. It does it only after a delay, however, and such offspring sometimes exhibit reduced viability (Barnes and Crisp, 1956; Barnes and Barnes, 1958). Barnes and Barnes (1958) suggested that facultative self-fertilisation might occur in all species of *Chthamalus*. Specimens of *C. montagui*, isolated before the beginning of the reproductive season, were found to carry broods and release larvae believed to be viable (F.P. personal observation; experiments to test this capacity, were carried out both in the field and in the laboratory). *Semibalanus balanoides* and *Elminius modestus* appear to be obligate cross-fertilisers, failing to reproduce in the absence of a mate (Barnes and Crisp, 1956).

I 1.4 BREEDING

Barnacles brood developing embryos within their mantle cavity before releasing larvae in the first of six naupliar instars.

Various studies have been carried out on the breeding habits of *Chthamalus montagui* and *stellatus*. Unfortunately many were before the two species were split by Southward (1976). Patel and Crisp (1960) observed that *Chthamalus* would only breed at temperatures equal to or above 15°C; this implies that the length of the breeding season should vary with latitude, becoming progressively shorter with distance north. Crisp (1950), Crisp and Davies (1955), Le Reste (1965), Achituv and Barnes (1976) all suggested that *Chthamalus* produces several broods each year. Burrows *et al.* (1992) were the first to study in detail the reproductive biology of the two species of *Chthamalus* after they had been separated by Southward (1976). From fieldwork observations in the south-west of England, they found that both species bred between the beginning of May and the end of September. *In vivo* laboratory experiments, allowed determination of the length of time taken from oviposition to hatching. In agreement with results already found by Barnes and Crisp (1956) it was estimated that at 15°C the total brood development required about three weeks to be completed. Furthermore, *C. montagui* required 23 days to arrive to maturation, while *C. stellatus* required 26. By observing the amount of time each individual spent brooding every year, and dividing this time by the number of days each brood was retained in the mantle cavity, it was possible to estimate the number of broods released each year. They calculated that both species had an average of between one to two broods per year at high shore levels, two to three broods at mid shore and two and a half to four broods at low shore. A similar study (O'Riordan *et al.*, 1992) carried out on experimental plates in Lough Hyne, south-west Ireland, indicated that the number of broods not only differed with shore levels, but also with year classes (older barnacles producing more broods). Barnacles of both species were found to produce from 0 to 2 broods already in their first year of life. Similar behaviour had already been observed by Southward and Crisp (1954). The minimum size at which barnacles belonging to class 0+ were found to breed was 4.5 mm of rostro-carinal diameter for *C. montagui* and 4.0 mm for *C. stellatus*. In the case of *C. stellatus* though, older individuals of size smaller than 4.0 mm were also found to breed. This indicates that the capacity to breed is not always size-dependent. The main breeding season in south-west Ireland was observed between June and August, sporadic winter breeding, with production of embryos which appeared to be viable, was also observed in this area for both *Chthamalus* species (O'Riordan, 1992).

A probable explanation for the increase in number of broods with decreasing shore level, is related to the variation in food supply to different shore heights. The lower the shore level, the more the food, the more energy available for growth and reproduction (e.g. work by Bertness *et al.*, 1991 on *S. balanoides*). Differences in number of eggs per brood was also found to be correlated to food supply. In the Burrows *et al.* study (1992) barnacles from sheltered or moderately exposed shores, where particulate matter is abundant, were larger and also produced more eggs. They also found that the number of eggs per brood was very variable and was estimated to be between 500 and 1500 for an animal of 0.5 mg body weight. If food supply is so important for the reproductive output, *C. stellatus* which is mainly found on the low shore, should have an advantage over the high shore species *C. montagui.* On the other hand as *C. montagui* favours more sheltered areas where suitable food might be more abundant, its capacity to produce larvae should still be considerable. Differences in larval production, associated with differences in coastal topography and hydrography would have important implications for the larval supply and recruitment to a certain area.

The breeding system of *Semibalanus balanoides* differs considerably from that of the two *Chthamalus* species. *Semibalanus*, like other species with a boreo-arctic distribution, produces a single brood per year (Barnes and Barnes, 1968). For gonad maturation, this species requires several weeks below the critical temperature of 12°C (Crisp and Patel, 1969). In late autumn all or nearly all individuals in the same locality become fertilised within a few days of one another (Stubbings, 1975). The eggs are then incubated through the winter until the spring algal bloom when they are released in the water (Stubbings, 1975). Due to the danger of losing the egg masses while moulting, *S. balanoides* suspends quite substantially its moulting cycle during the breeding season (Crisp and Patel, 1958; Patel and Crisp, 1961). After the laying down of the egg masses the barnacle enters in a debilitated condition which results from the discharge of reproductive material; this in turn leads to an uptake of water with a subsequent reduction of feeding activity (Barnes, 1962). Growth consequently slows down. The term 'anecdysis' is used to indicate this particular physiological condition (Barnes, 1962). At the end of the breeding season, when larvae are released in the water, all the normal activities are restored and the barnacle moults. In general, larval production is carried out by individuals belonging to class 1+ but a small percentage of one year old barnacles are also capable of breeding (Arnold, 1977). Arnold also showed that barnacles with a shell length of at least 7 mm in their first year of life were producing broods. For barnacles of this age class, the number of eggs per individual was found to be less than 10% of those produced by older individuals of the same size. Individuals belonging to class 1+ but with a shell length of 6 mm were also found breeding. This result is in agreement with that observed for *C. stellatus* and implies that reproductive capacity is not only dependent on age but also on size.

According to Crisp (1959) there are differences between time of fertilisation at different levels on the shore, different grades of exposure and different latitudes. Specimens high on the shore, in sheltered situations and in higher latitudes become fertilised before those near low water, on exposed coasts or in low latitudes. Some of this information was confirmed by Bertness *et al.* (1991). These authors found that growth and reproductive output of *S. balanoides* were much more pronounced in embayed situations where primary production was extremely high. Hence, they suggested that sites with exceptionally high secondary production may serve as major suppliers of larvae and recruits within a region, as long as larval transport among sites works in an efficient way. When comparing different habitats, they also found great differences in barnacle secondary production at different levels on the shore. According to them, and in partial disagreement with Crisp (1959), at low tidal heights in exposed habitats, barnacles had higher growth and reproduction than those at higher tidal heights.

Boreo-arctic species are characterised by large brood size, large egg size and prolonged embryonic development (Crisp and Davies, 1955). Large eggs give rise to large nauplii that are probably better adapted to eating the large planktonic organisms typical of cold waters (Barnes and Barnes, 1968). A significant direct correlation between brood size and aperture length was observed by King *et al.* (1993). Barnes and Barnes (1968) also studied the relationship between body weight and egg numbers and found that there was considerable variability from one individual to the other. The number of eggs produced by one individual could vary from as few as 400 to as many as 8000. In their study, egg numbers were found to be lower at upper shore levels as a consequence of the lower food intake and consequently smaller size of the individuals at this tidal height. In terms of wave action they also discovered that very exposed conditions were not favourable to high egg numbers and growth. This is probably a consequence of the detrimental effect of the waves on the cirral activity and of the poorer food supply characteristic of open coasts.

Of the four species found on the intertidal zone in Devon, *Elminius modestus* is certainly the one with the highest reproductive output. Breeding starts at a very early age: two month old animals were found to possess fertilised eggs (Crisp and Davies, 1955). As a consequence of the fact that *Elminius* is able to breed over a wide temperature range (from 6 to 20°C and above), its breeding season can last all year around (Crisp and Davies, 1955). Under excellent nutrient conditions this species can produce a brood in 14 days. It follows that the total number of broods produced in a year could potentially be 26 (Barnes and Barnes, 1968). Nevertheless, within the littoral zone, *Elminius* seems to produce broods much less frequently, possibly in relation to the nutritional status of the environment. In spring and summer the fecundity is probably limited by the rate of development of the embryos (oviposition never occurs until the previous brood is liberated); in autumn and winter by nutrition

(Crisp and Davies, 1955). *Elminius* eggs are very small and therefore more nauplii are produced per unit weight of tissue. This species has a significant advantage over boreal species like *Semibalanus* which produce only a few eggs once a year. This efficiency in egg production, probably accounts for the rapid spread of *Elminius*. Although not as productive as *Elminius*, the *Chthamalus* species also have an advantage over *Semibalanus* thanks to their production of smaller eggs and successive broods.

It is worth pointing out that both *Semibalanus* and *Elminius* can become infected with the parasite *Hemioniscus balani*. Naylor (1972) noted up to 90% infection in *Semibalanus* specimens from the south-west coast of England. Southward and Crisp (1952) suggested that in the British Isles, *Chthamalus* was never infected with the parasite. However Healy (1986) who worked in south-east Ireland observed the infection in both species. The infection prevents maturation of the ovary and so renders barnacles sterile.

I 1.5 PLANKTONIC PHASE

Another peculiarity of barnacles is the capacity to control the time at which larvae are released into the plankton. The so-called hatching substance, discovered in 1956 (Crisp, 1956), has been found in no organisms other than thoracican barnacles. This substance, a prostaglandin-like compound (Clare *et al.*, 1982; 1985), is produced by adult tissue only at a certain time of the year and is capable of stimulating larval release. In *S. balanoides* during the period between June and October, its production is largely reduced (Clare and Walker, 1986). During the winter months, its biosynthesis is restored and when environmental conditions become suitable for larval release, the substance is secreted into the mantle cavity. The resumed feeding activity of the parent after winter starvation, is the stimulus for secretion of the hatching substance. It is in this way that adult boreo-arctic barnacles tune the timing of larval release with the beginning of the spring algal bloom (see Crisp *et al.*, 1991 for review).

On the south coasts of England, barnacle larval release takes place at different times of the year depending on the species. *S. balanoides* releases larvae generally in March (Crisp, 1959), *C. montagui* and *C. stellatus* start in May/June and go on until September (Burrows, 1988; Burrows *et al.*, 1992), while *E. modestus* produces larvae all year around as long as the sea temperature does not fall below 6°C (Crisp and Davies, 1955). The released nauplii swim, feed and grow in the plankton, before metamorphosing into a non-feeding cyprid stage which settles on a solid surface to metamorphose into the juvenile barnacle. The periods of larval development in the plankton are generally influenced by water temperature. In the case of both *S. balanoides* and *E. modestus*, the length of larval development decreases with increasing temperature (Harms, 1984). In *S. balanoides* it is reduced from 30 days at the beginning of the hatching season (beginning of March) to 18 days at the end. In *E. modestus* larval development lasts 30 days in spring, but when the water temperature reaches its maximum in August it takes only 14 days (Harms, 1984). From laboratory investigations, Burrows (1988) estimated that the length of the larval development in the two *Chthamalus* species (in conditions of excess food and 19°C temperature) lasted 14 days in *C. montagui* and 22 in *C. stellatus.* From the analysis of plankton samples collected in the south of England, the same author discovered that late stages of *Chthamalus* larvae were abundant only when the sea temperature exceeded 15°C. Hence, he concluded that larvae released in May and June could fail to develop beyond the first two naupliar stages as a consequence of the low sea temperatures. Again according to Burrows, the longer larval phase of *C. stellatus* appeared to allow a greater dispersal for larvae of this species than for those of *C. montagui.* The larval dispersal range of barnacles can vary, in some cirripedes it has been recognised to be in the order of 15 to 20 km (Strathmann, 1974; Crisp, 1976). The dispersion of larvae has been viewed as a mechanism for partitioning the risk of mortality in a spatially uncertain environment (Strathmann, 1974; Crisp, 1976; Barnes and Barnes, 1977; Wethey, 1985a).

I 1.6 SETTLEMENT and RECRUITMENT

As a general definition, while the term settlement is used for those barnacles which just attached to the substratum, the expression recruitment is used to indicate those which survived the settlement period. Connell further extended this concept by indicating with the term recruits all those barnacles which not only survived the first few months of life, but also became part of the adult breeding population (Connell, 1985). Even in areas of dense barnacle cover, recruitment has been shown to reflect settlement quite well, especially low on the shore (Holm, 1990).

Several studies investigated the mechanism responsible for the exploration and the attachment of the cyprid to the substratum (see Walker (Walker, 1970; 1971; 1973; Walker and Yule, 1984) and Yule (Yule and Crisp, 1983; Yule and Walker, 1984a; 1984b; 1985; 1987) for details).

In recent years, considerable attention has been paid to the study of settlement and recruitment and their importance in structuring intertidal communities. Settlement in barnacles varies locally, regionally and temporally, but the causes of this variation are uncertain (Hawkins and Hartnoll, 1982; Caffey, 1985; Gaines *et al.*, 1985; Kendall *et al.*, 1985; Wethey, 1985b; Raimondi, 1990).

Variation in supply of competent larvae from adult barnacles into the plankton can be a first cause of variation in intensity of settlement. Often though, competent larvae are released in the plankton, but changes during the planktonic phase modify the larval supply to a particular area. Kendall *et al.* (1985), observed great annual changes in number of cyprids reaching the coast, but no changes in the reproductivity of adults living on that coast. It was then concluded that something happening during the planktonic phase was responsible for the variation in larval supply.

Variation in larval supply to the shore is believed to play an important role in influencing settlement intensity and consequently community structure. Studies on this subject have been carried out by several authors. Gaines *et al.* (1985), working in California on a strong upwelling system, found a perfect relationship between cyprid concentration in the water column and settlement, demonstrating that larval supply controls benthic communities. Sutherland (1987) confirmed this point by showing the link between the decline of a population and scarce larval supply to the area. Larval supply to a certain area can be influenced by various phenomena. Direction and intensity of the wind during the settlement season have been proved to influence the larval supply to a particular shore and modify the settlement rate on that shore. Onshore winds are generally associated with higher settlement (Hawkins and Hartnoll, 1982). In contrast, Shanks (1986), did not find a correlation between settlement intensity and wind speed or direction, but between settlement and maximum daily tidal range. He observed that peaks in the settlement occur from one to four days before spring tide. The pattern in which planktonic cyprids come in contact with the surface is very important in determining the pattern of settlement. Vertical swimming of the larvae in the water column can determine the depth of spatfalls (Hui and Moyse, 1987). Bertness *et al.* (1992) suggest that larval supply to a shore increases with wave exposure but decreases with increasing level on the shore (see also Hawkins and Hartnoll, 1982; Wethey, 1984; 1985b; Minchinton and Scheibling, 1991).

Variation in larval development can cause temporal patterns in settlement. Peaks in settlement have been attributed to possible waves in larval release (Wethey, 1984), but synchronous release has instead been observed. Larval behaviour has then been used to explain periodicity: larvae could synchronise settlement to the cycle of the moon (see Pineda, 1994 for an example). Various hypotheses have been put forward to explain peaks in settlement, but the real causes are not yet understood (Wethey, 1985b).

Cyprid behaviour has been proved to be responsible for part of the settlement pattern on the shore. Cyprid density appears to be directly correlated to the amount of bare rock available in conditions of high settlement (Minchinton and Scheibling, 1993a). When high settlement occurs, the rock surface looks like a mosaic of dense aggregates of cohorts punctuated by gaps of bare rock; in low settlement situations, organisms belonging to different cohorts are mixed together and vacant space is abundant everywhere (Roughgarden *et al.*, 1985). Under high settlement densities barnacles can adopt 'spacing-out' behaviour from other individuals (Hui and Moyse, 1987).

Gregarious settlement of cyprids has been observed in several species of barnacles, notably *S. balanoides* and *E. modestus* (Knight-Jones and Stevenson, 1950; Knight-Jones, 1953; Barnett and Crisp, 1979; Wethey, 1984). Cyprids tend to settle in the proximity of conspecifics or on barnacle bases left behind on the substratum. An exception is *E. modestus* whose larvae settle readily in contact with adult barnacles in the field, and do not discriminate between conspecific and alien adults (Hui and Moyse, 1982). Raimondi (1988) succeeded in inducing settlement of *Chthamalus anisopoma* above its upper limit on the shore by using conspecific extract. Chemical cues are thought to be responsible for this type of behaviour (see Gabbott and Larman, 1987 for review). Cues left by dead barnacles have been proved to be short-lived (less than 2 months) and unless the settlement season occurs very soon after the death of the adults the effect of these is minimal (Wethey, 1984). Chemical cues to barnacle settlement are also provided by non-barnacle organisms which share the same habitat. *Chthamalus anisopoma* was found to be attracted to surfaces where *Acanthina angelica* was present (Raimondi, 1988); *Acanthina* is the major predator of adult *Chthamalus* in California. The occurrence of this species, against which barnacles can build a predator-resistant morphology, was probably considered a reliable indicator of the presence of *Chthamalus* (Lively, 1986).

Certain characteristics of the substratum also act as cues for settlement (Bourget, 1988). Cracks and pits constitute a great attraction to cyprids (Barnes, 1956b), but at least in the case of *S. balanoides*, settlement on these surfaces does not provide any survival advantage (Wethey, 1984). In very exposed situations though, living in pits and crevices provides shelter and increases survivorship (Connell, 1961a). Regarding the orientation of the barnacle, in *S. balanoides* and *E. modestus*, this has been proved to be dependent on the direction of the incident light and on the direction of flow at the time of settlement (Barnes *et al.*, 1951).

The non-overlapping vertical distribution of two species, can result from the interaction of two factors: a restricted settlement pattern of each species to a particular height on the shore (generally the height on the shore reflects the position of the cyprids in the water column) and post-settlement mortality which affects the two species in different ways (Grosberg, 1982). Mortality at the early stages of life is often associated with desiccation problems (Foster, 1971a; 1971b). Desiccation affects high shore *S. balanoides* and *E. modestus* on both sheltered and exposed coasts. As already mentioned in the species zonation section, cyprids of *Semibalanus* settle at high shore but do not survive, limiting in this way the distribution of *S. balanoides* to lower levels on the shore. Adults of *Elminius* can occasionally be found higher on the shore than those of *Semibalanus*. This is probably a consequence of the longer period over which this species settles. A longer settlement season increases the chances of favourable climatic conditions during settlement, allowing the survival of some individuals. In the case of the two *Chthamalus* species, high post-settlement mortality was observed at low shore in *C. montagui* and at high shore in *C. stellatus* (Burrows, 1988). *C. montagui* morphology is believed to confer a better resistance to desiccation stress which allows this species to colonise the upper shore. In addition, it has been suggested (Burrows, 1988) that C. *montagui* could need a certain amount of exposure to the air in order to harden its shell plates, this characteristic would make low sites on the shore not very suitable for this species. *C. stellatus* instead, does not have great resistance to desiccation and survives better at lower sites on the shore. A later study, carried out by O'Riordan (1992), suggested that the pattern of vertical distribution of *Chthamalus* at exposed sites may be the result of the pattern of larval distribution in the water column. At sheltered sites, post-settlement mortality due to differential desiccation stress in the two species, is instead believed to be responsible for the zonation of the adults of the two species.

When distributions overlap, direct competition between two species occurs, but only in areas with a high settlement rate (this is the case of Californian species studied by Gaines and Roughgarden, 1985). The stronger competitor for space will eventually exclude the other species and, according to Hui and Moyse (1987), in evolutionary time this behaviour will influence the biogeographical distribution of the two. It can be dangerous though, to consider competition directly responsible for setting a species' geographical limits. A good example of this comes from the studies on the interactions between *S. balanoides* and *C. montagui.* Connell (1961b) suggested that *C. montagui*'s northern limit was set by competition with *Semibalanus.* Later Kendall and Bedford (1987), discovered that in Wales *C. montagui* breeds and settles over a much shorter period than in the south of England. As a consequence of this, the low level of larval supply more than the direct competition with *S. balanoides*, has to be blamed for the thinning of the *Chthamalus* populations towards its northern limit.

Separation of the season of settlement of different species can have important effects upon potential competitive interactions. *S. balanoides* settles during the spring and colonises bare surface made available by winter storms. *E. modestus* settles all year around, but mainly during the summer, and is therefore given ample opportunities for colonising bare area as it comes available (Crisp and Davies, 1955; Hui and Moyse, 1987). Temporal separation of settlement could reduce most of the competition between the fast growing juveniles of the two species. In Britain the two *Chthamalus* species settle simultaneously towards the end of the summer but, thanks to the limited overlapping of their vertical distribution, do not very often enter into direct competition (Burrows, 1988).

I 1.7 GROWTH

Studies on growth of intertidal barnacles have shown that growth rate can be influenced by abiotic (temperature, light, current, exposure, tidal level and surface contour) and biotic (food supply, population density, competing organisms, parasites, reproduction, size, species and age) factors (see Crisp and Bourget, 1985 for review).

The first studies on the growth of *S. balanoides* were carried out by Moore (1934) who observed variation in growth rate in relation to age, season and tidal level. In general the growth rate of *Semibalanus* has been recorded to be higher in younger barnacles and to increase with decreasing level on the shore in the first year of life. From the second year onward growth was observed to be faster at higher shore levels (Moore, 1934). Maximum growth has been described to take place during the summer months and slow down during the winter. Most of the factors affecting growth rate, act by modifying the food intake. For instance, individuals exposed to high flow speeds feed more frequently and grow to larger sizes than those exposed to low flow speeds (Bertness *et al.*, 1991; Sanford *et al.*, 1994). Algal cover inhibits growth by limiting access to food or by interfering with the cirral beat and hence with the feeding activity (Barnes, 1955).

The growth rate of *Chthamalus* had already been studied prior to their separation into two species (Hatton, 1938; Moore and Kitching, 1939; Barnes, 1956a). Later Burrows (1988) carried out a detailed study of the growth of the two species separately and observed that the rates of the two species were remarkably consistent over both gradients of tidal height and exposure to wave action. The only significant difference observed between the two, was that *C. stellatus* grew faster than *C. montagui* at the low levels of the most exposed shores, while *C. montagui* overtook *C. stellatus* at the most sheltered ones. These differences could be explained by differences in setation of the cirri of the two species (Southward, 1976) which would lead to differing feeding efficiencies in the different planktonic environments (Crisp *et al.*, 1981). These observations further support the hypothesis that *C. stellatus* is better adapted to low shore wave beaten habitats and *C. montagui* to mid-high shore embayed situations. *Chthamalus* growth was found to virtually cease during the winter and begin again the following spring (Fischer-Piette, 1941; Barnes, 1956a; Burrows, 1988). This winter reduction could be explained through the slowing effect of low temperatures on cirral activity and consequently on food intake.

Elminius modestus was showed to have a very fast growth rate. In only two months individuals were capable of reaching a shell length of 5 to 7 mm (Crisp and Patel, 1961). Growth was observed to be linear during this initial period and then slowed down in correspondence with the onset of maturity.

I 1.8 MORTALITY

There are various causes of mortality in intertidal barnacle communities. Many recent studies have focused on the importance of disturbance, competition and predation as causes of mortality and hence causative agents in setting distribution patterns and structuring communities (Connell, 1983; Schoener, 1983; Sih *et al.*, 1985).

In the life of a barnacle, the pelagic phase is a period of drastic morphological changes and of the highest mortality at any time of the life history. Predation by other marine organisms or failure to reach a suitable substratum in time (Lucas *et al.*, 1979), are probably the most common causes of death during planktonic life.

At the sessile stage, various abiotic (desiccation and wave action) and biotic factors (inter and intraspecific competition, predation, parasitism and human disturbance) can influence mortality.

Among abiotic factors, I have already mentioned the catastrophic effect of desiccation on the early stages of settlement of the four species in this study (refer to section on settlement and recruitment). Physiological and morphological adaptations are the only defences that sessile animals can adopt against this type of environmental stress; a reduction in the overall permeability to water loss is the secret for success.

Cyprids and spat, have been recognised to be the most vulnerable to desiccation, owing to their greater surface area to volume ratios (Foster, 1971a). Besides desiccation, very low winter temperatures can decimate marine organisms (Crisp, 1964a; Barnes and Barnes, 1966). In barnacles, low temperatures and freezing can inhibit cirral activity and consequently cause starvation (Southward, 1955; 1964). Very intensive wave action has also been shown to cause great mortality among intertidal barnacles, especially on exposed sites. Along the Ligurian coast (Italy), an exceptional storm wiped off up to 92% of the total *Chthamalus* populations living on the exposed sites (Relini and Galizia, 1991). It is worth pointing out that barnacles organised in dense colonies are more sensitive to wave action than isolated ones (Barnes and Powell, 1950). If the barnacles at the edge of the colony are damaged, the rest of the group is very likely to break up. This phenomenon has been observed particularly in 'hummocks', dense aggregates of barnacles (generally *S. balanoides* specimens) with long tubular shells, very small basal attachment and large contact surface with neighbouring barnacles (Barnes and Powell, 1950). Shanks and Wright (1986) demonstrated that breaking waves can throw rocks and pebbles against organisms on the shore and cause fatal damage to them.

In dense settlement, growth soon causes the barnacles to touch one another and become crowded, resulting in intense inter and intraspecific competition for space and food which occasionally leads to death. Barnacles have been observed to grow on top of each other, covering the opercular plates of the lower individuals and restricting their feeding activity (Barnes and Powell, 1950; Crisp, 1961). Limpets and older barnacles (Connell, 1961a; Dayton, 1971; Hawkins, 1983; Miller and Carefoot, 1989) have also been seen to displace younger barnacles or to grow over them. Algal overgrowth can provoke death too, by limiting the barnacle cirral activity and therefore the ability to feed (Barnes, 1955). Removal of barnacles by *Fucus* plants can also occur (Burrows and Lodge, 1950; Hawkins *et al.*, 1992).

The dog-whelk *Nucella lapillus* is considered the main predator of intertidal barnacles along the British coasts. According to Connell (1959; 1961a) in areas where this gastropod is abundant, the lower limit of *S. balanoides* distribution is set by *Nucella* predation. Predation by this dog-whelk tends to affect larger individuals rather than smaller ones (Dunkin and Hughes, 1984). The same authors observed two different methods used by *Nucella* to attack barnacles: smaller individuals tended to be prised open, larger ones are drilled. Most of the studies carried out in Britain have aimed to investigate the effect of predation by *Nucella* on *S. balanoides* (Connell, 1961a; Hughes and Burrows, 1990). Recently, some work has also been done on predation on the two *Chthamalus* species (Burrows, 1988). *Nucella* not only selects prey on the basis of size within species (as shown in *Semibalanus*) but also between species, Burrows (1988) discovered that dog-whelks fed preferentially on *C. stellatus* which is the largest species available. Regarding the predation technique used, *Nucella* was found to prise the smaller *C. montagui* and drill the larger *C. stellatus*. Predation of *Nucella* on *E. modestus* was found to be carried out by means of prising (Barnett, 1979). It is important to remember that due to the effects of tri-butyltin (TBT) pollution, during the seventies dog-whelks on British coasts declined. Their role in controlling population structure through predation may therefore be lower than in the past (Hawkins *et al.*, 1994). Other organisms such as small crabs, fish (such as blenny and wrasse), nudibranchs, polychaetes, flatworms (Hurley, 1975) and nemertean worms (Paine, 1981) could be important predators of barnacles, but their effect on barnacle populations is probably limited when compared with dog-whelks.

The main barnacle parasite, *Hemioniscus balani*, can have a weakening effect on barnacles, increasing their risk of mortality. All four species in this study, and in particular *S. balanoides* and *E. modestus*, have been shown to be affected by this parasite (Naylor, 1972; Healy, 1986).

Human disturbance can affect shore communities in different ways. Human trampling on rocks has been showed to have immediate effects on various intertidal organisms, but no long-term implications (Bally and Griffiths, 1989). Devastating effects have instead been caused by oil-spill disasters and consequent use of toxic dispersants (Southward and Southward, 1978; Southward, 1979; Hawkins and Southward, 1992).

I 1.9 LONGEVITY

S. balanoides has been recognised to live for approximately three years at low shore and five to six years at higher levels (Moore, 1934). The two *Chthamalus* species are believed to live much longer. Photographic monitoring over a twenty year period of selected groups of *Chthamalus* along the Ligurian coast (Italy), showed that on relatively sheltered shores, specimens of *C. montagui* survived for at least 10 years and *C. stellatus* for 17 (Pannacciulli, 1991). Southward (pers. comm.) suggests that both species of *Chthamalus* could live for several decades. In contrast the life span of *E. modestus* is believed to be very short. According to Southward (1991), very few *Elminius* survive beyond the first year of life in South Devon.

I 1.10 OBJECTIVES

Barnacles have been shown to be good indicators of climate changes. The change in ratio of southern (*Chthamalus*) and northern (*Semibalanus*) species correlates well with temperature (see previous section on barnacles as bio-indicators of long-term climate changes). However, in order to better understand long term variation in the balance of the *Chthamalus* and *Semibalanus* populations, shorter term detailed studies of their population biology and interactions are required in the south-west of England. This was started by Burrows (1988), whose study was mainly concerned with comparisons of the biology of *C. montagui* and *C. stellatus*, following their separation by Southward (1976). Interactions between *Chthamalus* and *Semibalanus* had been studied by Connell (1961b) who showed the effect of competition in setting vertical zonation patterns. At Millport, where Connell's study was carried out, *C. montagui* is the only *Chthamalus* species present. In south-west

Britain, *C. montagui* and *C. stellatus* intermingle with *S. balanoides* making interactions more complex. *S. balanoides* is also less abundant than at Millport, and its settlement density is much less (Hawkins and Hartnoll, 1982). The presence, of the Australian species *E. modestus* on much of the south coast, increases even further the potential complexity of interactions. Therefore study of the population biology of *Elminius* is also needed.

The barnacle populations at Cellar Beach, (South Devon, see section on study site in methods for details) along the transect monitored by Southward during the past forty years (1991), were selected for this study. Over the forty year period, Cellar Beach showed most variation in the relative proportions of the different species of intertidal barnacles. During Southward's long-term study, the site could only be visited once or twice a year, as part of a wide-ranging survey of south-west England. In the present study monitoring was carried out fortnightly over one year, with subsequent less frequent follow up visits.

The period of intensive monitoring was aimed to:

- investigate changes in availability of bare surface, space occupancy by limpets and algae, and above all monitor changes in the barnacle cover and abundance and hence community structure;
- carry out a detailed investigation of settlement and subsequent recruitment in *C. montagui*, *C. stellatus, S. balanoides* and *E. modestus* during the 1991/1992 settlement season;
- determine growth rates for each species, in relation to age class and shore level;
- analyse differential post-settlement mortality in connection with timing of settlement (early versus late settling within a season), and examine variation in mortality rates at different shore levels and among age classes.

The information gathered allows consideration of whether space is limiting, what factors directly influence the balance of the species at various shore levels and how influential the invading species (*E. modestus*) has been. During the survey (see also Southward, 1991), it also became apparent that the *S. balanoides* population markedly declined. The reasons for this are discussed.

I 2. GENERAL METHODS

I 2.1 DESCRIPTION of the STUDY SITE

All the fieldwork was carried out in South Devon (England), on a shore 15 miles east of Plymouth (see Fig I 2.1). The selected site was Cellar Beach (National Grid reference SX 531477) at the mouth of the River Yealm. The freshwater input in this area is very small and consequently the fauna is fully marine. The rocky part of Cellar Beach is characterised by steeply sloping rocks made of Dartmouth slates of Devonian age. This area of the beach receives minimal sunshine almost all year around as it faces north-north-west. Desiccation does not seem to be a major problem here for the organisms which live on the rocks. Wave action is also relatively moderate on this shore; the shelter created by Wembury reef and a sand bar visible at low tide reduces the fetch and consequently the wave amplitude. Occasionally though, in conditions of high tide and strong inshore wind from the west, the shore becomes exposed to waves. Its location allows reasonable chances of working throughout the year.

I 2.2 SAMPLING DATES

The study began in September 1991, just as *C. montagui* and *C. stellatus* were beginning to settle from the plankton. Between September 1991 and the end of September 1992, the fieldwork was carried out twice a month on the lowest spring tides, with the exception of one sampling date at the beginning of January 1992.

Following this year of intensive monitoring, sampling was undertaken on three subsequent occasions over the following two and a half years. During 1993, sampling was carried out in May (at the end of *Semibalanus* settlement) and November (at the end of *Chthamalus* settlement). The final survey took place at the beginning of January 1995, almost three and a half years after the beginning of the study.

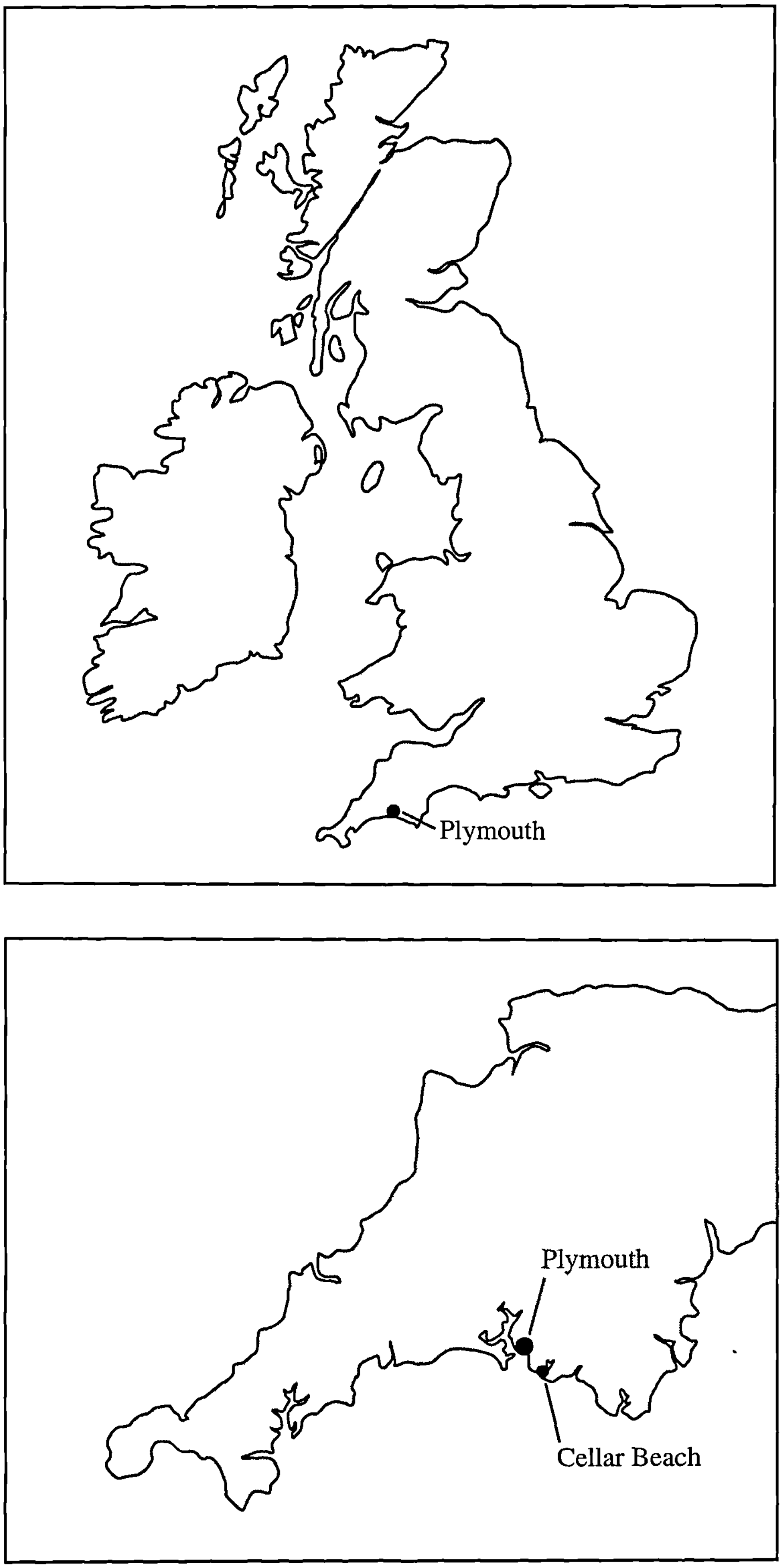


Figure I 2.1: Map showing the location of the study site, Cellar Beach.

A total of thirty sampling sessions was accomplished.

I 2.3 PHOTOGRAPHIC SURVEY

A series of small quadrats was selected at Cellar Beach on the same transect surveyed by Southward (1991) for forty years. Six replicates about 20 cm apart were chosen at high, mid and low shore. As some of the quadrats on the mid shore were partially covered with algae, another extra four were selected at this tide level. These extra four quadrats were located about 4 metres away from the original group of 6. See Table I 2.1 for details.

Table I 2.1: Details of tide levels and replicates used in the photographic survey

Shore level	Tide level	Tidal height (above C.D.)	n° of replicates	code for replicates
HIGH	MHWN	4.3 m	6	H1, H2, H3, H4, H5, H6
MEDIUM	MTL	3.3 m	6 4	M1, M2, M3, M4, M5, M6 MA, MB, MC, MD
LOW	MLWN	2.2 m	6	L1, L2, L3, L4, L5, L6

In order to locate the camera over exactly the same area on repeated visits, small holes were drilled in the rock to position a tripod. The tripod consisted of a chemical retort stand which carried a frame in which the camera was placed. A bracket was attached to the stand to carry a side mounted flash. A perspex plate supported by an aluminium frame was also attached to the tripod and placed between the flash and the photographed area to diffuse the light and help fill shadows.

Close-up photographs were taken on a black and white "Panatomic-X" film with a SLR camera fitted with a 50 mm lens in macro mode. Each photograph covered an area of approximately 9 x 6 cm^2 which was taken as the quadrat size. Unfortunately, due to movements of the camera in the tripod frame, which at the time went undetected, some of the photographs covered only half of the original quadrat. This problem affected a few sets of photographs taken half way through the study. I will refer to these as "half photographs". The problem was solved by screwing the camera firmly onto the tripod.

On every sampling session two photographs were taken of each quadrat: one with a five pence coin used as a reference scale, the other one without a coin to avoid loss of information due to the coin presence. Photographs without the coin were enlarged to approximately 24 x 16 cm^2 for easier analysis and printed out on Ilford Multigrade III photographic paper.

To determine the scale of each photograph it was necessary to measure the coin on the negative, refer this to the size of the negative and the size of the negative to the one of the enlargement. In order to get the most accurate measurement of the coin, each negative was enlarged on an Image Analyser and the coin diameter measured. The scale for each one of the 660 prints was determined. The total number of 660 was obtained by photographing the 22 fixed replicates on each one of the 30 sampling sessions. In the text, I will use the term "set of photographs" to refer to the photographs taken of each one of the 22 replicates on a single sampling session. With the expression "series of photographs" I will mean instead the sequence of 30 photographs taken of each single replicate.

I 2.4 COLLECTION OF ROCK SAMPLES

Additional destructive sampling was undertaken at corresponding tide levels. Five random rock samples bearing barnacles and being approximately 40 cm^2 each in size, were collected from the three shore levels on each sampling session (with the exception of January 1995). Care was taken to select samples from all over Cellar Beach in order to get a representation of the entire shore. All samples were carried back to the laboratory, analysed (see below) and left to dry out naturally. They were then stored for subsequent reference. To avoid development of mould, the rock chips were wrapped in paper envelopes instead of plastic bags.

I 3. METHODS and RESULTS

I 3.1 CHANGES in the COMMUNITY STRUCTURE

METHODS

I 3.1.1 Changes in amount of bare rock, percentage cover of barnacles, algae and limpets from the analysis of photographs

Initial analysis of photographs concentrated on establishing patterns of space occupancy by the major components of the community. The natural changes in percentage cover of barnacles, bare rock, algae and limpets, were determined throughout the period of study by analysing photographs from each one of the sampling sessions.

A sheet of acetate paper on which a regular array of 100 dots had been plotted was placed over each photograph. This method is known as the "point intercept" method (Meese and Tomich, 1992). The number of points covering barnacles, bare rock, algae and limpets was recorded. To limit the generation of noise in the data due to analysing each time different parts of the photograph, the grid was placed always in the same position on each photograph of the time series. In this way it was possible to follow more or less exactly the real changes in the percentage cover of that particular replicate through the time.

The "half photographs" were left out from the percentage cover analysis as it was thought they would have not supplied all the necessary information.

Six out of the ten mid-shore replicates had to be left out too. This was due to algal overgrowth on these six replicates. The general measures of percentage cover in fact, would have been affected by the predominance of the algal cover.

I 3.1.2 Changes in species composition of barnacles from the analysis of photographs

In the analysis of barnacle species composition two techniques were adopted. Both percentage cover and abundance of individuals were assessed. Because of differences in size between species these two measurements give differing results but both give ecologically useful information. Percentage cover indicates the area of space occupied by each species. Large species may occupy a large area but be of low overall abundance.

I 3.1.2.1 Percentage cover

The percentage cover of the four species of barnacle present at Cellar Beach was determined using the point intercept method described above. Photographs of each replicate were analysed monthly.

I 3.1.2.2 Absolute abundance

Changes in number of individuals belonging to each of the four species was estimated by sub sampling the area in question. This method is the one which has been used by other authors (Moore, 1936; Southward, 1985) to investigate this problem in barnacles. Its application in this study will enable direct comparisons with the previous work.

The following approach was then used. All photographs were analysed at the beginning of the study to see if there was any marked difference in species ratio between replicates at each tide level. Only small differences were noted. Four replicates were then randomly chosen at each of the three tide levels. An area of 10 cm^2 (2 x 5 cm) with a dense barnacle cover was selected on each of the 12 replicates. This area was carefully chosen in that part of the quadrat which was common to all the photographs, including the "half photographs". All barnacles in this area were counted and scored for species. When species were scarce, such as *E. modestus* on the high shore, the sub sampled area was increased from 10 to 25 cm^2. The same area was analysed on each photograph of the time series. An overall picture of the changes in barnacle community structure over the three and a half years period of study was finally obtained.

I 3.1.3 Changes in species composition from the analysis of rock samples

For every sampling date, all barnacles found on the rock samples were identified and counted. All rock samples were also measured and the amount of bare rock available estimated. The last two measurements were taken by placing a piece of acetate paper on which a grid had been plotted over the samples. The use of a sheet of acetate paper instead of a rigid grid presented its advantages, the sheet in fact could be shaped to adhere to the rock contour allowing a more accurate estimate of size. The grid was placed over the samples and the number of cm^2 covering bare and colonised rock recorded separately. Variation in species density was so estimated. As the samples were collected always from the same tide levels but from different parts of the shore, a certain amount of variation was observed among them.

Only two years of data are available for this particular study because, as mentioned before, no rock samples were collected in January 1995.

RESULTS

I 3.1.4 Changes in amount of bare rock, percentage cover of barnacles, algae and limpets from the analysis of photographs

Results for this section are presented in Table I 3.1.1 and Figs I 3.1.1a-7a.

In September 1991 at the beginning of the study, barnacles occupied the majority of space at all three tidal levels on the selected transect at Cellar Beach (see Fig I 3.1.1a-

Table I 3.1.1: Changes in % cover of barnacles, bare rock, algae and limpets over the period of study from the analysis of photographs.
The data here reported is a summary of that showed in Fig I 3.1.1-I 3.1.3 and Fig I 3.1.5-I 3.1.7.
The percentage cover values presented, are the means of the data obtained from the various replicates.

	BARNACLES				BARE ROCK				ALGAE				LIMPETS			
	average % cover			n° of replicates showing change	average % cover			n° of replicates showing change	average % cover			n° of replicates showing change	average % cover			n° of replicates showing change
	Sept '91	Jan '95			Sept '91	Jan '95			Sept '91	Jan '95			Sept '91	Jan '95		
HIGH				-	8%	37%	↑	5/6				-				-
			=	1/6			=	1/6	0%		=	6/6	3%		=	5/6
	89%	61%	↓	5/6				-				-			↓	1/6
MIDDLE				-	12%	46%	↑	4/4				-			↑	1/4
				-				-	1%-12%		=	4/4	3%-7%		=	2/4
	84%	44%	↓	4/4				-				-			↓	1/4
LOW				-				-				-			↑	2/6
	60%-85%			-	10%-36%		=	6/6	0%		=	6/6	4%-9%		=	4/6
			↓	6/6				-				-				-

↑ increase
= constant (means constant over the three year study; if fluctuation is present in the data, the lower and higher values of % cover are reported, e.g. 60%-85%)
↓ decrease

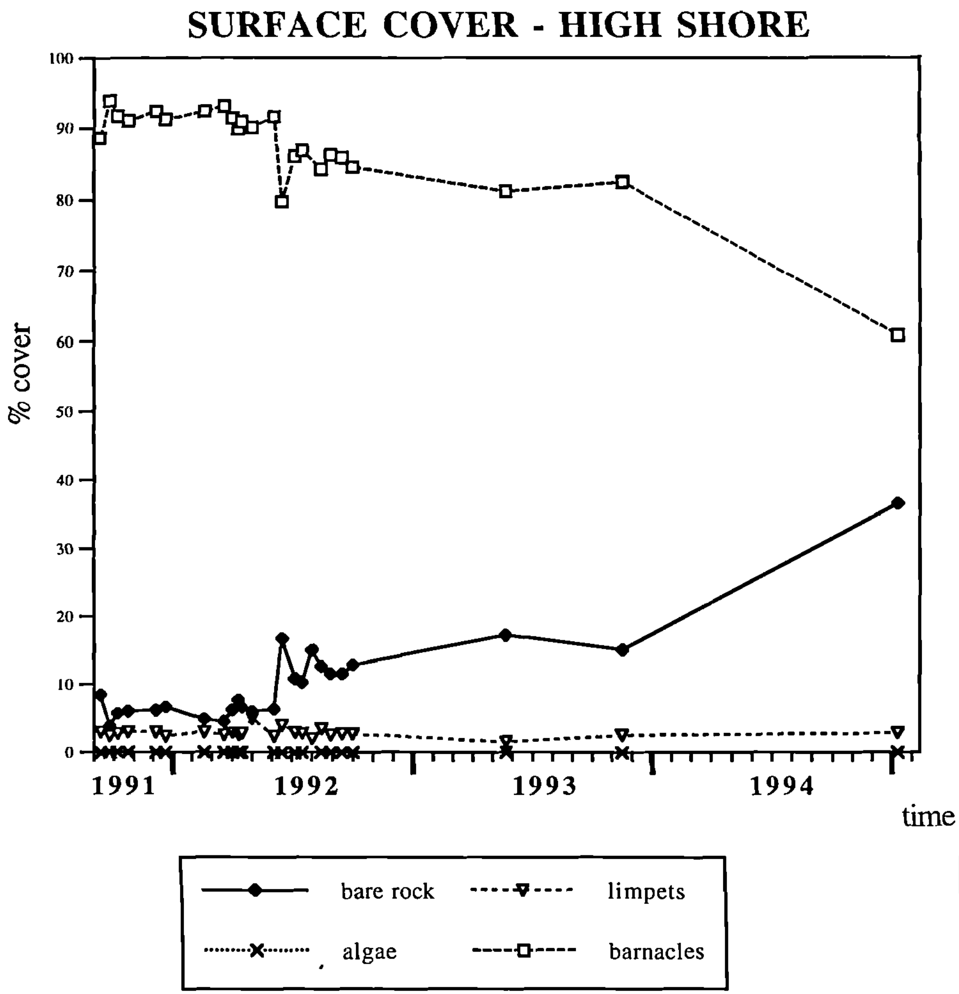


Fig I 3.1.1a: Changes in surface cover over the three years period from the analysis of photographs.
The % cover values here presented are means of the data obtained from six replicates. Error bars have been omitted for clarity.

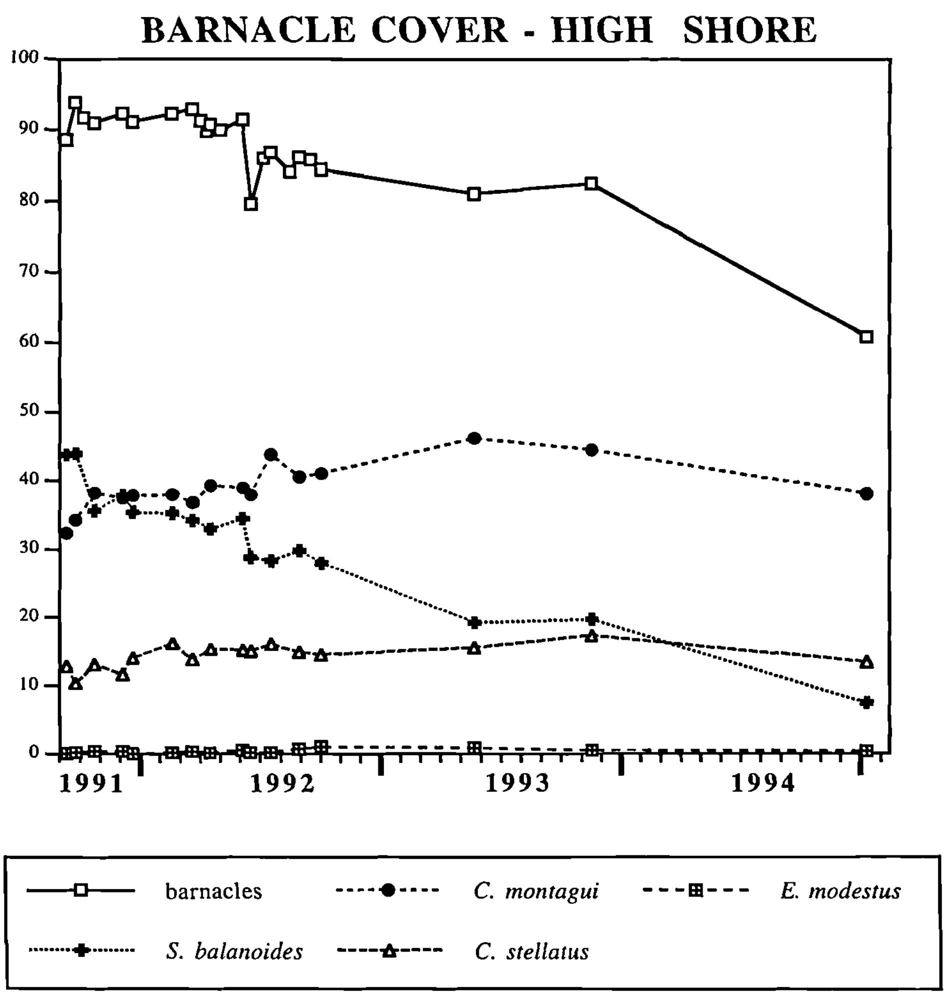


Fig I 3.1.1b: Changes in barnacle cover over the three years period from the analysis
of photographs.
The % cover values here presented are means values of the data obtained from six replicates. Error bars have been omitted for clarity.

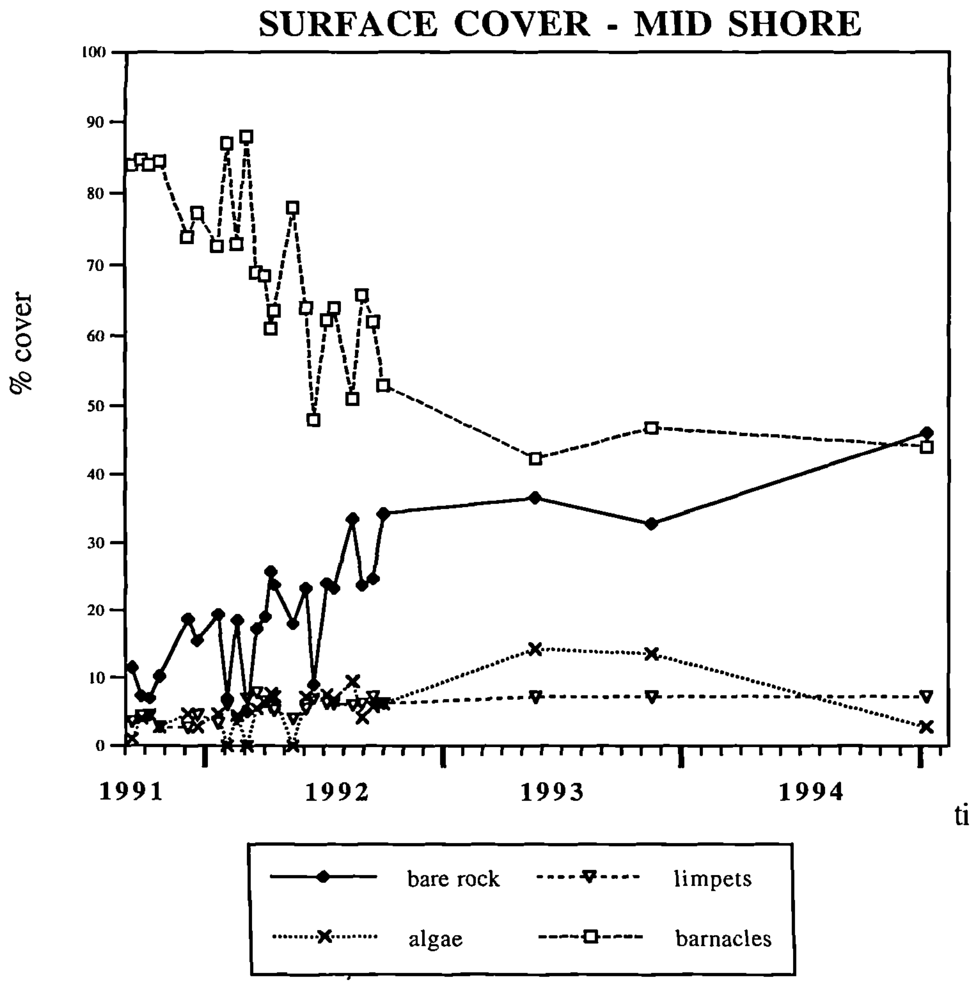


Fig I 3.1.2a: Changes in surface cover over the three years period from the analysis of photographs.
The % cover values here presented are means of the data obtained from four replicates (those without algal overgrowth).
Error bars have been omitted for clarity.

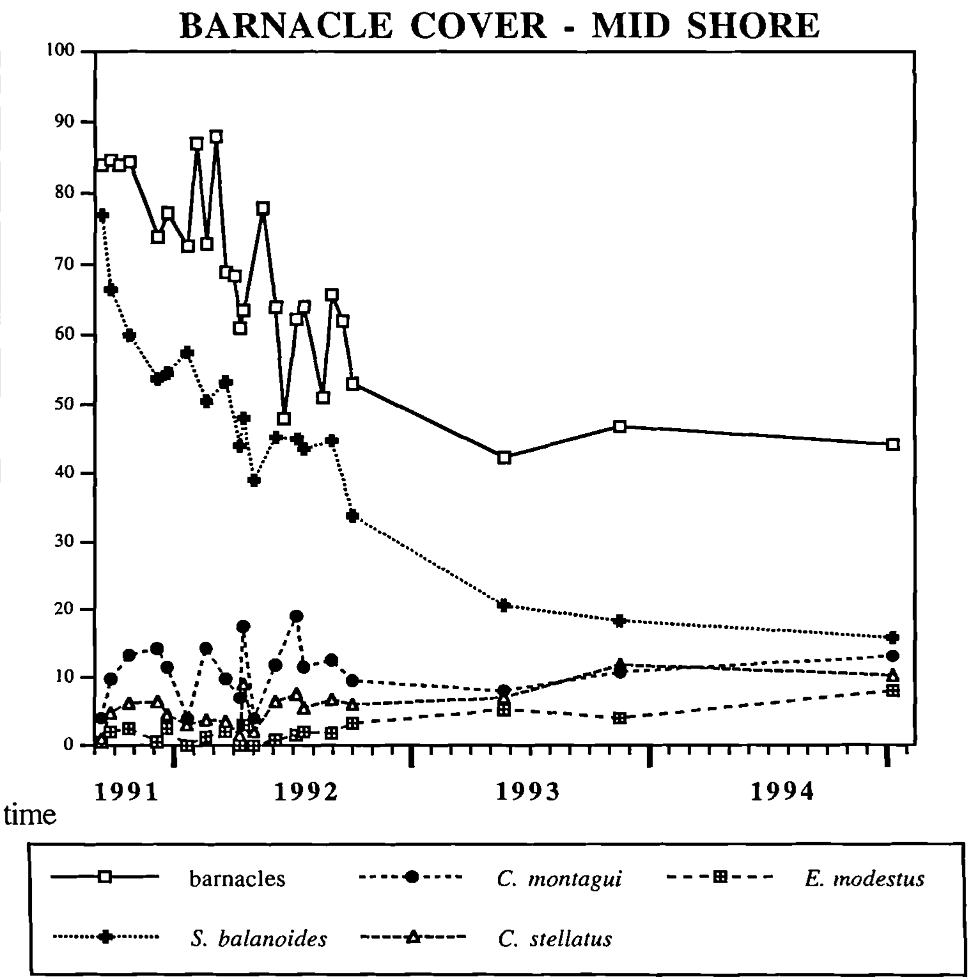


Fig I 3.1.2b: Changes in barnacle cover over the three years period from the analysis of photographs.
The % cover values here presented are means of the data obtained from four replicates (those without algal overgrowth).
Error bars have been omitted for clarity.

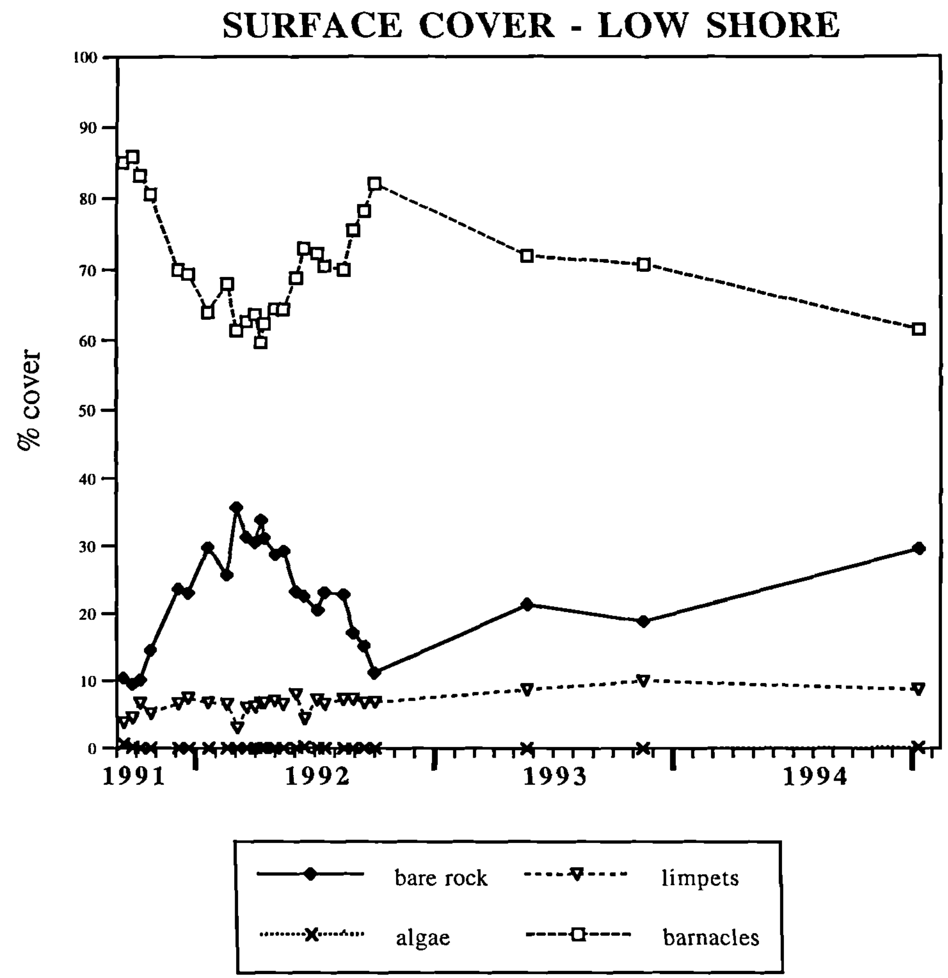


Fig I 3.1.3a: Changes in surface cover over the three years period from the analysis of photographs.
The % cover values here presented are means from six replicates.
Error bars have been omitted for clarity.

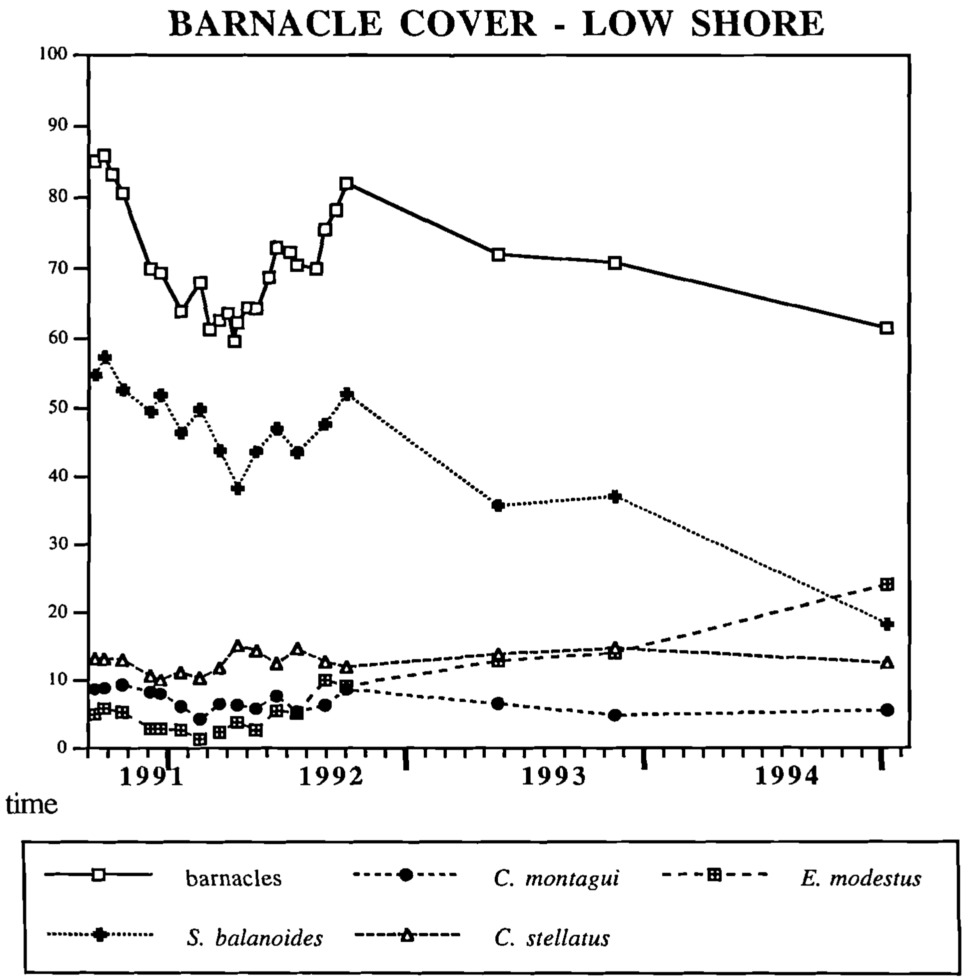


Fig I 3.1.3b: Changes in barnacle cover over the three years period from the analysis of photographs.
The % cover values here presented are means from six replicates.
Error bars have been omitted for clarity.

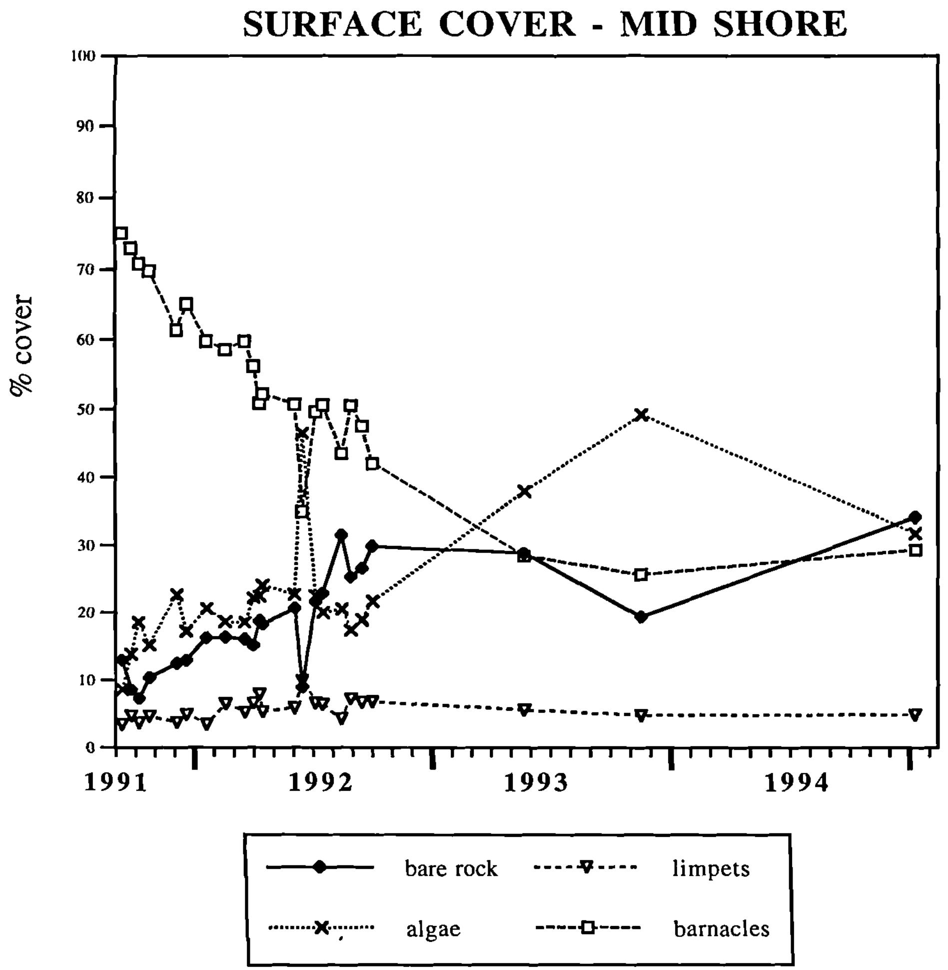


Fig I 3.1.4a: Changes in surface cover over the three year period from the analysis of photographs.
The % cover values here presented are means of the data obtained from ten replicates (those with and without algal overgrowth).
Error bars have been omitted for clarity.

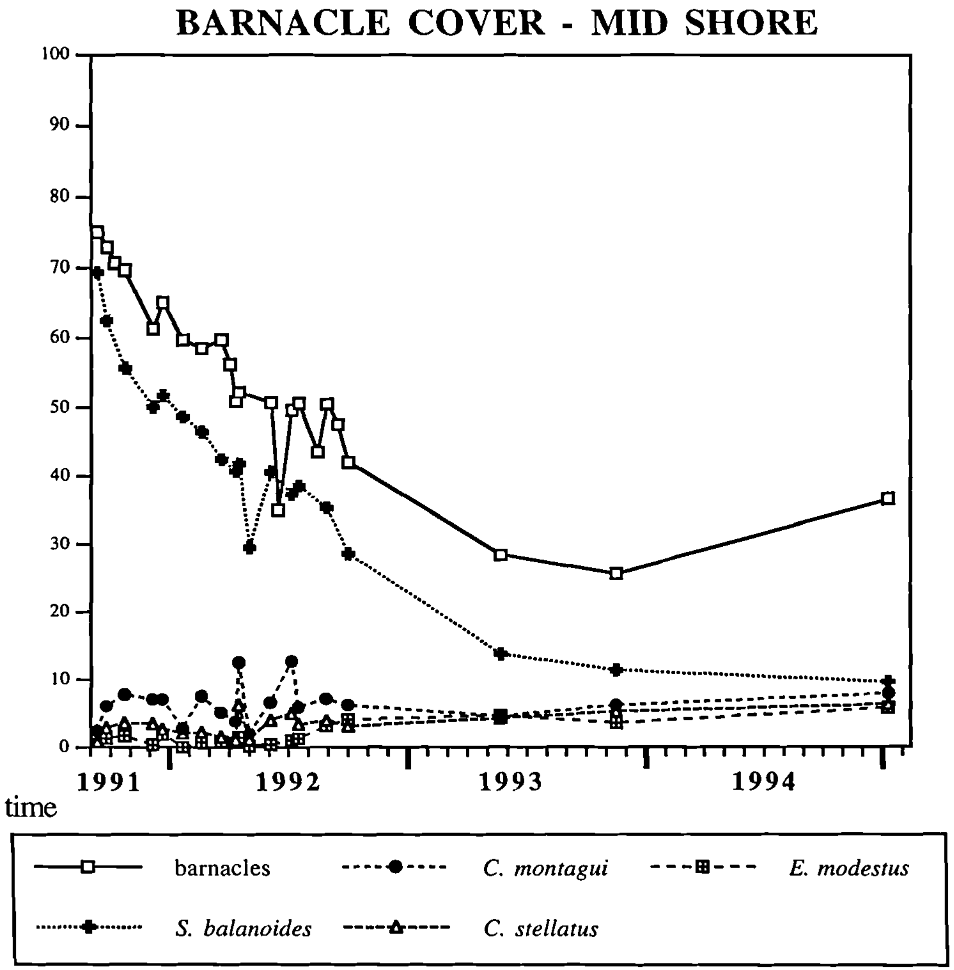


Fig I 3.1.4b: Changes in barnacle cover over the three year period from the analysis of photographs.
The % cover values here presented are means of the data obtained from ten replicates (those with and without algal overgrowth).
Error bars have been omitted for clarity.

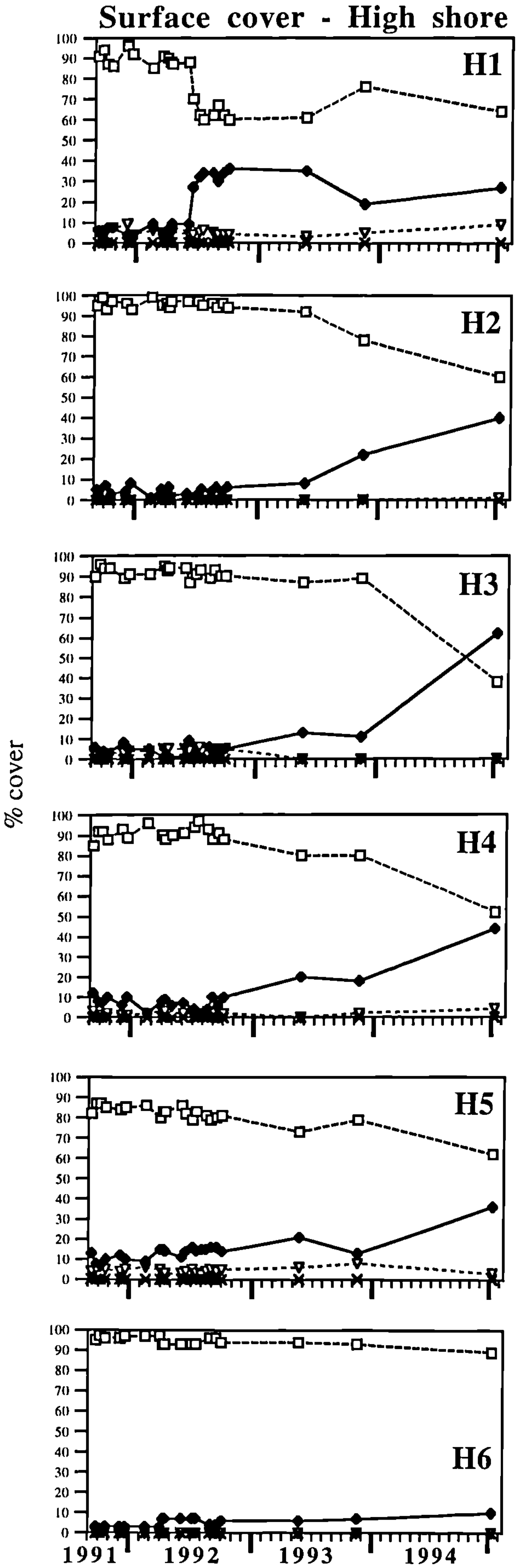


Fig I 3.1.5a: changes in surface cover from the analysis of photographs. In this case replicates have been plotted one by one. For mean values and legend see Fig I 3.1.1a.

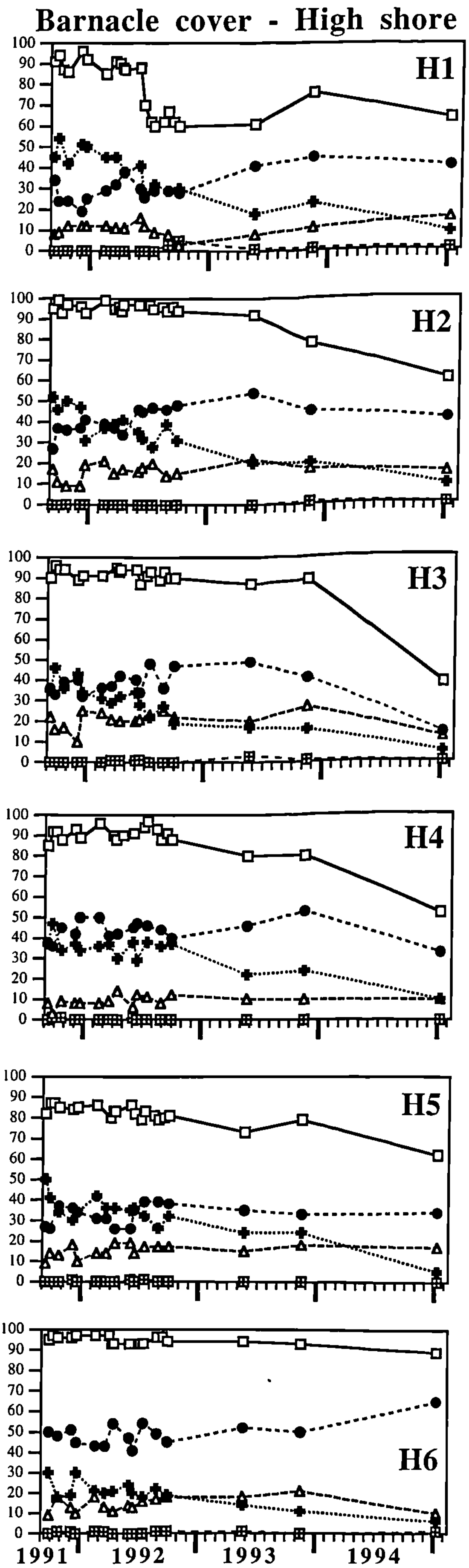


Fig I 3.1.5b: changes in barnacle cover from the analysis of photographs. In this case replicates have been plotted one by one. For mean values and legend see Fig I 3.1.1b.

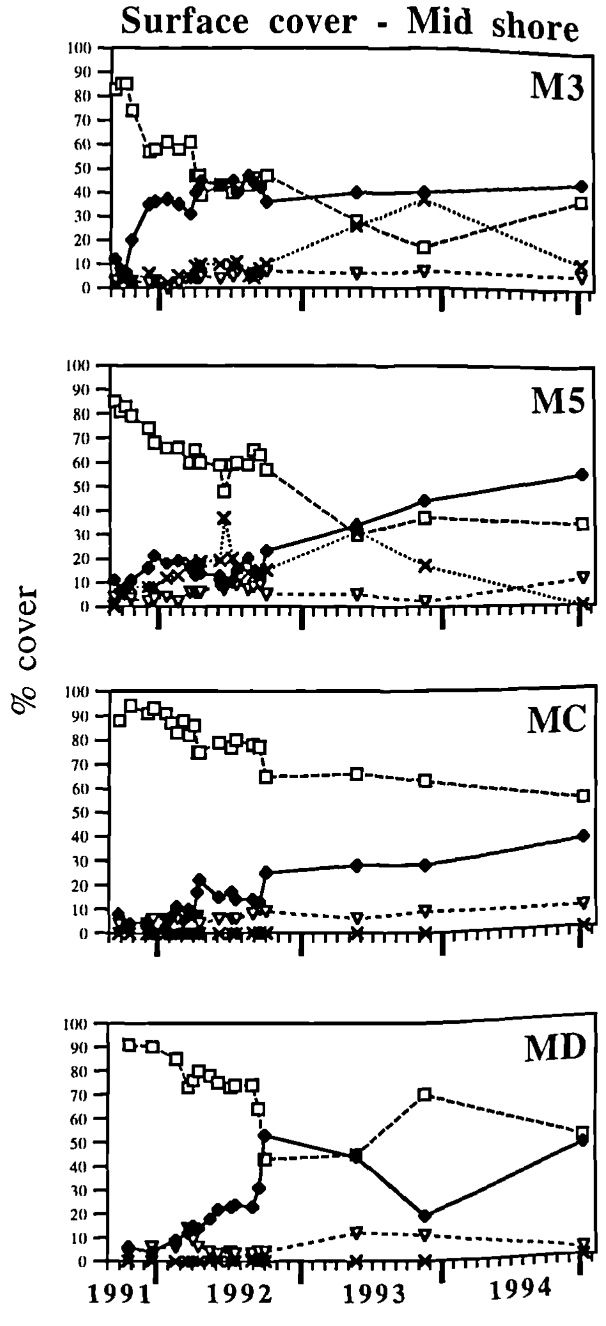


Fig I 3.1.6a: changes in surface cover from the analysis of photographs. In this case replicates have been plotted one by one.
For mean values and legend see Fig I 3.1.2a.

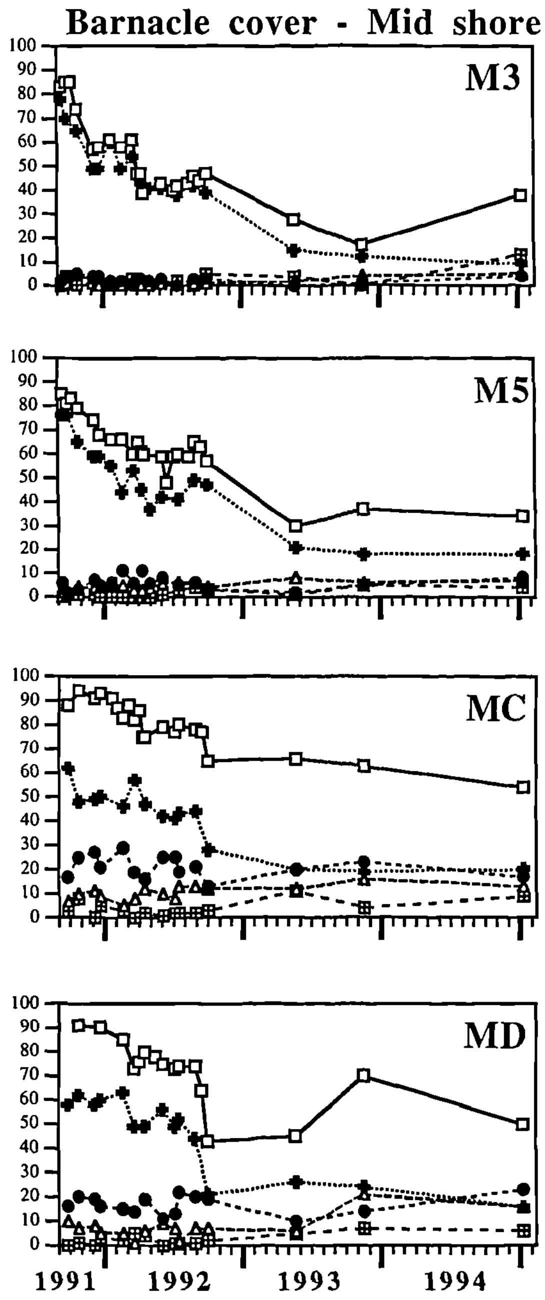


Fig I 3.1.6b: changes in barnacle cover from the analysis of photographs. In this case replicates have been plotted one by one.
For mean values and legend see Fig I 3.1.2b.

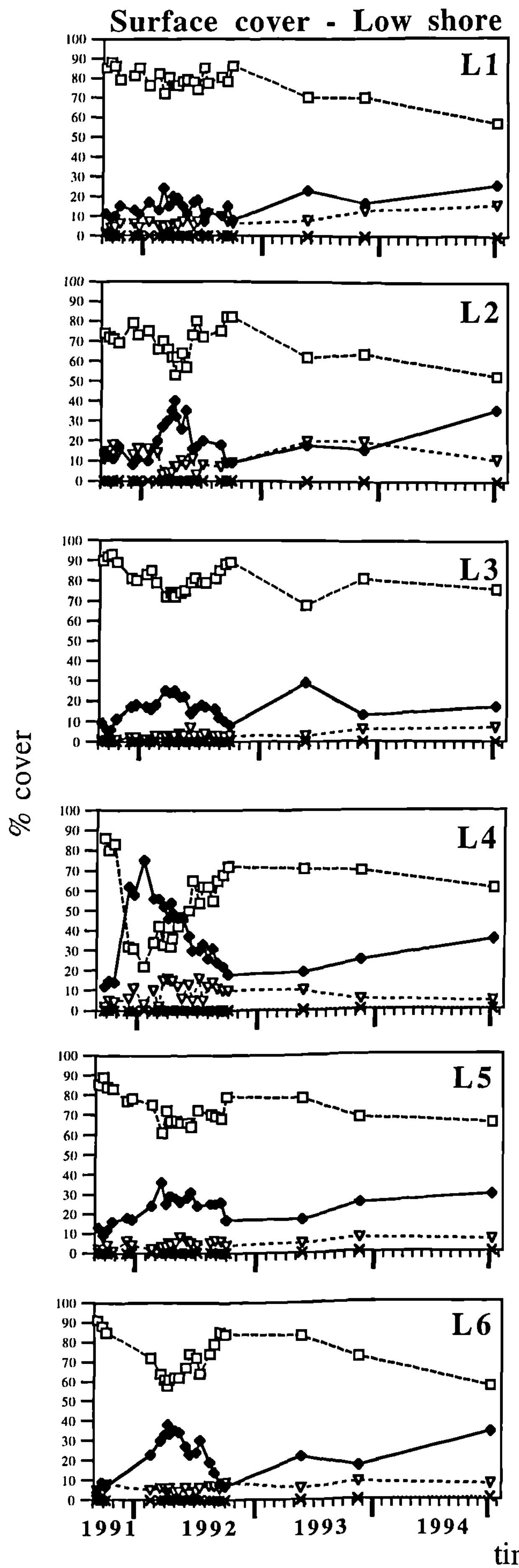


Fig I 3.1.7a: changes in surface cover from the analysis of photographs. In this case replicates have been plotted one by one. For mean values and legend see Fig I 3.1.3a.

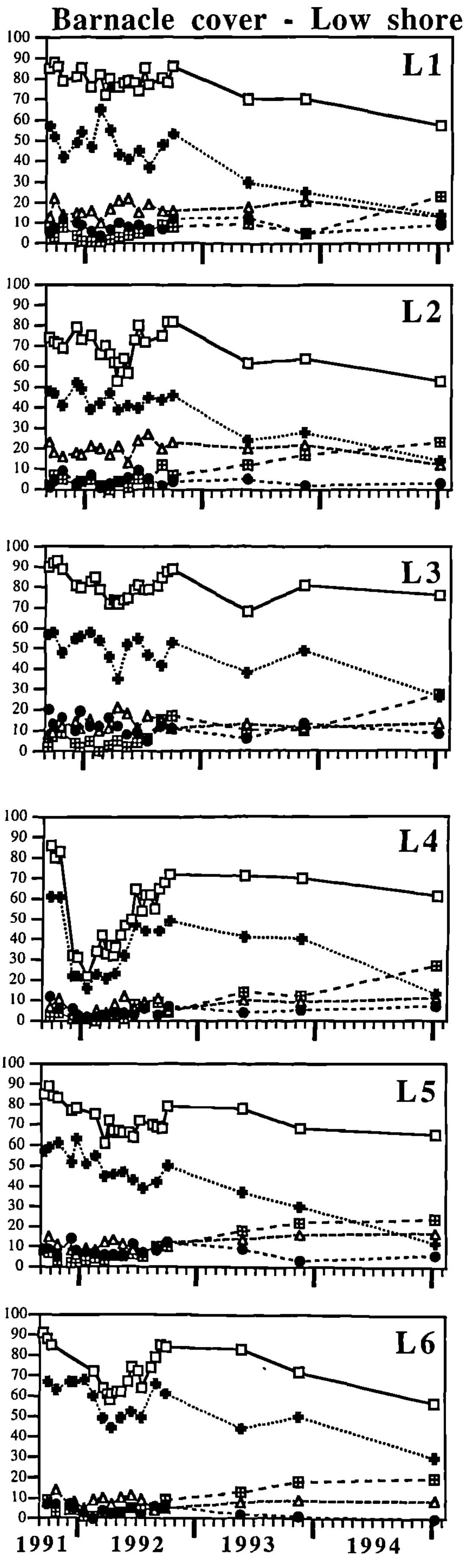


Fig I 3.1.7b: changes in barnacle cover from the analysis of photographs. In this case replicates have been plotted one by one. For mean values and legend see Fig I 3.1.3b.

3a and Table I 3.1.1). Barnacle cover was greater at the high level and lower at the other two levels. Bare rock occupied only approximately 10% of the total area at all shore levels at the start of the study. Limpets occupied relatively little space: at the low and mid-shore sites, observations over the period of study show percentage cover of between 3 and 9%. The area covered by limpets was even lower at the high shore site.

Replicates at all tidal levels were initially placed to avoid algae, in order to avoid possible loss of information on barnacles due to algal overgrowth. At the high and low shore sites, algae remained absent, but in the mid-shore *Laurencia pinnatifida* Lamour encroached into a number of the replicates. This did not create a problem in most aspects of the research, since for the study of barnacles, areas free of algae were selected on photographs. In the case of these more general measures of percentage cover though, six of the mid-shore replicates had to be rejected in order to allow comparisons among shore levels.

A general decrease in barnacle cover was observed at high and mid-shore sites throughout the period of study (Figs I 3.1.1a-2a). Cover decreased from 89 to 61% on the high shore and from 84 to 44% on the mid-shore. The majority of the decline on the mid-shore took place over the first year. At the low shore site barnacle cover showed an initial steep decline from 85% to 58% between September 1991 and April 1992 but then recovered to initial levels by September 1992. This pattern can be explained as follows: during the winter months the barnacle cover decreased, probably as a result of intensive wave action and consequent mortality. With the beginning of the *S. balanoides* settlement season, during spring time, the free area was then re-occupied. The heavy settlement of *E. modestus* during June 1992 (see section on settlement in results) also contributed to the increase in barnacle cover. This annual pattern was not evident at the high and mid-shore sites during the year of intensive monitoring. The decrease in barnacle cover resulted in a corresponding increase in bare rock (see Table I 3.1.1).

High and low shore replicates showed a constant absence of algae throughout the study. The four replicates chosen as 'algal free' at the mid-shore site did show some increase in algal cover up to a mean of 12% in 1993. Thus a small proportion of the decrease in barnacle cover at this shore level can be assigned to overgrowth by *Laurencia pinnatifida*. Fig I 3.1.4a shows the mean changes in percentage cover for the ten replicates with and without algal overgrowth. An increase in algal cover, from the initial 9% to 50% at the end of 1993, associated with a more marked decline in barnacle cover than in Fig I 3.1.2a can be seen.

To summarise, all shore heights showed a decline in barnacle cover which was a result of adult mortality not associated with algal overgrowth. The decline was gradual at mid and high shore sites. At the low shore the initial year of intensive study showed a rapid decline followed by recovery owing to high recruitment. Over the subsequent two years only three sampling dates were assessed. These were not sufficient to give an indication of this marked annual pattern.

I 3.1.5 Changes in percentage cover of *S. balanoides, C. montagui, C. stellatus* and *E. modestus* from the analysis of photographs

Results for this section are presented in Table I 3.1.2 and Figs I 3.1.1b-7b. This study of percentage cover shows the amount of space occupied by each one of the four species of barnacles. Changes in species composition have also been followed through the time.

I 3.1.5.1 High shore (Fig I 3.1.1b and Fig I 3.1.5b)

In Autumn 1991, *S. balanoides* together with *C. montagui* were the major space occupiers on the high shore. *C. stellatus* represented a minor proportion of the barnacle population (about 13%) while *E. modestus* was very rare or absent. The

Table I 3.1.2: Changes in % cover of the four barnacle species over the period of study from the analysis of photographs.
The data here reported is a summary of that showed in Fig I 3.1.1-I 3.1.3 and Fig I 3.1.5-I 3.1.7.
The percentage cover values are means of the data obtained from the various replicates.

	S. balanoides				*C. montagui*				*C. stellatus*				*E. modestus*			
	average % cover			n° of replicates showing change	average % cover			n° of replicates showing change	average % cover			n° of replicates showing change	average % cover			n° of replicates showing change
	Sept '91	Jan '95			Sept '91	Jan '95			Sept '91	Jan '95			Sept '91	Jan '95		
HIGH				-	32%	38%	↑	1/6				-				-
				-			=	4/6	13%		=	6/6	0%		=	6/6
	44%	7%	↓	6/6			↓	1/6				-				-
MIDDLE				-	4%	13%	↑	1/4	1%	10%	↑	2/4	1%	8%	↑	4/4
				-			=	3/4			=	2/4				-
	77%	16%	↓	4/4				-				-				-
LOW				-				-			↑	1/6	5%	24%	↑	6/6
			=	1/6	4%-9%		=	5/6	10%-15%		=	5/6				-
	55%	18%	↓	5/6			↓	1/6				-				-

↑ increase in average % cover

= constant (means constant over the three year study; if fluctuation is present in the data, the lower and higher values of % cover are reported, e.g. 4%-9%)

↓ decrease in average % cover

percentage cover of the various species, stayed relatively constant throughout the three year period, with the exception of *S. balanoides* whose cover decreased from 44 to 7%. At the final sampling date in January 1995, *C. montagui* was by far the most dominant species. Cover of *S. balanoides* had declined to lower than that of *C. stellatus.*

I 3.1.5.2 Mid-shore (Fig I 3.1.2b and Fig I 3.1.6b)

At the beginning of the study *S. balanoides* had a percentage cover of 77% and was by far the major space occupier on the mid-shore. Changes in total barnacle cover were therefore a direct reflection of the changes in the percentage cover of *S. balanoides.* The other three species were much less common, all having less than 10% cover. Percentage cover of *E. modestus* was consistently lower than that of either of the *Chthamalus* species. Over the three year period the *S. balanoides* population suffered a severe reduction. From the initial 77% cover in September 1991 it dropped down to a value of 16% cover recorded in January 1995. This great reduction was evident in all four replicates investigated. Between September 1991 and September 1992 the steady decrease of this species generated a large amount of bare rock as described above. Regarding the other three species, no major changes were observed over the three year period. The populations of these three species did not appear to rise with the increase in availability of bare rock. Fig I 3.1.2b shows fluctuations in percentage cover of *C. montagui*, which probably simply reflects sampling error. Fig I 3.1.4b shows the mean changes in percentage cover of the four species for the ten replicates with and without algal overgrowth. It is evident that the marked decline in barnacle cover is mostly due to the decrease of *S. balanoides.*

I 3.1.5.3 Low shore (Fig I 3.1.3b and Fig I 3.1.7b)

Once again *S. balanoides* represented the major component of the barnacle cover at the beginning of the study (55 out of the total 85%). At the beginning of the study, *C. stellatus* was found to be the second most abundant (12%) followed by *C.*

montagui (9%) and *E. modestus* (5%). Percentage cover of the two *Chthamalus* species stayed more or less constant through the three year study period while *S. balanoides* showed a marked decrease (from 55 to 18%). An interesting finding was the gradual but consistent increase in percentage cover of *E. modestus* from the initial 5% to 24% in January 1995. The distinct pattern of total barnacle cover over the first year (Fig I 3.1.3b) seems to be a result of changes in percentage cover of two species, *S. balanoides* and *E. modestus.* Both of these species show a decline in cover over the winter and an increase through spring and summer.

Overall, the most interesting result of this analysis of percentage cover was the decrease in *S. balanoides* at all tidal levels. This trend was observed in 21 out of the 22 replicates analysed. Another interesting finding was the correspondent increase in *E. modestus* at mid and especially low shore.

I 3.1.6 Changes in species abundance from the analysis of photographs

The data presented in the previous section expressed the amount of space occupied by each one of the four species. This method is a valid means of expressing species abundance, but the means of estimating percentage cover does introduce a certain amount of error. An alternative way of expressing abundance is to count the number of individuals of each species within a given area. The method employed, by choosing an area of relatively complete barnacle cover at the beginning of the study, introduced some uniformity among replicates. However, the large decrease in barnacle cover at all shore levels described below did create subsequently bare space for settlement.

Results are shown in Fig I 3.1.8-10 and are presented here separately for each level on the shore.

I 3.1.6.1 High shore (Fig I 3.1.8)

S. balanoides, with 5 animals/cm^2, was the most abundant species at the beginning of the study. Its density started to decline in February 1992. By the end of the study, in January 1995, less than 1 individual per cm^2 was found at this height on the shore. The two *Chthamalus* species stayed remarkably constant in abundance through the three year study. *C. montagui* showed an average density of 4 individuals/cm^2 and *C. stellatus* only 1.4 per cm^2. *E. modestus* was absent or very rare on the high shore. A very slight increase in its abundance (from 0 to 0.05 per cm^2) occurred from 1993 to 1994.

I 3.1.6.2 Mid-shore (Fig I 3.1.9)

Also at this shore level *S. balanoides* was the most abundant species at the beginning of the study. Its density was slightly higher than on the high shore, with 5.8 animals/cm^2. Around the beginning of 1992, this species underwent a great reduction in numbers so that by January 1995 less than 1 individual per cm^2 was present on the mid-shore. On this final sampling date *S. balanoides* was the least abundant species, being below even *E. modestus.* At mid-shore level the density of *C. montagui* was much lower than at high shore, being approximately 1.7 animals/cm^2 in late 1991. This species showed a small decrease in abundance (from 1.7 to 1) between September 1991 and November 1993. A complete recovery was achieved during the 1994 settlement season which brought the density up to 2.4 animals/cm^2, a value greater than the initial one. *C. stellatus* also showed an increase in abundance during the three years of monitoring. From the initial density of 1 animal/cm^2 it reached the value of 1.5 animal/cm^2 recorded at the end of the study. From what is shown in Fig I 3.1.9, it looks as if 1992 and 1994 were good years for the settlement of *C. stellatus*. The fourth species, *E. modestus*, was almost absent at the beginning of the survey. After a good settlement season in summer 1992, its density increased to approximately 0.5 barnacles/cm^2. Low settlement and/or mortality were probably the causes of the slight reduction in *E. modestus* abundance observed during 1993. The

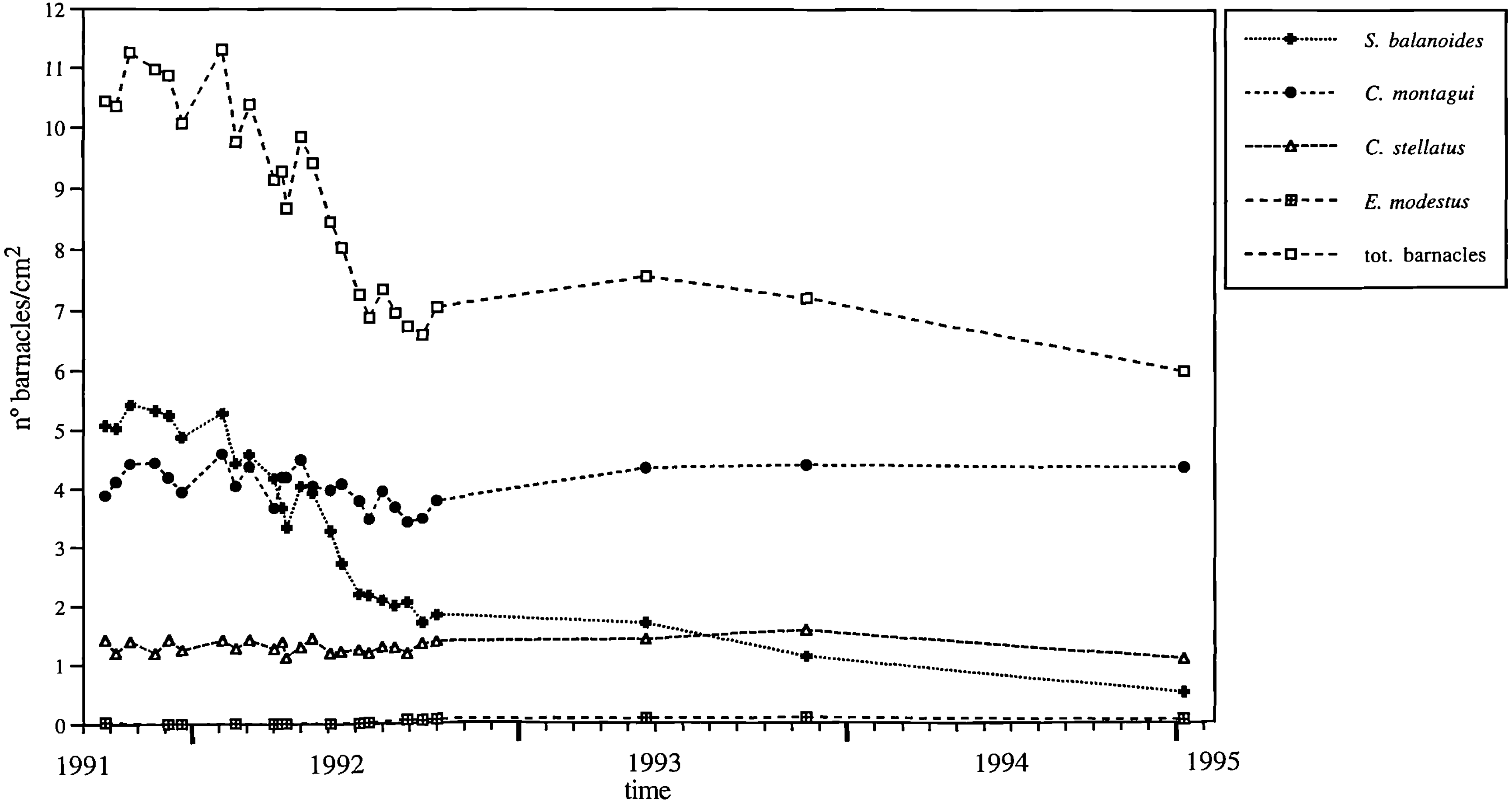


Fig I 3.1.8. High shore. Changes in species composition over a period of three years. Data obtained from the analysis of photographs. Four randomly chosen replicates were chosen at each shore level. On each replicate an area of 10 cm squared with dense barnacle cover was selected. When species were scarce (e.g. *E. modestus* at highshore) the sub sampled area was increased to 25 cm squared. The density values here presented are means of the data from the four replicates analysed at this shore level. Error bars are omitted for clarity.

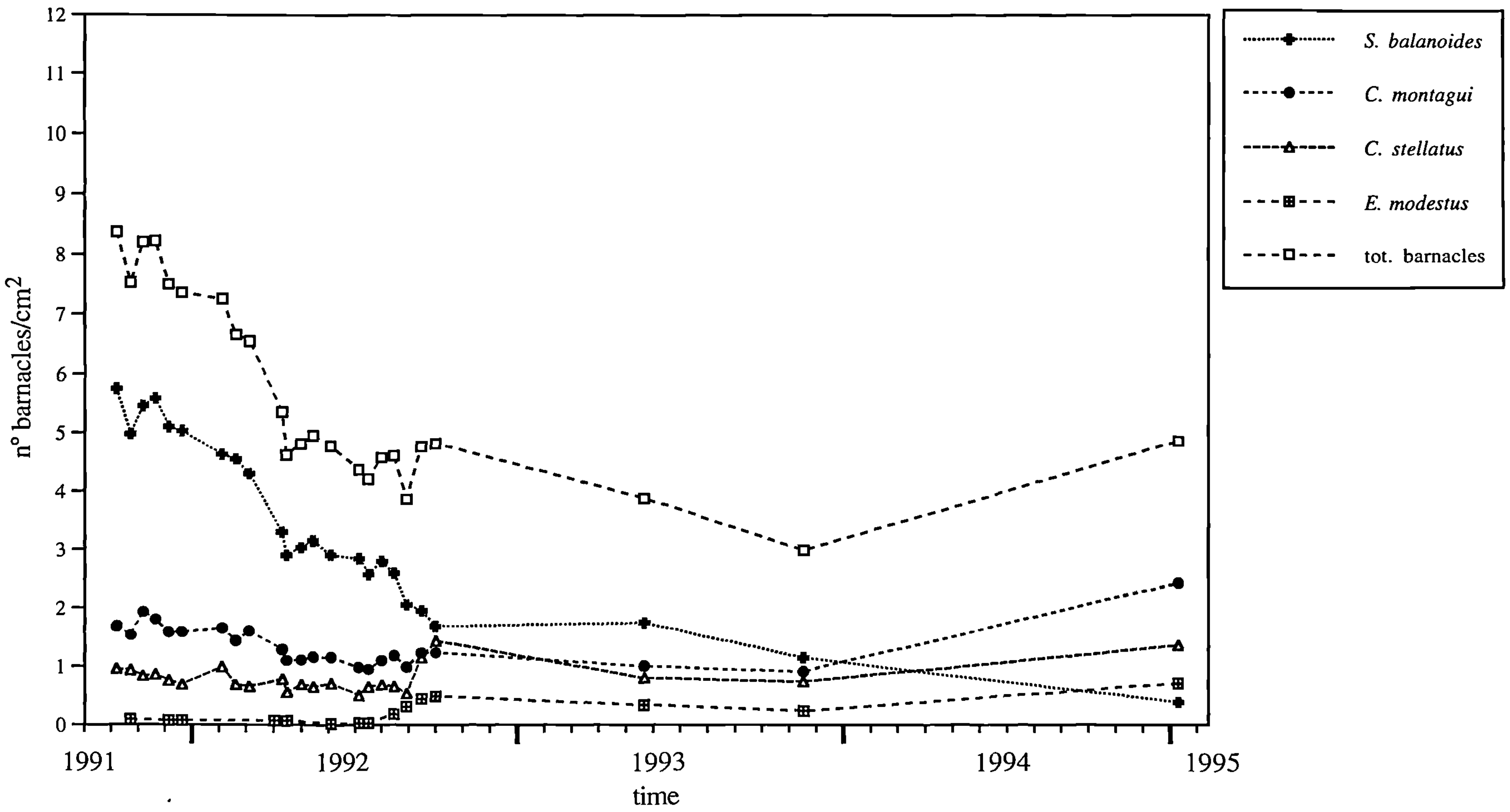


Fig I 3.1.9. Mid shore. Changes in species composition over a period of three years. Data obtained from the analysis of photographs. Four randomly chosen replicates were chosen at each shore level. On each replicate an area of 10 cm squared with dense barnacle cover was selected. When species were scarce (e.g. *E. modestus* at highshore) the sub sampled area was increased to 25 cm squared. The density values here presented are means of the data from the four replicates analysed at this shore level. Error bars are omitted for clarity.

year 1994 saw the density of this species increase again to reach approximately a value of 0.7 animals/cm^2, a great increase if compared with the initial value of 0.1 individuals/cm^2.

I 3.1.6.3 Low shore (Fig I 3.1.10)

The low shore pattern of changes in species composition, resembles quite closely that of the mid-shore. Here *S. balanoides* was even more abundant (6 animals/cm^2 at the beginning of the study) than at the upper levels. As at the high and mid-shore sites, at low shore, its abundance decreased consistently over the three year period to reach a density of less than 0.5 individuals/cm^2 in January 1995. Nevertheless, a small partial increase was observed in the spring of 1992, presumably corresponding to the settlement season. *C. montagui* showed quite a lot of variation in abundance over the three year period at the low shore site. This variation can be attributed to an increase in abundance associated with the good settlement seasons of 1992 and 1994, and the decrease during 1993. *C. stellatus* showed a similar pattern of abundance. The 1992 recruitment season seemed to be quite favourable to this species, allowing it to reach a density of 1.6 animals/cm^2. No such increases in density followed the settlement seasons of 1993 and 1994. *E. modestus* appeared to show an overall increase in density over the study period although its abundance showed obvious fluctuations with season. Large increases in density were observed following the settlement seasons of 1992 and 1994. Like the two *Chthamalus* species, the data suggest limited recruitment in 1993.

The major points of interest are as follows. The great decrease in mean density of *S. balanoides* at all three levels through the period of study·is of obvious importance. Although the other three species present may have shown some general increase in abundance, the large areas of bare space created by the decline of *S. balanoides* have not been colonised to any great extent.

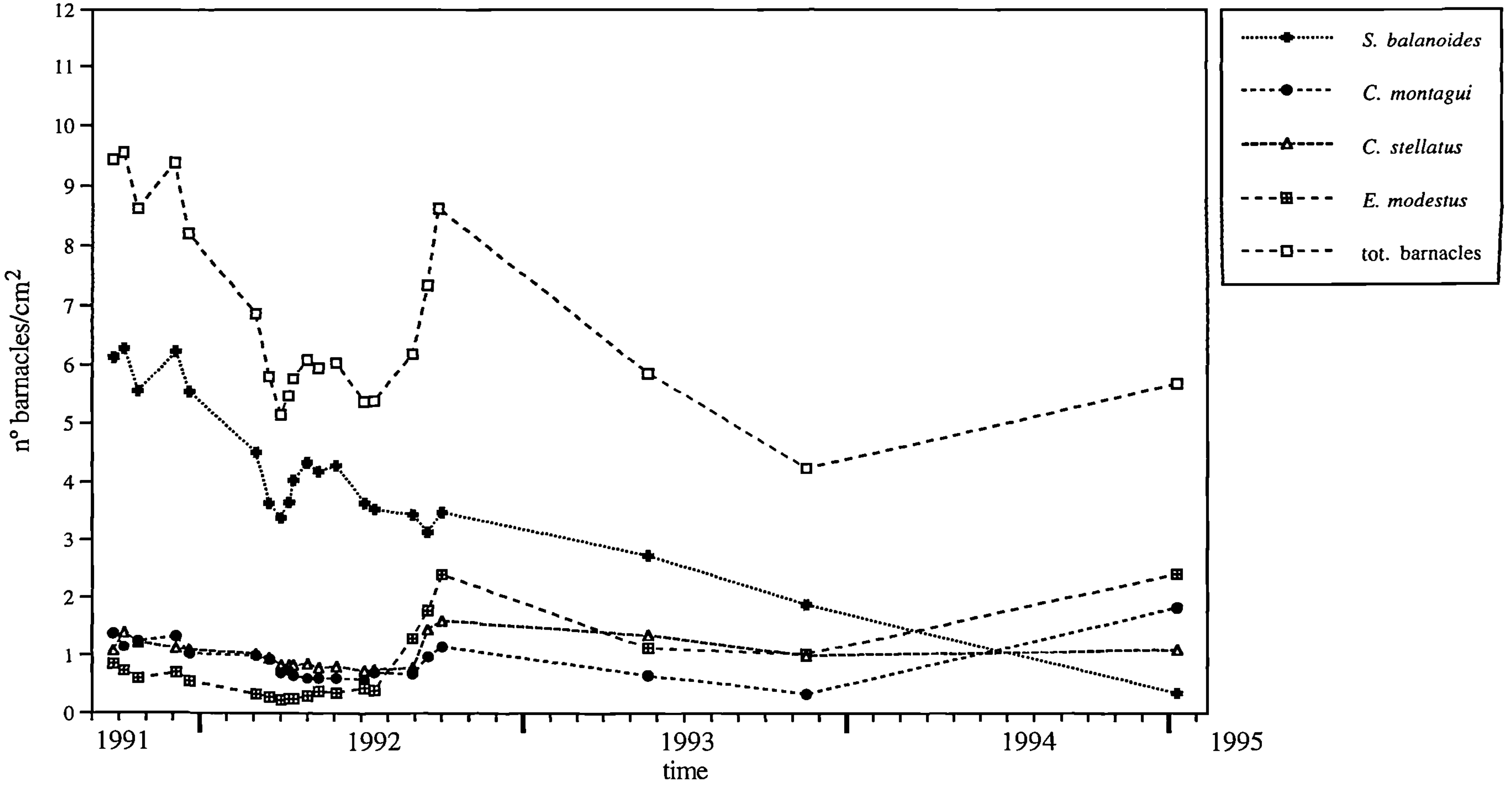


Fig I 3.1.10. Low shore. Changes in species composition over a period of three years. Data obtained from the analysis of photographs. Four randomly chosen replicates were chosen at each shore level. On each replicate an area of 10 cm squared with dense barnacle cover was selected. When species were scarce (e.g. *E. modestus* at highshore) the sub sampled area was increased to 25 cm squared. The density values here presented are means of the data from the four replicates analysed at this shore level. Error bars are omitted for clarity.

I 3.1.7 Changes in species abundance from the analysis of rock samples

The information presented so far from the analysis of photographs is representative of only a small area of the shore: the fixed quadrats selected along a transect at Cellar Beach. By collecting and analysing rock samples from all over Cellar Beach, one should expect to get more general information about the whole area. The question is then, how close are the results from the two studies and how representative is the small scale analysis of photographs of the general changes occurring on Cellar Beach?

In Fig I 3.1.11-13 two graphs for each shore level are shown. The first graph represents changes in species abundance from the analysis of rock samples collected from all over the shore. The second one, shows the same type of study carried out on photographs of fixed quadrats. Unfortunately no rock samples were collected on the last sampling session so that the comparison between the two data sets is not complete. As expected, the series of data generated from the analysis of rock samples, shows much more variation than the one obtained from the photographs. Photographs were always taken in the same fixed area and thus variation between sampling data is minimised. In addition, although an effort was made to collect rock chips from the same tidal levels where the photographs were taken, it is likely that some of the rock samples were collected from slightly different heights on the shore. If the variation is ignored, a similar trend can be seen. For mid and low shore sites this trend resembles quite closely the one from the small scale photographic analysis (Fig I 3.1.12-13). For the high shore the two graphs do not appear to be comparable (Fig I 3.1.11). The data on density of *S. balanoides* and *C. montagui*, are similar only for the first sampling session. After that, in the rock samples graph, *C. montagui* becomes the predominant species while *S. balanoides* starts to decrease. It is quite difficult to decide if this decrease in *Semibalanus* density is a genuine result or an artefact of the sampling strategy. My interpretation is that after the first sampling

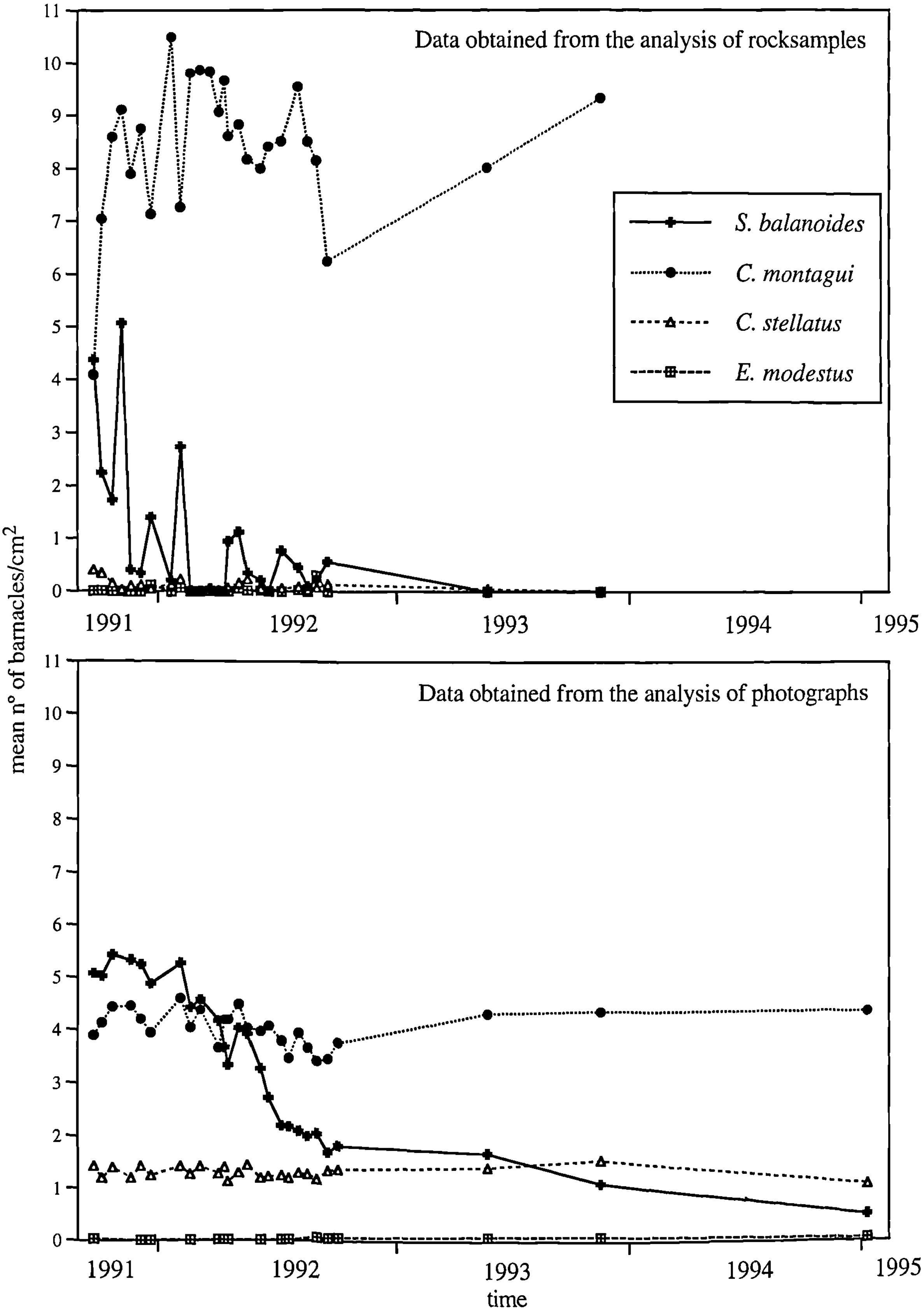


Fig I 3.1.11: High shore: changes in species abundance over a period of three years. Comparisons between data obtained from the analysis of rock samples and that from photographs. The density values are means of the data from the various replicates. Error bars are omitted for clarity.

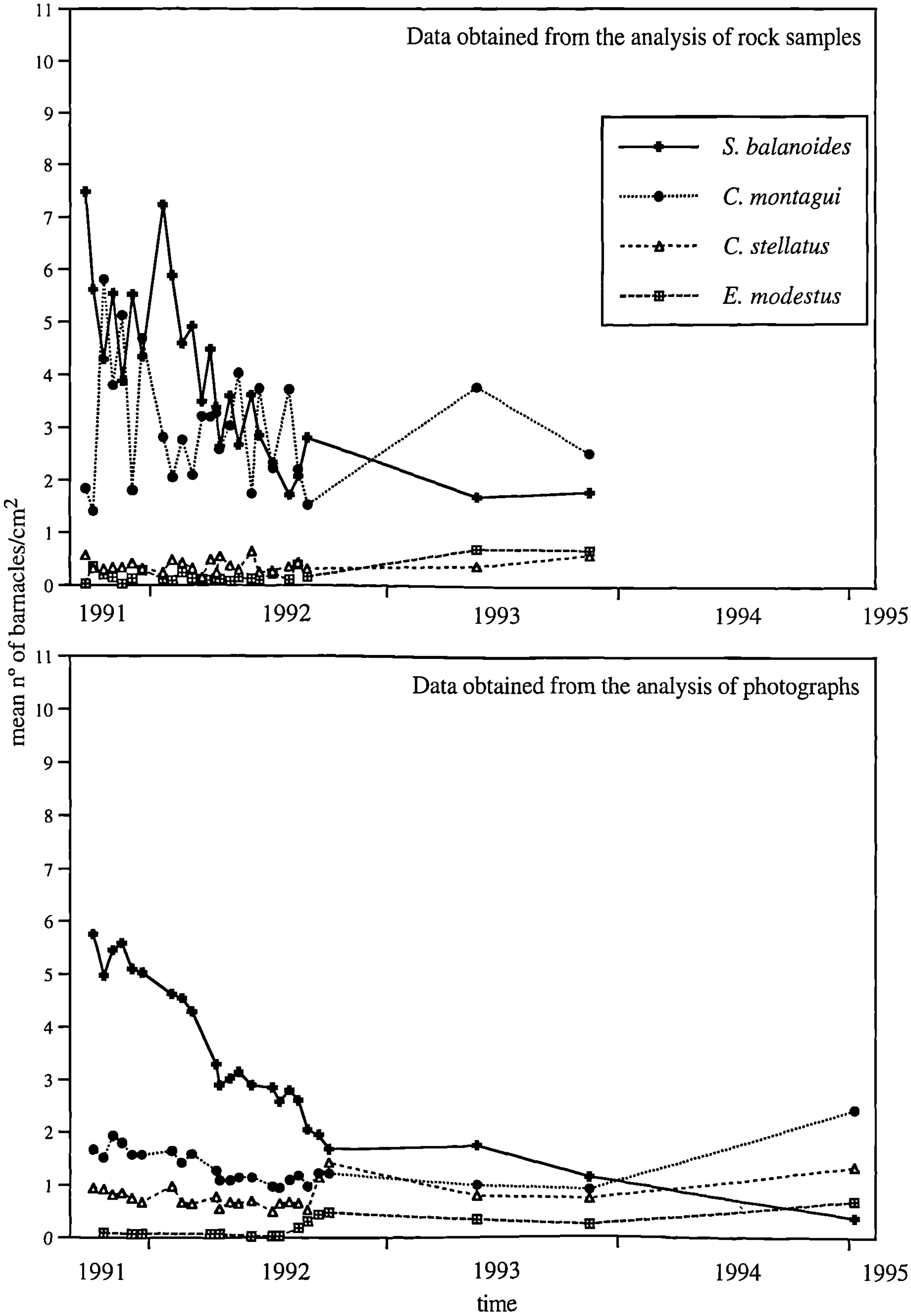


Fig I 3.1.12: Mid shore: changes in species abundance over a period of three years. Comparisons between data obtained from the analysis of rock samples and that from photographs. The density values are means of the data from the various replicates. Error bars are omitted for clarity.

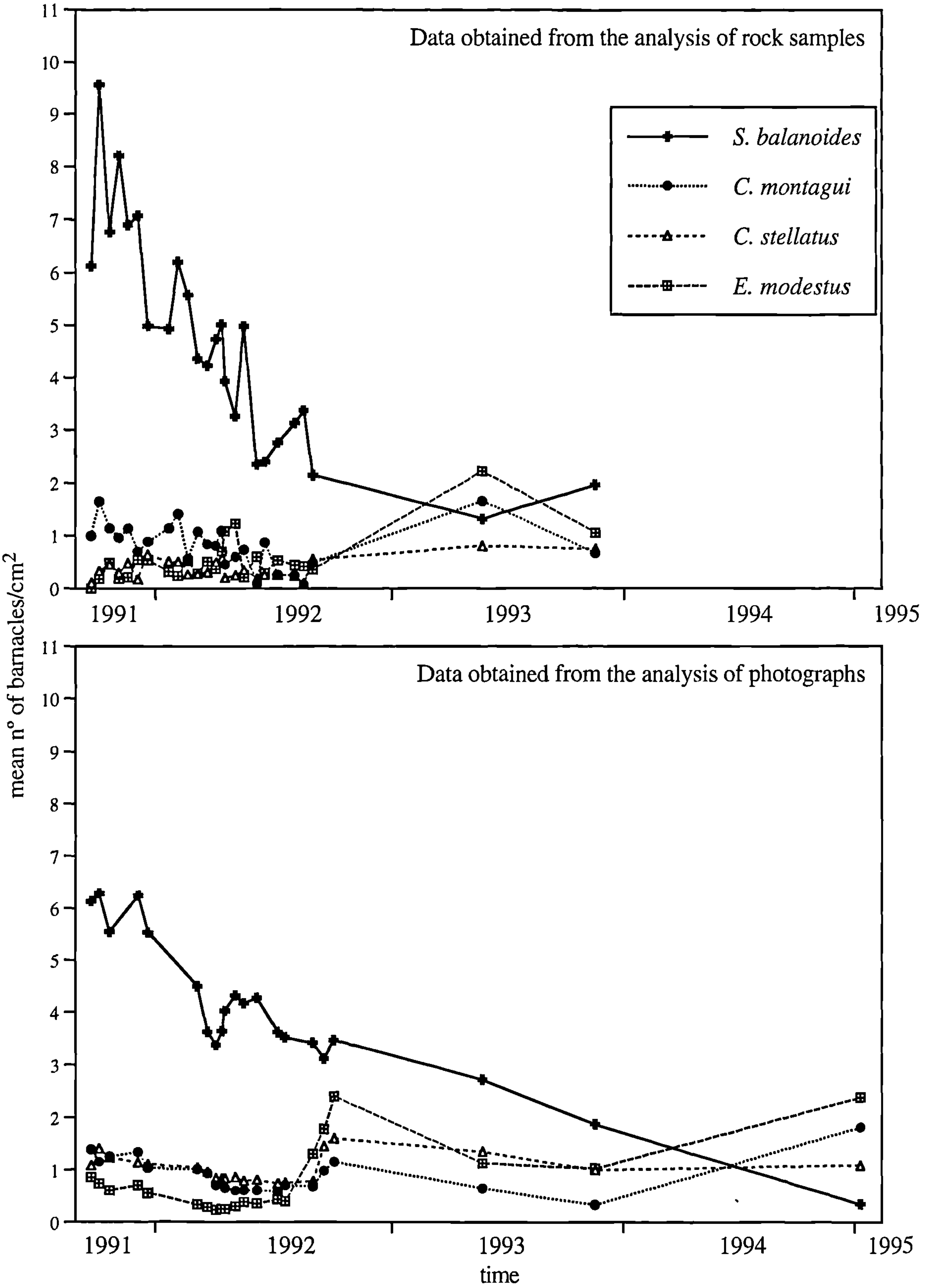


Fig I 3.1.13: Low shore: changes in species abundance over a period of three years. Comparisons between data obtained from the analysis of rock samples and that from photographs. The density values are means of the data from the various replicates. Error bars are omitted for clarity.

session, high shore rock chips were collected from a slightly higher tidal level where *C. montagui* was the most abundant species and *S. balanoides* was not very common. The decrease in *S. balanoides* was then possibly due to a change of the tidal height at which samples were collected probably combined with a natural decline of this species.

On the basis of these observations on changes in species abundance from rock samples, I think it is possible to suggest that the small scale study is quite representative of what is happening on the whole shore.

Some of the changes in community structure and species composition over the three year period can be seen in Plate I 1 (high shore) and Plate I 2 (low shore).

Plate I 1

Photographic record of the change in barnacle populations in a selected quadrat over a three year period on the high shore

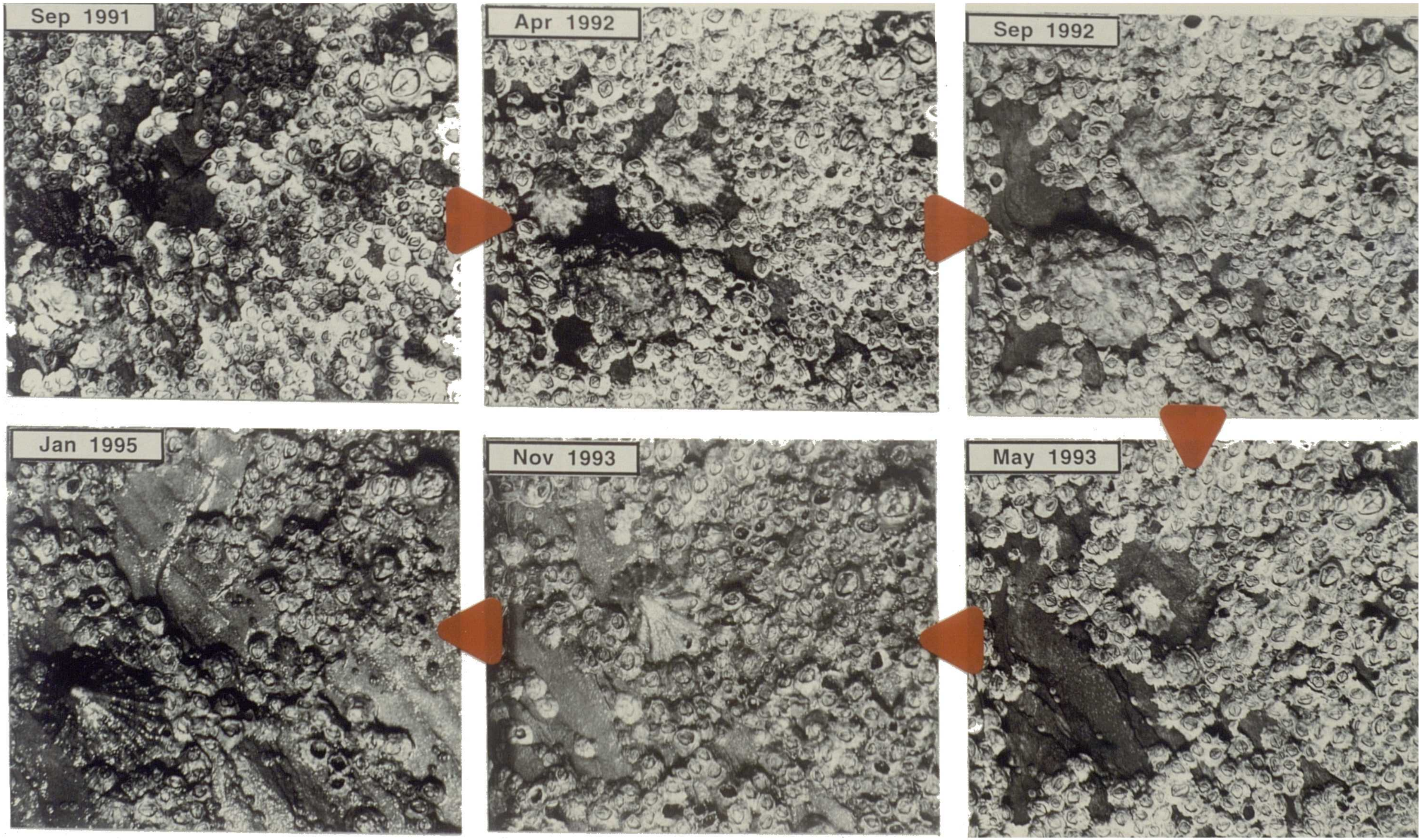
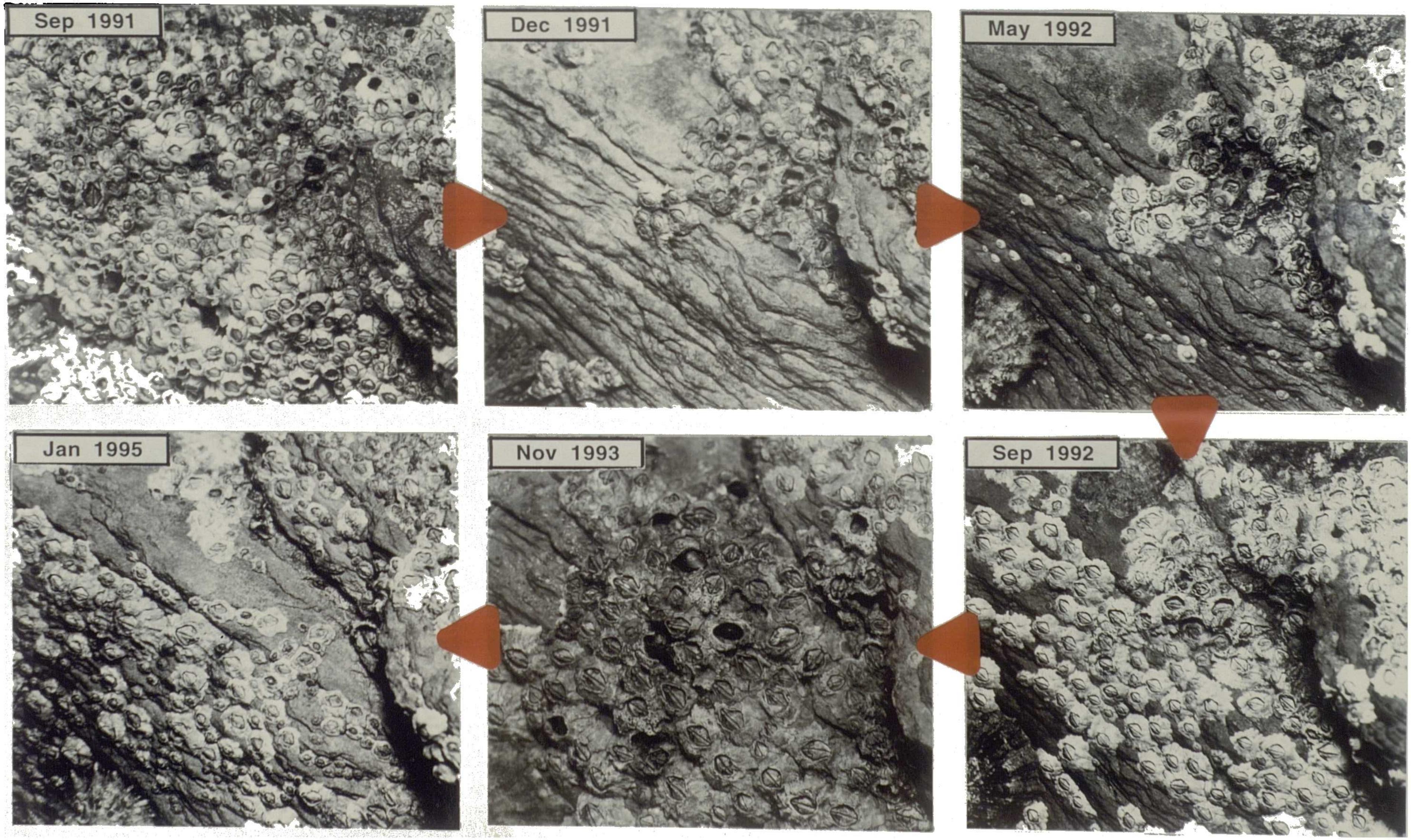

Sep 1991
Apr 1992
Sep 1992
May 1993
Nov 1993
Jan 1995

Plate I 2

Photographic record of the change in barnacle populations in a selected quadrat over a three year period on the low shore.


Sep 1991
Dec 1991
May 1992
Sep 1992
Nov 1993
Jan 1995

I 3.2 BREEDING

METHODS

I 3.2.1 The reproductive state of *Semibalanus* and *Chthamalus*

In order to estimate the time period between the first release of larvae into the plankton and the beginning of the settlement season, the following analysis was carried out. On every sampling occasion, between the beginning of February 1992 and the beginning of May of the same year, a number of specimens of *Semibalanus balanoides* were collected on rock chips and taken back to the laboratory. This collection was made to allow the analysis of the egg masses stored in the barnacles' mantle cavity. Twenty-five individuals from each of the three shore levels were measured along the length and breadth of the base of the shell. They were then separated from the rock surface and from adjacent individuals taking care not to rupture egg masses. If present, the egg masses (lamellae) were removed and put in vials containing 200 µl sea water. They were subsequently examined under a microscope. As the main aim at this stage was to determine the percentage of barnacles ready to release larvae, the specimens of *S. balanoides* were only scored as ready to release larvae or not ready.

The same type of study was carried out between the middle of June and the end of September 1992 for *C. montagui* and *C. stellatus*. Again, the aim of the study was to estimate the percentage of barnacles ready to release their broods. However in addition the developmental stages were scored using the method outlined by Achituv and Barnes (1976):

stage 1: early development from newly laid to a few cells;
stage 2: multicellular;
stage 3: from the appearance of limb buds to the presence of limbs and spines;
stage 4: from the presence of the naupliar eye to hatching;

stage 4h: those which hatched upon removal from the mantle cavity.

The term "stage 0" was added to the list and used to describe those barnacles showing no reproductive characters. For this study, stage 1, 2 and 3 were grouped together as well as 4 and 4h.

Elminius was not analysed because of the low numbers present.

I 3.2.2 Number of eggs per brood in *Semibalanus balanoides*

A detailed analysis of the number of eggs per brood of the two species of *Chthamalus*, was carried out by Burrows (1988) and Burrows *et al.* (1992). Barnes (1992) related the number of eggs to the dry body weight of specimens of *Semibalanus balanoides* collected from different levels on the shore. A brief investigation of the relationship between number of eggs and size of the animal in *S. balanoides* was carried out also in this study. The same animals which had been measured and scored as stage 4-4h of embryonic development were used. Their eggs, previously placed in vials with 200 µl of sea water, had hatched and released nauplii. Once all the larvae were well separated and started to swim, 10 µl of Formaldehyde (10 % in sea water) were added to the vial. The Formaldehyde was used to fix the larvae and stop them swimming. The contents of the vial were then pipetted into a Petri dish and the larvae counted under a microscope.

RESULTS

I 3.2.3 Reproductive state

The aim was to investigate the timing of larval release at Cellar Beach during the year of intensive monitoring. With this information, it should be possible to relate larval release with the timing of settlement. By looking at the time gap between these two events, it should be possible to understand if the larvae which settle at a certain time of the year are produced locally or are coming from elsewhere.

I 3.2.3.1 *Semibalanus balanoides* (Fig I 3.2.1)

High shore At the beginning of February 1992, when the first samples were collected, less than 5% of the barnacles from the high shore were ready to release their broods. This percentage increased slowly during the breeding season and reached the highest value at the beginning of April when about 40% of the barnacles analysed at high shore were able to release their larvae.

Mid-shore At mid-shore the peak of the larval release season was reached in mid February. On this sampling date, 45% of the barnacles analysed showed eggs ready to hatch. The last release on the mid-shore at Cellar Beach, took place around mid April.

Low shore At the beginning of the analysis, approximately 70% of the *Semibalanus* specimens from the low shore were ready to release their larvae. This contrasted sharply with the situation at the high shore and mid-shore sites where the percentage of individuals reaching stage 4h was only 4% and 16% respectively. The peak of the hatching season lasted for at least a month, from the beginning of February until probably early March. After this date a consistent decrease in number of barnacles carrying broods ready to be released, was noticed. By the end of April all larvae had been released into the plankton and no more barnacles with reproductive characters were found among the ones analysed. Barnacles were all categorised as stage 0.

In conclusion, in 1992 the major part of the larval release by *S. balanoides* at Cellar Beach, took place during the months of February and March. Larvae were first ready to be released on the low shore and then progressively at the mid and high shore sites. Overall low shore barnacles seemed to be the most productive: their breeding

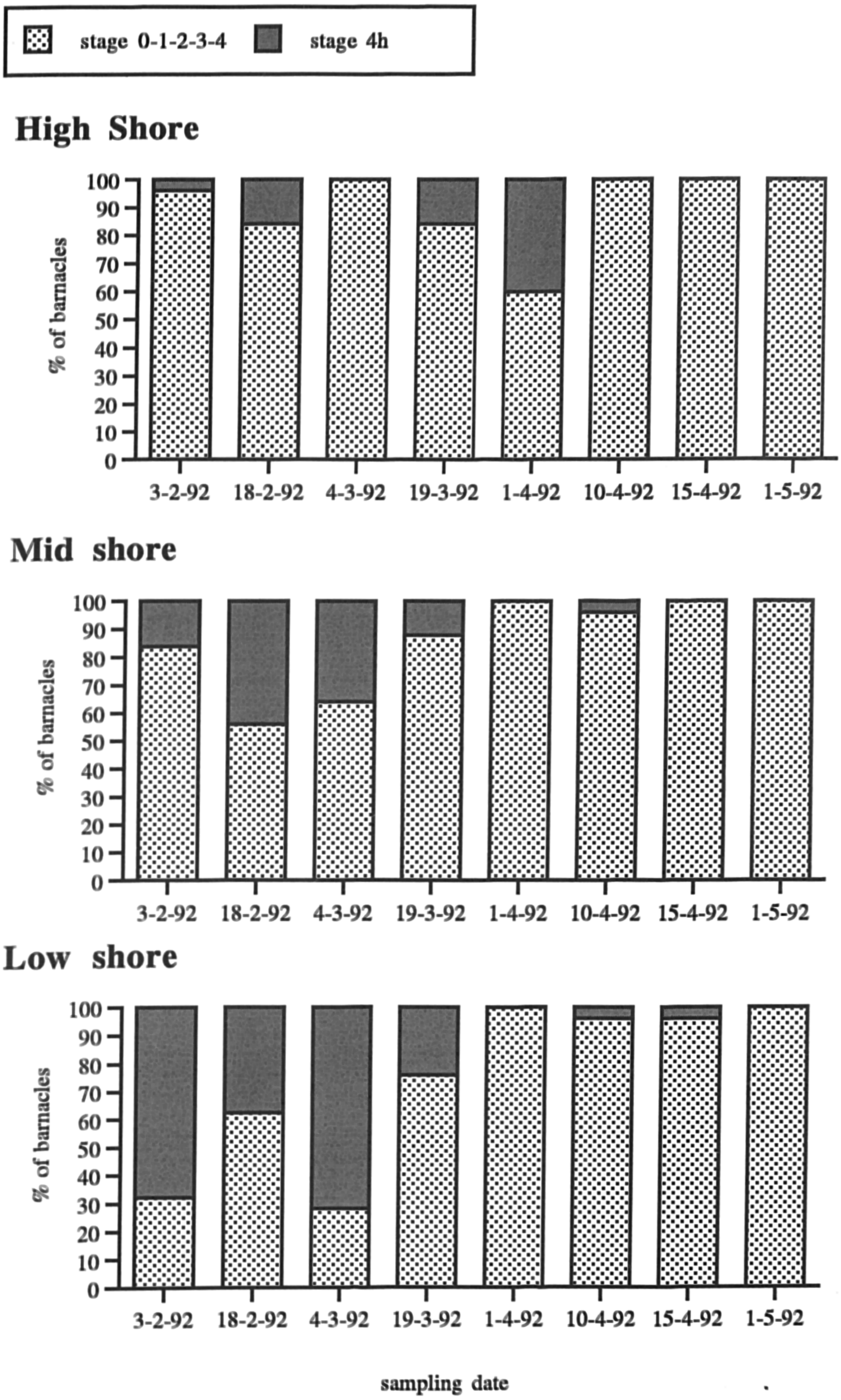


Fig I 3.2.1: Reproductive state of *S. balanoides* through the breeding season. The data plotted represent the % of barnacles ready to release broods (stage 4h) versus barnacles not ready (other stages). See section on breeding in the methods for explanations of larval stages. N=25 in all cases except high on the shore on the 4-3-92 (N=20) season was the longest and the number of individuals ready to release broods was the highest.

I 3.2.3.2 *Chthamalus montagui* (Fig I 3.2.2)

High shore On the high shore larval release started in mid July and finished at the end of August. Only a small proportion of the barnacles analysed, a maximum 30% at a given sampling session, were found to be ready to release larvae into the plankton. The vast majority of individuals displayed no reproductive characteristics, being scored as stage 0.

Mid-shore At this shore level, larvae started to be released during mid July. Mid August was the last date on which *C. montagui* was found to be carrying broods which were scored at stage 4h. However, it was likely that the releasing season lasted at least until the end of August, as it did at the other two shore levels, since there were still a few barnacles at stage 4 in mid August.

Low shore On the low shore, larval release started at the same time as the other two shore levels but probably went on for longer. The last larvae were released around mid September. In addition, slightly larger number of barnacles than at the other two shore levels, (35% at the end of July) were found to be ready to release larvae. The number of individuals of *C. montagui* in a reproductive state was similar at the low and mid-shore sites, much less on the high shore.

In conclusion, during the year of intensive monitoring, larvae of *C. montagui* were released into the plankton between mid July and the first half of September. Among the three tidal levels, the low shore was the one which showed the longest releasing season with a peak around the end of July.

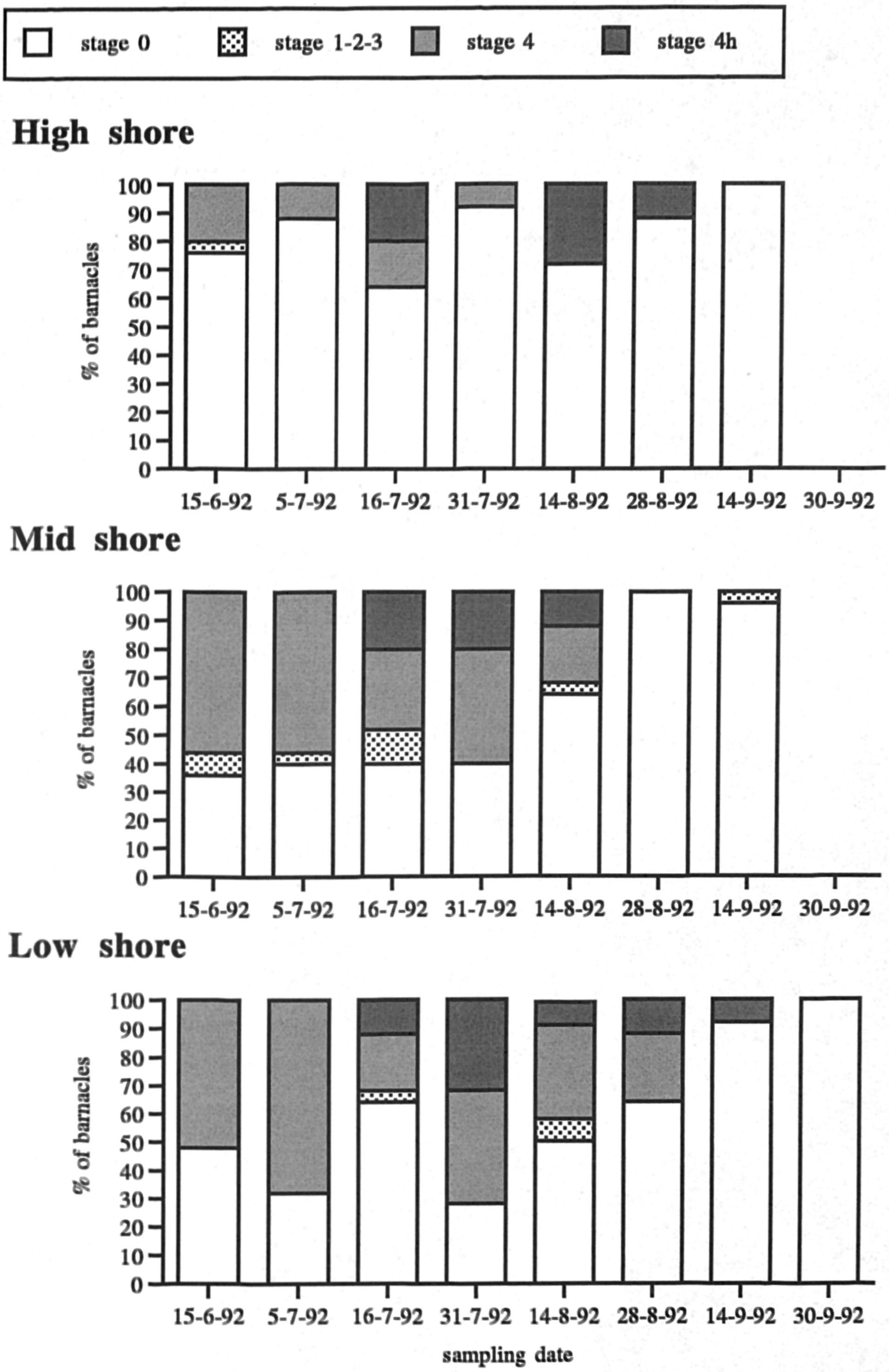


Fig I 3.2.2: Reproductive state of *C. montagui* through the breeding season.
The data plotted represent the % of barnacles ready to release broods (stage 4h) and the % of barnacles found in other stages. See section on breeding in the methods for explanations of larval stages.
N=25 in all cases except low on the shore 14-8-92 (N=12)

I 3.2.3.3 *Chthamalus stellatus* (Fig I 3.2.3)

High shore Due to scarcity of *C. stellatus* on the high shore, only few animals could be analysed from this tidal height. At the end of July only one barnacle was found to be ready to release its brood.

Mid-shore As for *C. montagui, C. stellatus* began release of its broods in mid July. Larval release went on until at least mid September.

Low shore Once again the larval release started in mid July and ended probably around mid September. However, at the end of September, only one of the 25 barnacles analysed was still carrying a brood at the early stages of development. Hence, if the egg masses were allowed to complete their development, further larval release would have taken place some time in the Autumn. It is interesting to notice that the peak of the larval release of *C. stellatus* appears to be reached around the end of August/beginning of September. *C. montagui* in contrast, was releasing most of its broods a month earlier, around the end of July.

Although no data were available for high shore, analysis of barnacles from the mid and low sites showed that the release of larvae of *C. stellatus* took place between mid July and probably mid September. As for *C. montagui* the highest number of barnacles ready to release broods, was found on the low shore. Unlike *C. montagui*, *C. stellatus* reached the peak of the releasing season at the beginning of September.

I 3.2.4 Number of eggs per brood in *S. balanoides*

In barnacles, as well as in many other crustaceans, the number of eggs produced is a function of the size of the parent (Barnes and Barnes, 1968). For each of the three levels on the shore, brood sizes of *S. balanoides* were plotted against base area (shell length x shell breadth). The resulting Model I regression slopes are presented in Fig I 3.2.4. At mid and low shore heights a significant relationship of egg number against base area was obtained (Table I 3.2.1). Fig I 3.2.4 shows

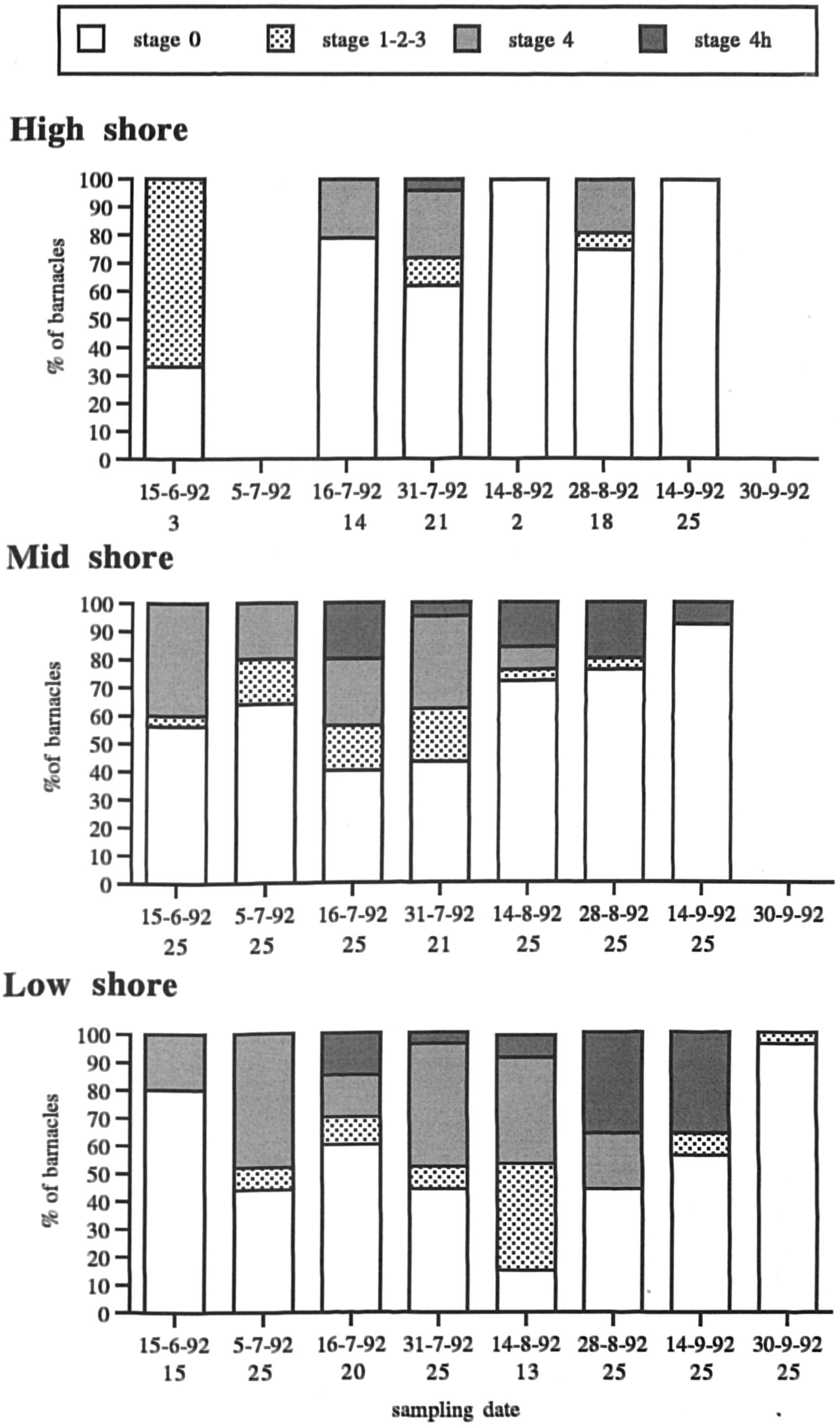
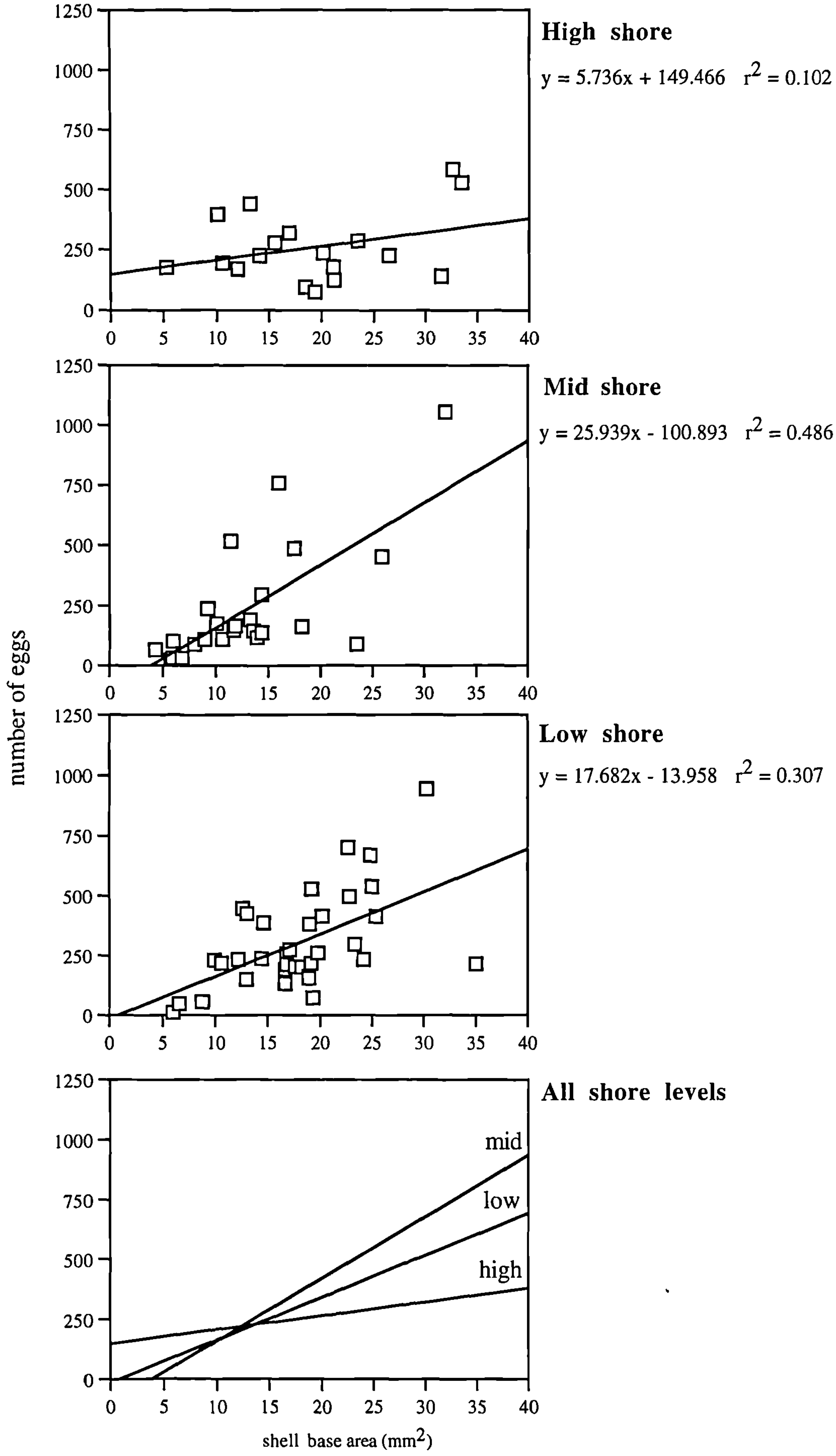


Fig I 3.2.3: Reproductive state of *C. stellatus* through the breeding season. The data plotted represent the % of barnacles ready to release broods (stage 4h) and the % of barnacles found in other stages.
See section on breeding in methods for explanations of larval stages.
The numbers below the sampling dates represent the nº of barnacles analysed on that particular day.



High shore

$y = 5.736x + 149.466 \quad r^2 = 0.102$

Mid shore

$y = 25.939x - 100.893 \quad r^2 = 0.486$

Low shore

$y = 17.682x - 13.958 \quad r^2 = 0.307$

All shore levels

Fig I 3.2.4: Regression slopes of n° of eggs against shell base area in *S. balanoides* at three shore levels.

Table I 3.2.1: Regression equations (Model I: (Sokal and Rohlf, 1995)) of the number of eggs per brood against shell area for samples of *S. balanoides* collected from the three shore levels during the breeding season of 1992.

a) High shore

Regression equation $Y = 149 + 5.74\,X$ $r^2 = 10.2\%$
(Y = n° of eggs X = shell area)

Source	df	Mean Square	*F*-Value	*P*-Value
Regression	1	36214	1.82	0.196
Error	16	19922		
Total	17			

b) Mid shore

Regression equation $Y = -101 + 25.9\,X$ $r^2 = 48.6\%$
(Y = n° of eggs X = shell area)

Source	df	Mean Square	*F*-Value	*P*-Value
Regression	1	699849	20.8	<0.001
Error	22	33639		
Total	23			

b) Low shore

Regression equation $Y = -14.0 + 17.7\,X$ $r^2 = 30.7\%$
(Y = n° of eggs X = shell area)

Source	df	Mean Square	*F*-Value	*P*-Value
Regression	1	419997	14.65	0.001
Error	33	28674		
Total	34			

differences between shore heights in the slope of the regression lines. The lowest increase in brood size with increase in base area occurred on the high shore (although no significant regression was obtained) and the highest increase occurred on the mid-shore. Analysis of covariance was used to compare regression slopes. Owing to the lack of a significant regression on the high shore only the mid and low shore were compared. No significant difference in the slopes or intercepts was found (Table I 3.2.2).

Table I 3.2.2: Analysis of Covariance testing for a significant difference in brood size between mid and low shore levels. Levels of significance : * $P < 0.05$, ** $P < 0.01$

Source	df	Mean Square	*F*-Value	*P*-Value
Shore height	1	15913	0.52	0.474
Shell area	1	1115475	36.38	<0.001 **
Shore height*Shell area	1	39967	1.30	0.259
Error	55	30660		
Total	58			

N.B. The *P*-value of 0.259 for the interaction between shore height and shell area indicates no significant difference in the slopes of the two regressions.

I 3.3 SETTLEMENT and RECRUITMENT

METHODS

I 3.3.1 Monitoring settlement and recruitment on photographs: assessment of numbers and species of spat and presence of cyprids

All photographs taken during the year of intensive monitoring (Sept. 1991-Sept. 1992) were analysed for the presence of cyprids and spat. Observations were made for each species at each shore level. In the case of *E. modestus*, however, settlement and recruitment (for definition of terms, see section on settlement and recruitment in introduction) were only analysed on the low shore. This decision was justified by the fact that *E. modestus* is mainly a low shore species and therefore analysis of settlement and recruitment at mid and high shore levels would have not provided adequate data.

With the exception of *Elminius*, all the other species were characterised by a relatively short and well defined settlement season which allowed a very detailed analysis of this stage of their life history. Because *E. modestus* settles all year around, such a detailed analysis of the photographs over the whole year period would have been prohibitively time consuming. Data on the settlement and recruitment of this species were already available from the analysis of the rock-samples. This analysis over the whole year indicated the existence of a peak when settlement intensified greatly. A detailed analysis of this period was then carried out on the photographs.

A problem was encountered while analysing *Chthamalus* species: it was not possible to separate on the photographs cyprids and newly-metamorphosed spat of *C. montagui* from those of *C. stellatus*. This problem was due to the complete overlap of their settlement seasons, to the very small size of the spat and to the almost identical morphological features of the two species at this life stage. In addition, because the settlement and post-settlement seasons of *Chthamalus* occurred during autumn/winter when the rock surface rarely dries out, the photographic resolution was not very good. For all these reasons, no clear identification of the two species of *Chthamalus* which settled in Autumn 1991 could be made until the beginning of April 1992, five to six months after the end of the settlement season. Due to the high mortality at the early stages of life (see section on spat mortality), most of the spat died before this time, making it impossible to quantify the settlement of each one of the two species in autumn 1991. Hence the data provided were of the settlement of the two species combined.

For the detailed analysis of settlement from photographs, the following procedure was adopted. All photographs were screened to detect the presence of newly settled cyprids or spat belonging to the species of interest. Once the beginning of the settlement season was determined for a certain species, cyprids and spat of that species were marked on the relevant set of photographs. This generally involved the analysis of all replicates at each shore level. Only cyprids and spat found in the area common to the whole series of photographs were selected. Note that cyprids were recorded separately from newly settled spat on each sampling session. Barnacles belonging to the first settling sub-cohort were all labelled on the photographs with micro labels of the same colour. The same barnacles were then recognised on the following sets of photographs and labelled again maintaining the same colour of label. Barnacles which settled on the second set of photographs were labelled with labels of a different colour from the ones used for the first sub-cohort. The second settling sub-cohort was then recognised on the following sets of photographs, and marked again with its colour. The whole process was repeated again and again until the end of the settlement season. By using labels of different colours, it was possible to distinguish every new settling sub-cohort from previous ones. Hence, on each sampling date a very accurate estimate of the number of newly settled barnacles and of barnacles which survived from the previous sub-cohorts could be made.

It is acknowledged that this type of analysis provides an underestimate of the real intensity of settlement, because it does not take into account the number of cyprids and spat which settle and die between two sampling sessions.

By using the interception point method, estimates of the amount of bare rock available at the beginning of the settlement season, in each area analysed for settlement, were also obtained. With this data the amount of settlement could be expressed as density of cyprids and spat per cm^2 and compared directly with the data obtained from the analysis of rock-samples (see following paragraph).

I 3.3.2 Monitoring settlement and recruitment on rock samples: assessment of number, size and species of spat and juveniles and presence of cyprids

In order to confirm the results obtained from the analysis of photographs, rock samples were also analysed for settlement and recruitment. If similar results were obtained from the two studies, it could be suggested that the detailed analysis of a small area on the shore was providing results representative of the whole shore. Analysis of rock samples in the laboratory also allowed accurate measurements of spat and juvenile barnacles which provided data on early changes in the population structure of the newly settled cohort and on early growth.

All rock samples were analysed in the laboratory under a microscope for presence of cyprids, spat and juveniles. The number of these was related to the bare rock available on each sample. No attempt was made to measure or distinguish cyprids of different species.

The shell length along the rostro-carinal axis of each spat and juvenile found on the rock chips, was measured with a stereo microscope fitted with an eyepiece graticule.

At the beginning of the survey, in Autumn 1991 an attempt was made to distinguish the newly settled cohort of *Chthamalus* species. Due to initial inexperience, the larger individuals of this cohort could not be distinguished from the smaller individuals of the previous year settlers. Therefore, initially only individuals less than 1 mm in length were monitored. After two months, the ability to separate the newly settled cohort from older animals was acquired and all barnacles belonging to that cohort, independently from their size, were measured. Because the main morphological characters (e.g. colour and calcification of the shell) for separating new settled barnacles from old ones were only evident on fresh samples, no attempt was made to re-analyse the samples collected during the first two months.

Some problems were encountered while trying to identify to species level. Normally barnacles with a shell length less than 1.5 mm look very similar in the different species. However, as the period of settlement differs in the three genera studied, it was possible to understand which species was settling in relation to the time of the year the rock samples were collected. A major problem was encountered while trying to separate the two species of *Chthamalus* which settle during the same months. Burrows (1988; 1992) used electrophoretic techniques to calculate the proportion of each species in the total spat counts. Later in his study, by using a Scanning Electron Microscope, morphological differences were recognised that allowed separation of newly settled spat into the two species using field microscopy. In this study neither of the two techniques could be employed. The skills required to separate spat of the two species by simply looking at them through a microscope, were acquired only during the second settlement season. Due to the very scarce number of *C. stellatus* identified and the desire to have a consistent approach through the whole period of intensive monitoring, the measurements of *Chthamalus* spat were always combined for *stellatus* and *montagui.*

RESULTS

I 3.3.3 Settlement and recruitment from the analysis of photographs

I 3.3.3.1 *S. balanoides* (Fig I 3.3.1)

In 1992 settled *Semibalanus* began to appear in mid March on the mid and low shore and at the beginning of April on the high shore. Settlement finished around mid May on the high and low shore, while at mid shore it continued for at least another couple of weeks. *Semibalanus* started to release larvae in the plankton in January and finished in April/May. Hence, the time between naupliar release and cyprid settlement was just over thirty days. A similar time span was found by Harms (1984). The peak of the settlement season was reached around the second half of April at all shore levels. The 15-4-92 sampling session, revealed the greatest number of settling cyprids, while the following sampling date (1-5-92) showed clearly the highest density of newly settled spat. On the high shore the peak was reached slightly earlier, in mid April. Overall at this tide level the settlement season was shorter than at the other two.

At mid and low shore, the peak of recruitment matched that of settlement. Thus although settlement continued until 14-5-92 or 3-6-92, by these dates the rate of spat mortality was higher than the rate of settlement. Surprisingly on the high shore where one might expect high spat mortality, the peak of recruitment coincided with that of the end of the settlement season. Also the population density of settlers and recruits, showed differences between shore levels. The highest values were recorded on the low shore, followed closely by mid-shore. The lowest number of settlers was present high on the shore.

Summarising, *S. balanoides*' 1992 settlement season, started in mid March and lasted for just less than three months. Settlement took place mainly at mid and low shore, but early post-settlement mortality was quite high.

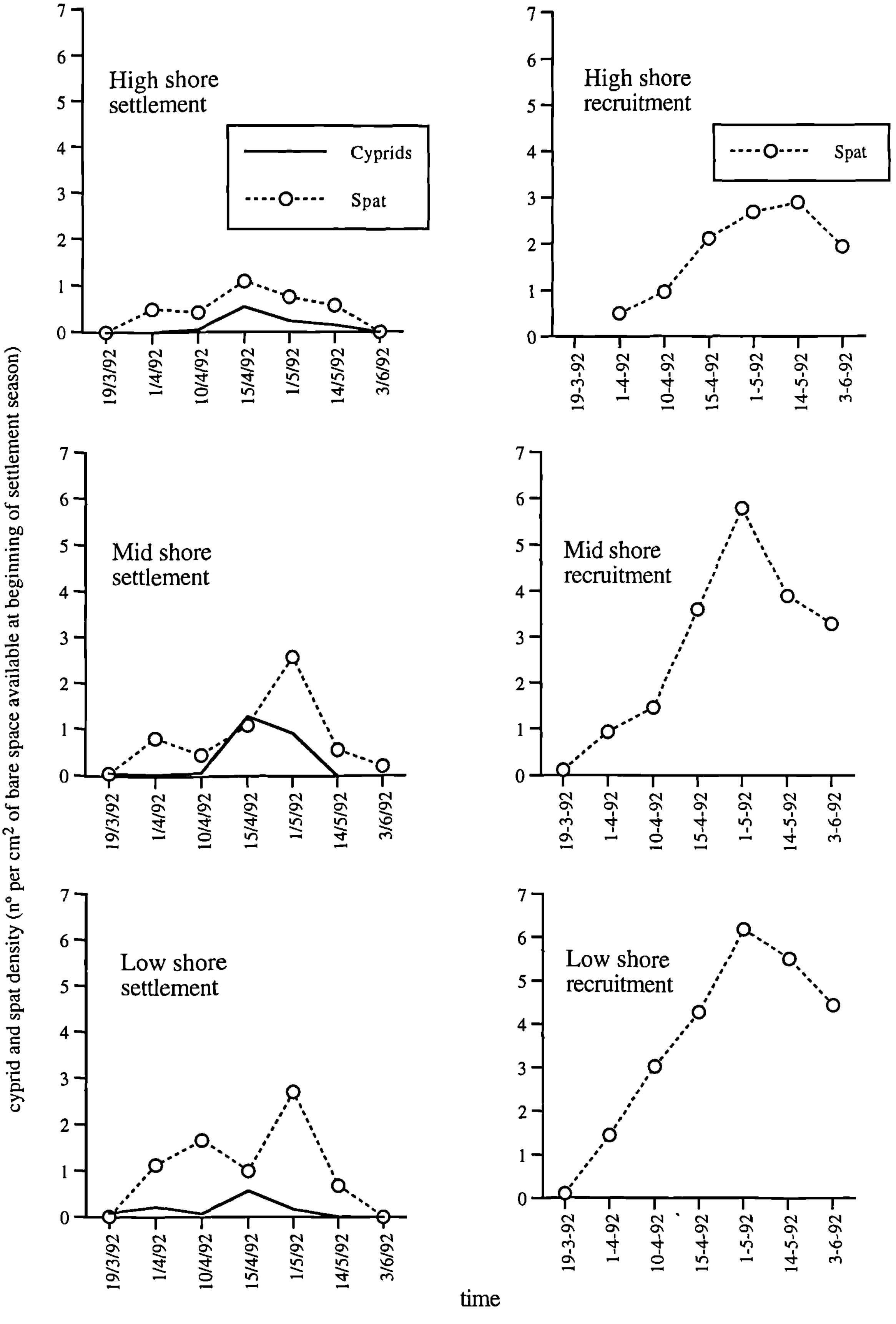


Fig I 3.3.1: Settlement and recruitment season of *S. balanoides* at three different shore levels. Data obtained from the analysis of photographs. Graphs on the left hand side (settlement) represent the density of newly settled cyprids and spat observed on a particular sampling date. Graphs on the right (recruitment) represent the cumulative density of spat which settled and survived between the beginning of the settlement season and the particular sampling date. Values are mean densities of the several replicates analysed at each shore level. Error bars are omitted for clarity.

I 3.3.3.2 *Chthamalus* (Fig I 3.3.2)

Due to the late starting time of the survey, the initial part of the settlement season of *Chthamalus,* went unmonitored. In a previous study (Burrows, 1988), the settlement season of *Chthamalus* was found to start at different times in different years, but always some time in July or August. In my study non-quantitative observations of photographs taken during the 1992 settlement season, indicated that in that year settlement began in mid August (in 1992 larval release started in mid July, just a month before the beginning of settlement). In 1991, settlement continued until approximately mid November, although after mid October very few settlers were recorded at all tide levels. The peak of the season was reached around the end of September, but by that time, a decline in the number of settlers at high shore was already taking place. It is interesting to note that most of the newly settled *Chthamalus* were found in the metamorphosed form, while only very few were recorded at the cyprid stage. This result contrasts with that found for *S. balanoides*, which showed quite a high proportion of the newly settled barnacles still at the cyprid stage. This argument is made even stronger by the higher density of *Chthamalus* settlers in comparison to *Semibalanus.*

Although the highest density of settlers was recorded on the low shore, the largest recruitment was certainly taking place on the high shore. It appears from the recruitment data that mortality of newly settled spat was much lower on the high shore; the increase in the number of recruits continued much later at this tide level and the decline after 6-11-91 occurred at a much lower rate.

The observed very low number of newly settled *Chthamalus* at the cyprid stage and the high recruitment at high shore but not at mid and low shore, seem to be the most interesting points coming out from this study of settlement and recruitment in *Chthamalus* species.

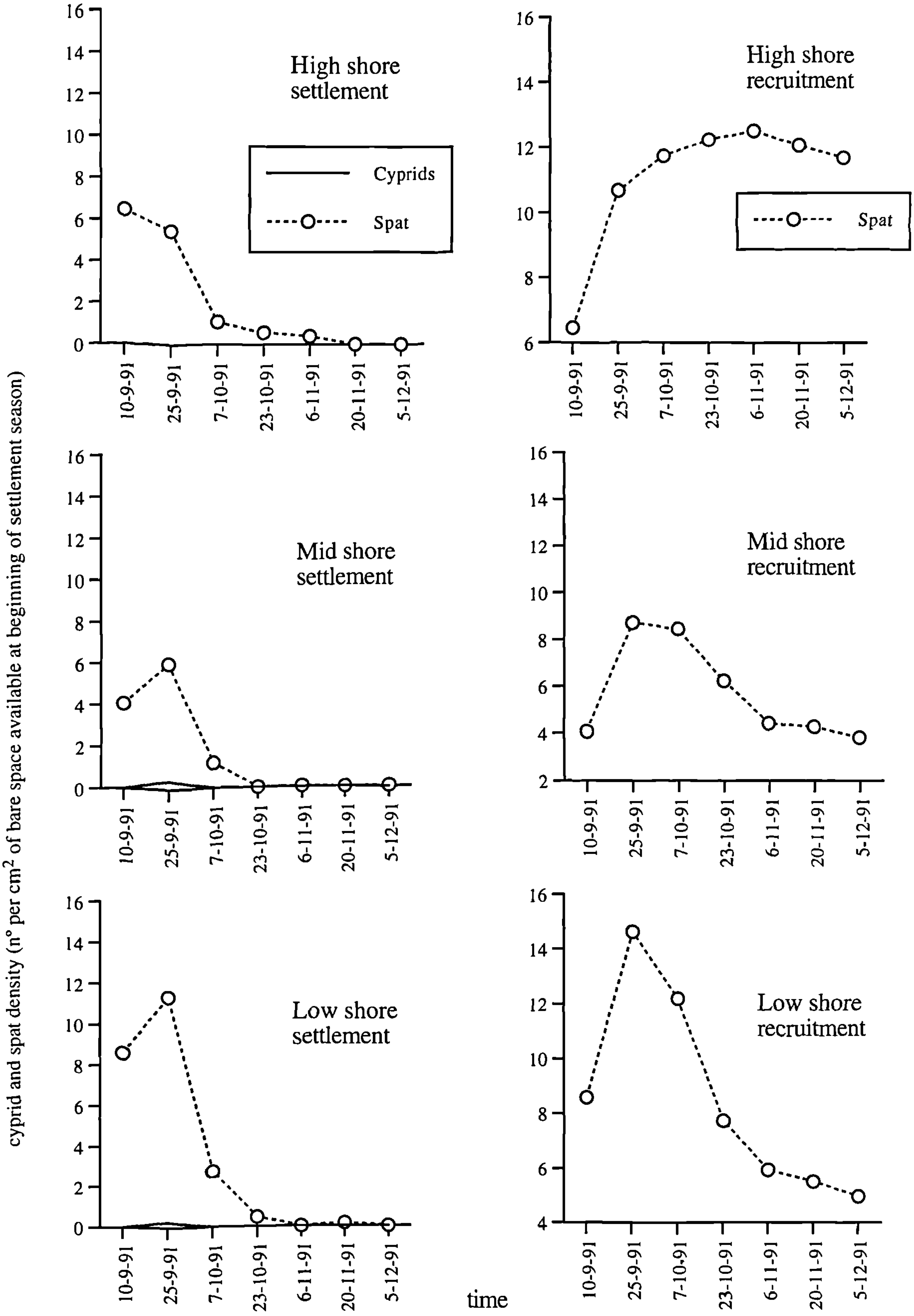


Fig I 3.3.2: Settlement and recruitment season for the two *Chthamalus* species combined together at three different shore levels. The survey started on the 10-9-91 when *Chthamalus* settlement had already started. Data obtained from the analysis of photographs. Graphs on the left hand side (settlement) represent the density of newly settled cyprids and spat observed on a particular sampling date. Graphs on the right (recruitment) represent the cumulative density of spat which settled and survived between the beginning of the settlement season and the particular sampling date. Values are mean densities of the several replicates analysed at each shore level. Error bars are omitted for clarity.

I 3.3.3.3 *E. modestus* (Fig I 3.3.3)

E. modestus settlers were sporadically observed throughout the year of intensive monitoring. A distinct settlement did not occur until the beginning of July 1992. Between the 5th of July and the 14th of September, *E. modestus* showed a quite intensive settlement on the low shore. A few settlers were also observed at mid-shore, while no newly settled barnacles were found on the high shore. At low shore the settlement showed two main peaks, one at the beginning of July and another one just before the end of the season, in mid August. Spat density was similar to that of *Semibalanus* on the low shore, but much lower than that of *Chthamalus* at the same level on the shore. Also for this species, only a few barnacles were found at the cyprid stage. Newly settled *Elminius* appeared to show a high survival rate which was reflected in continued accumulation of recruits over the period of monitored settlement.

In conclusion, *Elminius* showed a peak of settlement during the summer months. Settlement occurred mainly on the low shore and the main period of settlement lasted approximately two and a half months. The density of recruits at the end of the settlement was higher than that of *Semibalanus* and *Chthamalus* on the low shore.

I 3.3.4 Settlement, recruitment and early growth from the analysis of rock samples

I 3.3.4.1 *S. balanoides* (Fig I 3.3.4-5)

No data on *Semibalanus* settlement was gathered from the high shore. This was due to the fact that high shore rock samples, were collected from just above the upper limit of this species.

The first settlers, both at mid and low shore, were observed at the beginning of April 1992. In agreement with observations from the photographs, the peak of

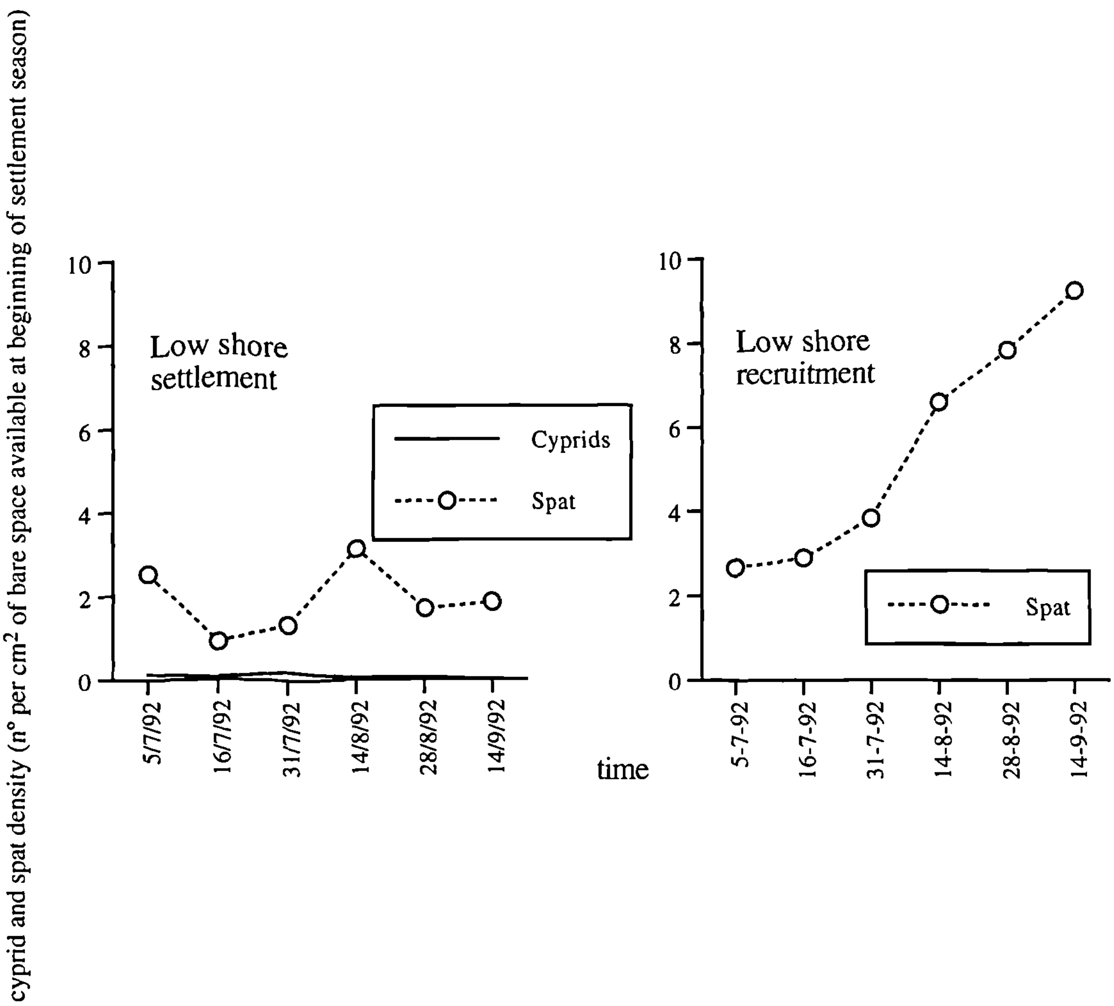


Fig I 3.3.3: Although *E. modestus* settles all year around a peak in its settlement was noticed during the summer of 1992. Heavy settlement was recorded from the beginning of July until mid September.
Due to scarcity of settlers, no study of settlement and recruitment was carried out at high and mid shore.
Data obtained from the analysis of photographs. Graphs on the left hand side (settlement)represent the density of newly settled cyprids and spat observed on a particular sampling date. Graphs on the right (recruitment) represent the cumulative density of spat which settled and survived between the beginning of the settlement season and the particular sampling date. Values are mean densities of the several replicates analysed at low shore.
Error bars are omitted for clarity.

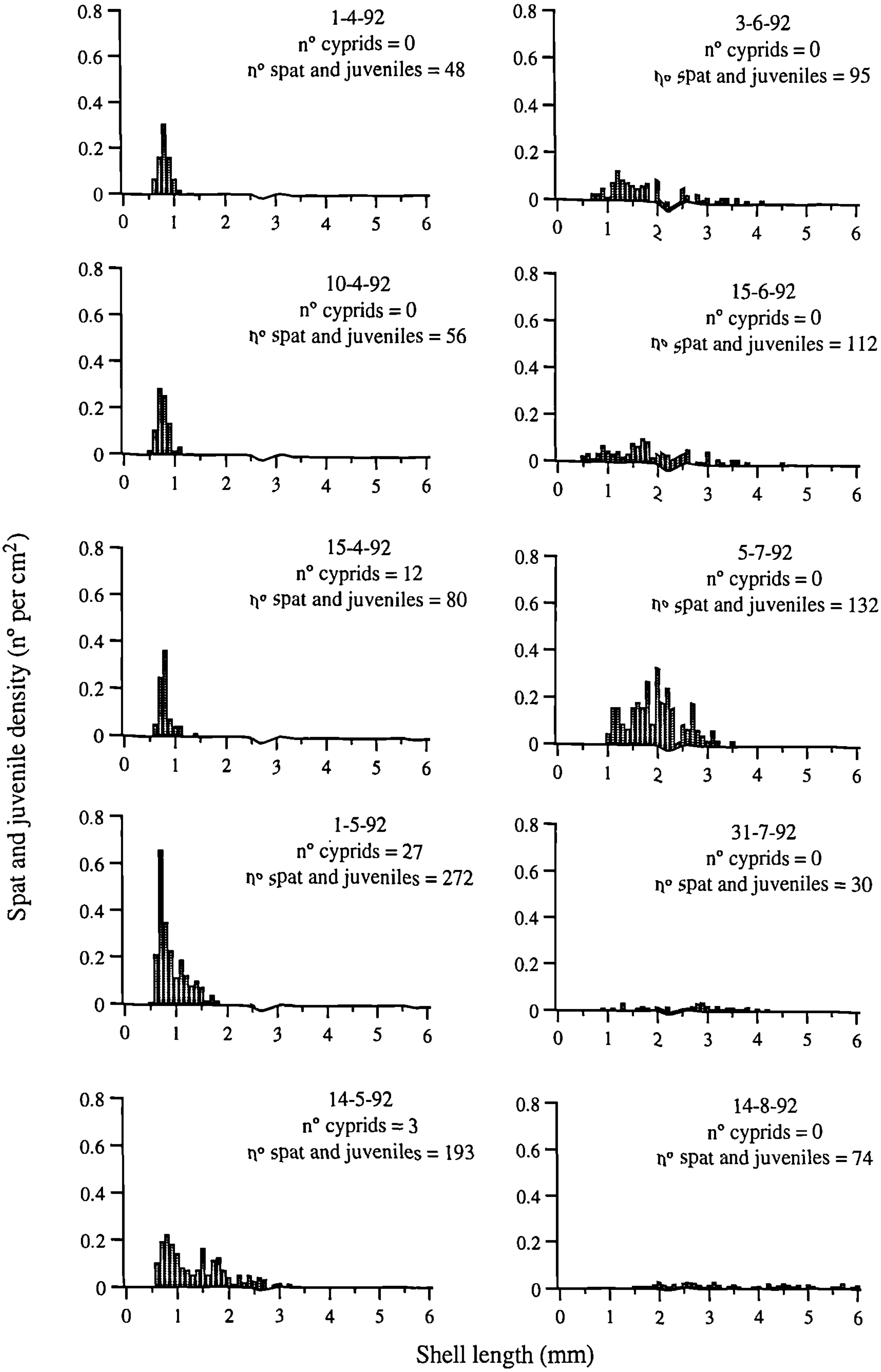


Fig I 3.3.4: Settlement and recruitment of *Semibalanus balanoides* at mid shore.
The histograms show changes in density of various size classes of spat and juveniles over time. The number of animals analysed for this purpose is reported on each graph.
Cyprids are not plotted, but the number of those observed is also reported.
All the data were obtained from the analysis of five rock samples collected on each date at each shore level. The density values are from the five replicates pooled together.

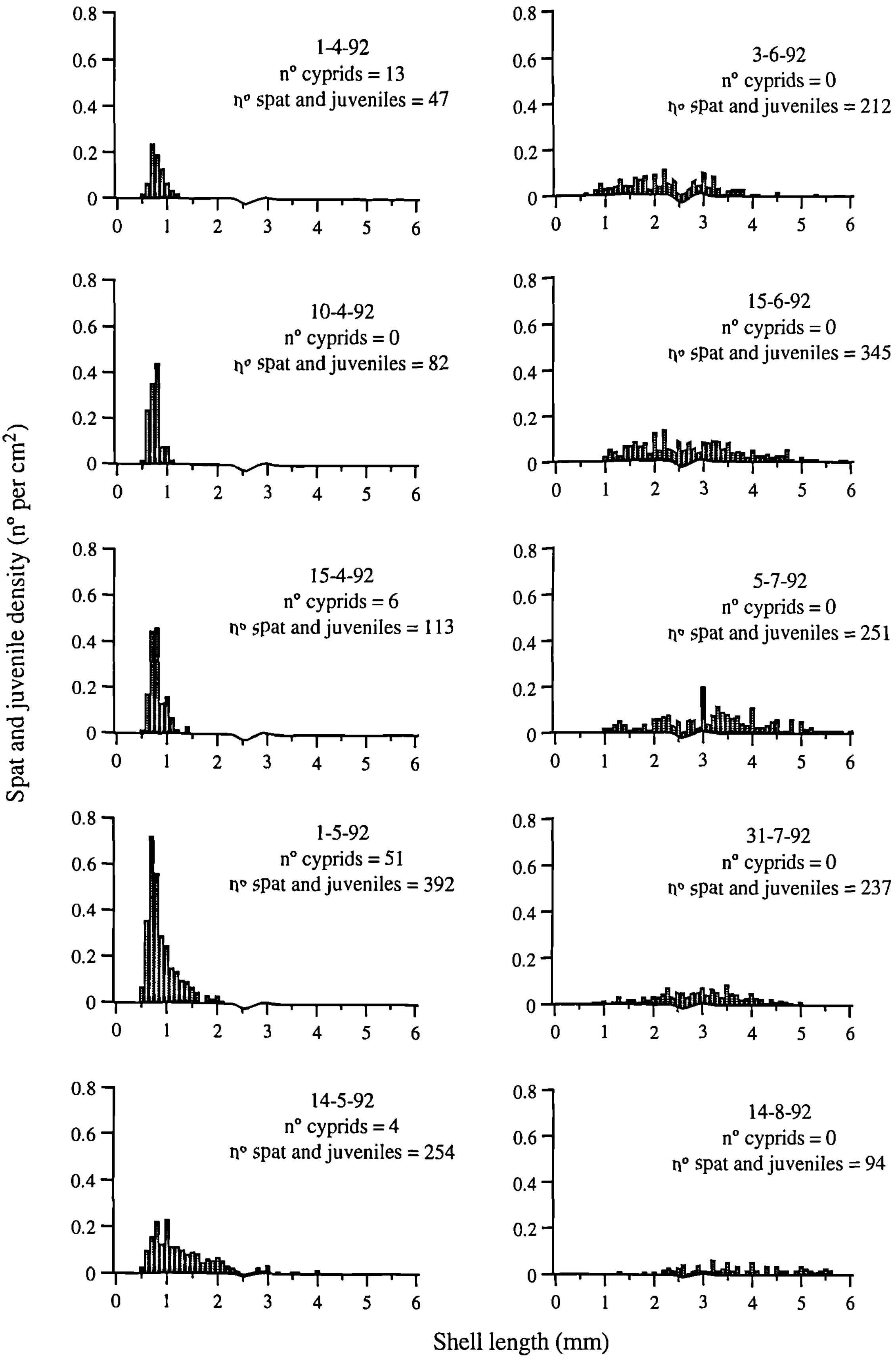


Fig I 3.3.5: Settlement and recruitment of *Semibalanus balanoides* at low shore.
The histograms show changes in density of various size classes of spat and juveniles over time.
The number of animals analysed for this purpose is reported on each graph.
Cyprids are not plotted, but the number of those observed is also reported.
All the data were obtained from the analysis of five rock samples collected on each date at each shore level. The density values are from the five replicates pooled together.

settlement was reached around the end of April/beginning of May. The following sampling date, 14-5-92, also showed the same trend as that from photographs with a decrease in the settlement rate. The additional information provided by the graphs (Figs I 3.3.4-5) was related to the growth of the newly settled barnacles through the year of intensive monitoring. It is interesting to see how the shell length measurements were at first narrowly distributed, with sizes ranging between 0.5 and 1 mm, and then, in less than 4 months from the beginning of settlement, gradually expanded to cover a much larger size range, such as the one on the low shore which ranged from 1 to 5 mm. Over this range of sizes no obvious peak occurred. Mid shore barnacles, showed a slightly slower growth. With time, it is expected that the variation in size among barnacles which settled in the same year but belonged to different sub-cohorts, would gradually decrease leading to a narrower size distribution.

I 3.3.4.2 *Chthamalus* (Fig I 3.3.6-8)

The final part of 1991 *Chthamalus'* settlement season and the initial part of that of 1992 were both monitored during the first year survey. Data on growth of spat larger than 1 mm shell length, were recorded only from the beginning of December (see methods for explanation). For this reason the first five histograms from each shore level appear truncated on the right hand side.

On the first sampling date (25-9-91), the peak of the settlement had already occurred at mid and low shore, while it was just taking place on the high shore. Due to the slow growth of the new settlers, it is hard to tell when the settlement season ended. Certainly no more peaks were noticed after the end of October. *Chthamalus* cyprids seemed to metamorphose in most of the cases into spat .of 0.5 mm shell length. On some occasions, however, spat of 0.4 mm size were also recorded. In general, *Chthamalus* specimens showed a smaller initial size than that of *Semibalanus* and *Elminius*. Newly settled spat of these last two species, were normally found to be 0.6/0.7 mm in length.

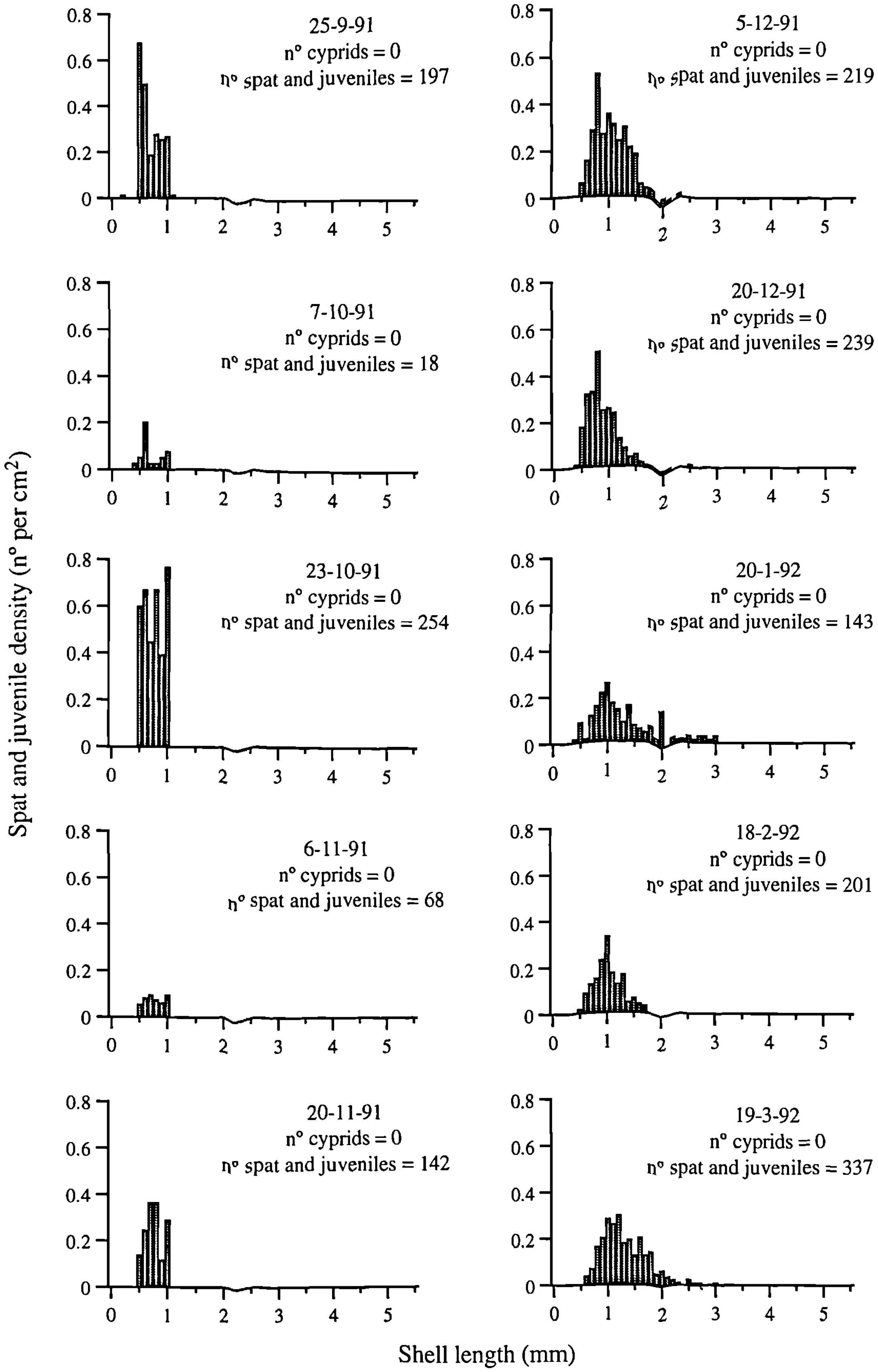


Fig I 3.3.6: Settlement and recruitment of *Chthamalus* at high shore.
The histograms show changes in density of various size classes of spat and juveniles over time.
The number of animals analysed for this purpose is reported on each graph.
Cyprids are not plotted, but the number of those observed is also reported.
All the data were obtained from the analysis of five rock samples collected on each date at each shore level. The density values are from the five replicates pooled together.

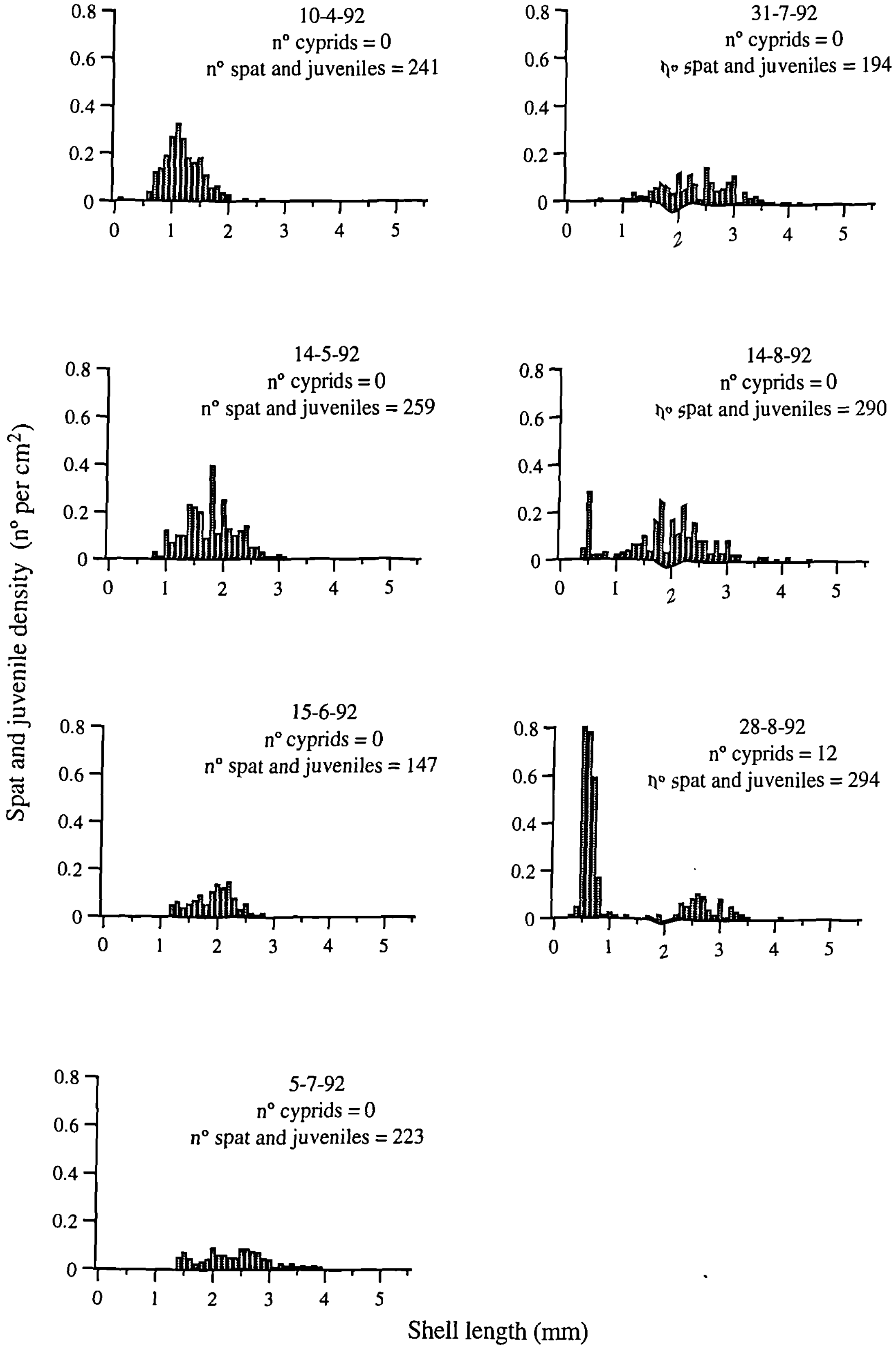


Fig I 3.3.6 (continued): Settlement and recruitment of *Chthamalus* at high shore.
The histograms show changes in density of various size classes of spat and juveniles over time.
The number of animals analysed for this purpose is reported on each graph.
Cyprids are not plotted, but the number of those observed is also reported.
All the data were obtained from the analysis of five rock samples collected on each date at each shore level. The density values are from the five replicates pooled together.

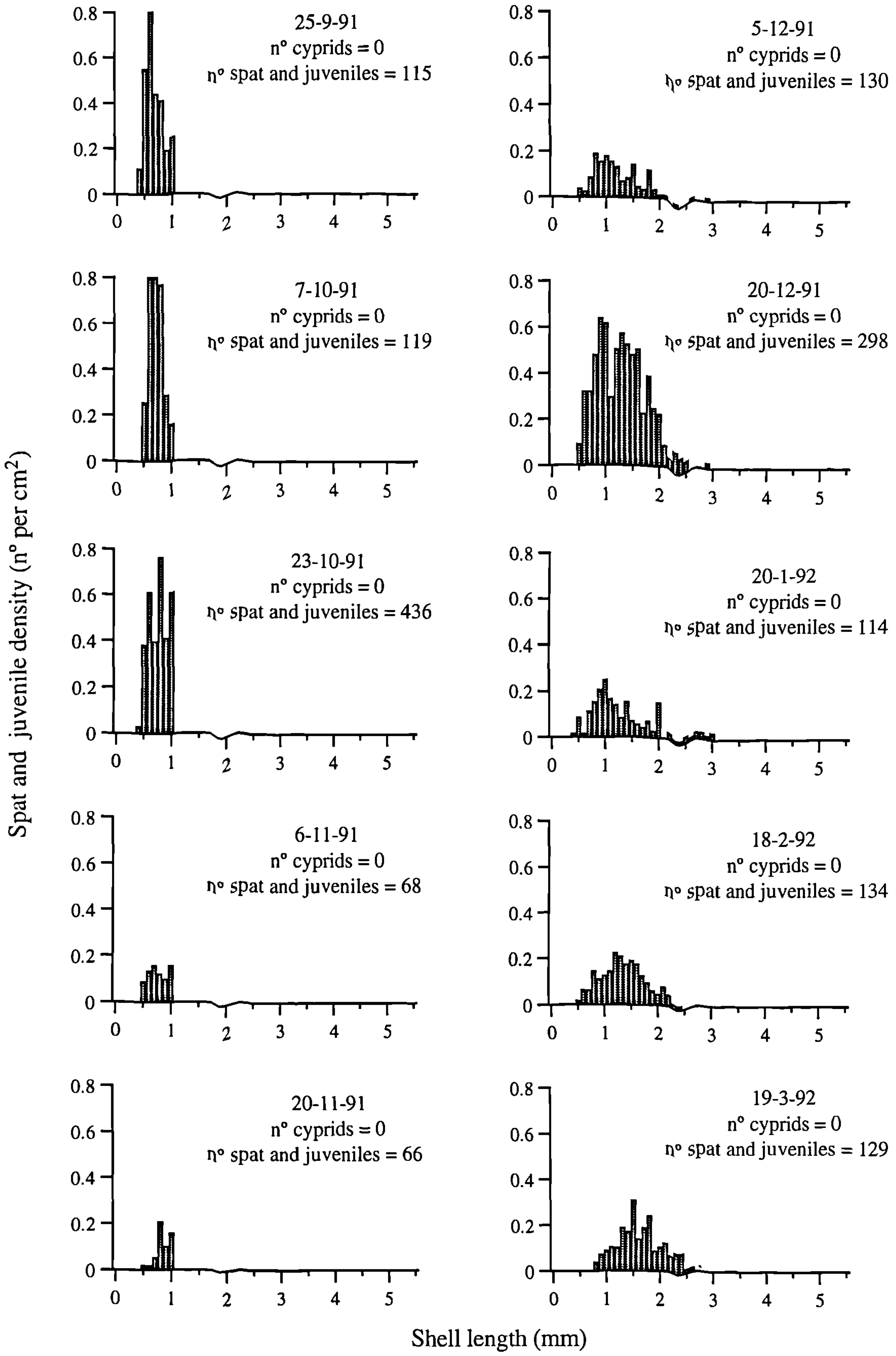


Fig I 3.3.7: Settlement and recruitment of *Chthamalus* at mid shore.

The histograms show changes in density of various size classes of spat and juveniles over time. The number of animals analysed for this purpose is reported on each graph.

Cyprids are not plotted, but the number of those observed is also reported.

All the data were obtained from the analysis of five rock samples collected on each date at each shore level. The density values are from the five replicates pooled together.

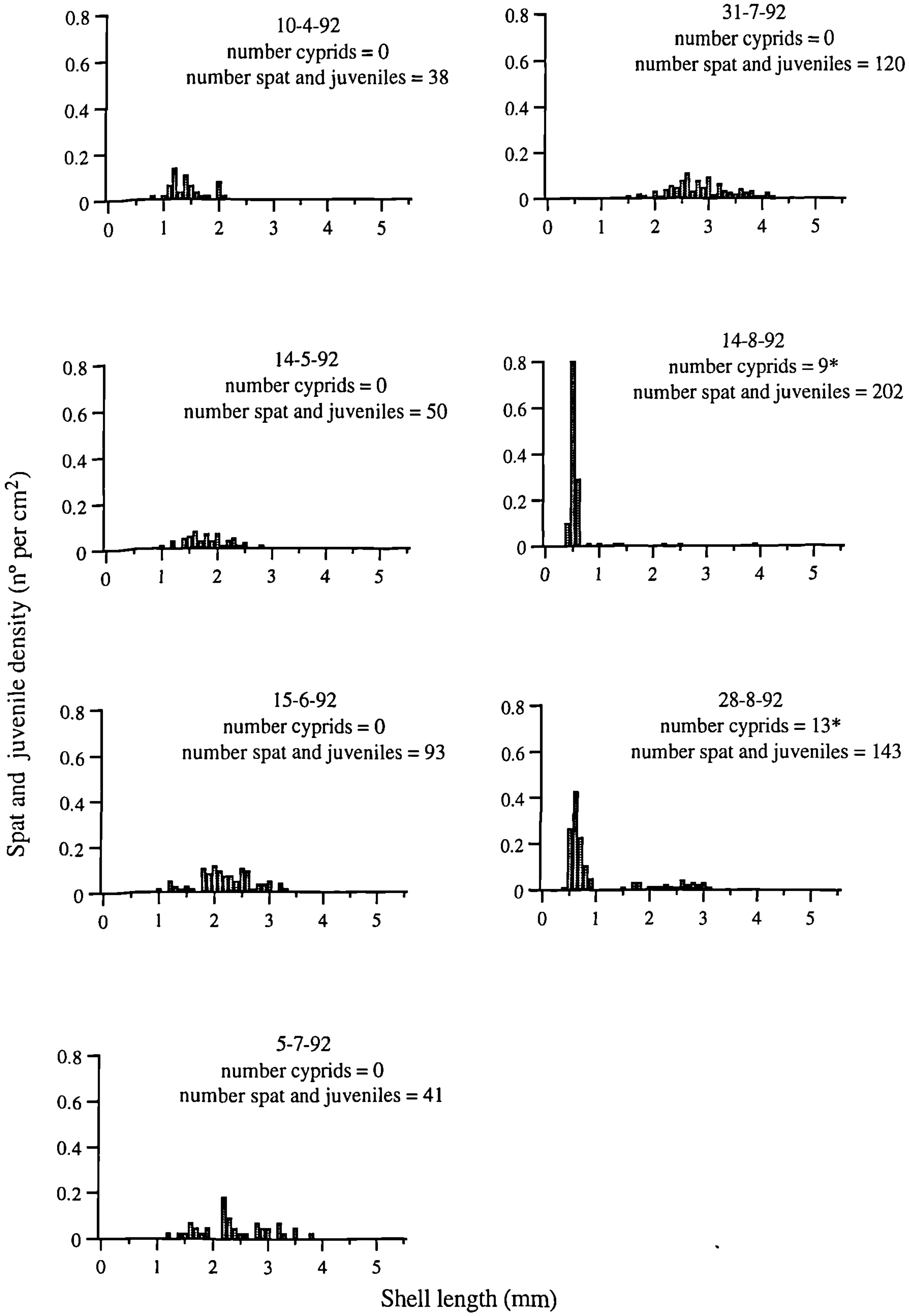


Fig I 3.3.7 (continued): Settlement and recruitment of *Chthamalus* at mid shore.
The histograms show changes in density of various size classes of spat and juveniles over time.
The number of animals analysed for this purpose is reported on each graph.
Cyprids are not plotted, but the number of those observed is also reported.
All the data were obtained from the analysis of five rock samples collected on each date at each shore level. The density values are from the five replicates pooled together.

* Some could be *Elminius* cyprids

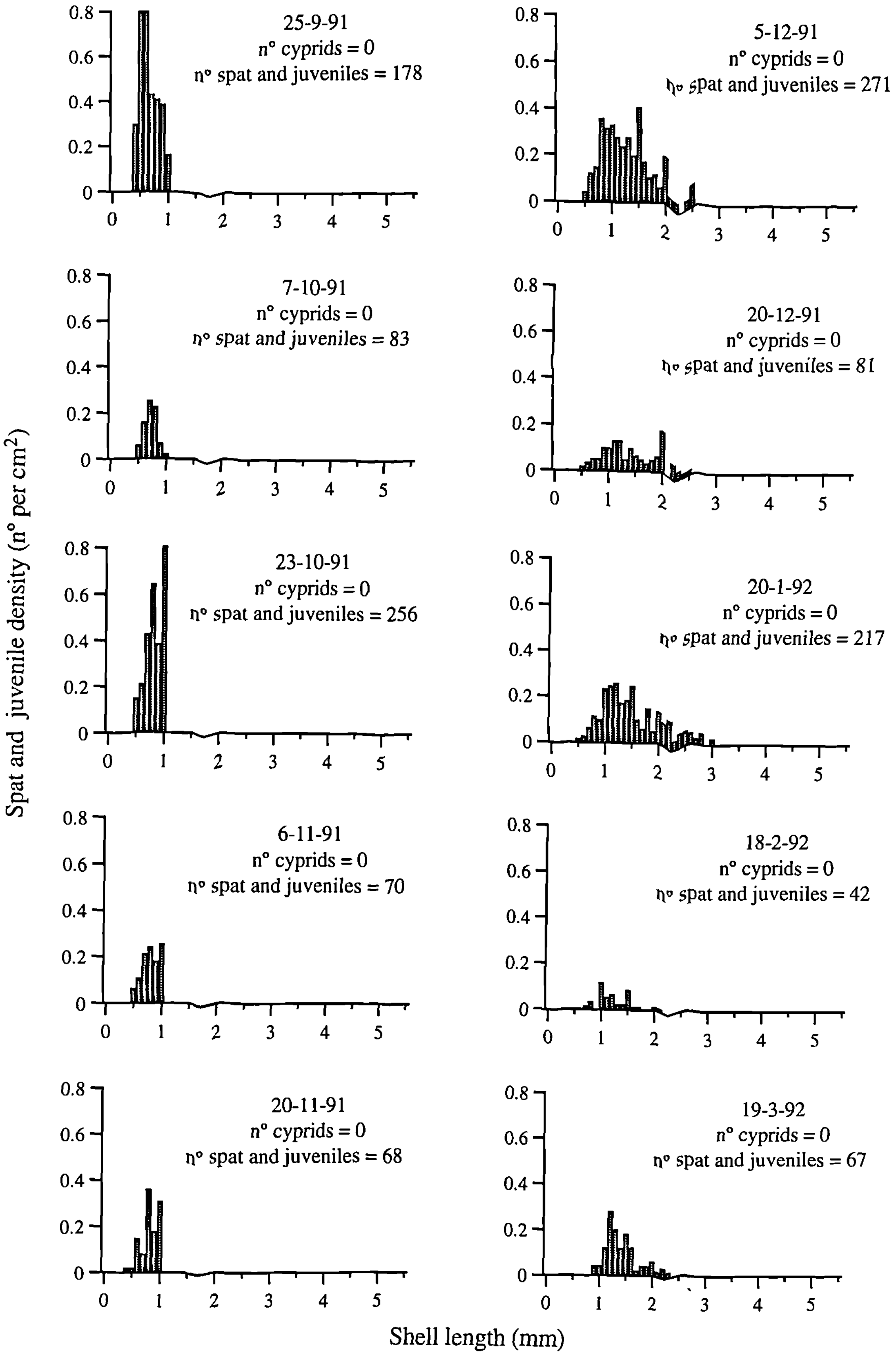


Fig I 3.3.8: Settlement and recruitment of *Chthamalus* at low shore.
The histograms show changes in density of various size classes of spat and juveniles over time.
The number of animals analysed for this purpose is reported on each graph.
Cyprids are not plotted, but the number of those observed is also reported.
All the data were obtained from the analysis of five rock samples collected on each date at each shore level. The density values are from the five replicates pooled together.

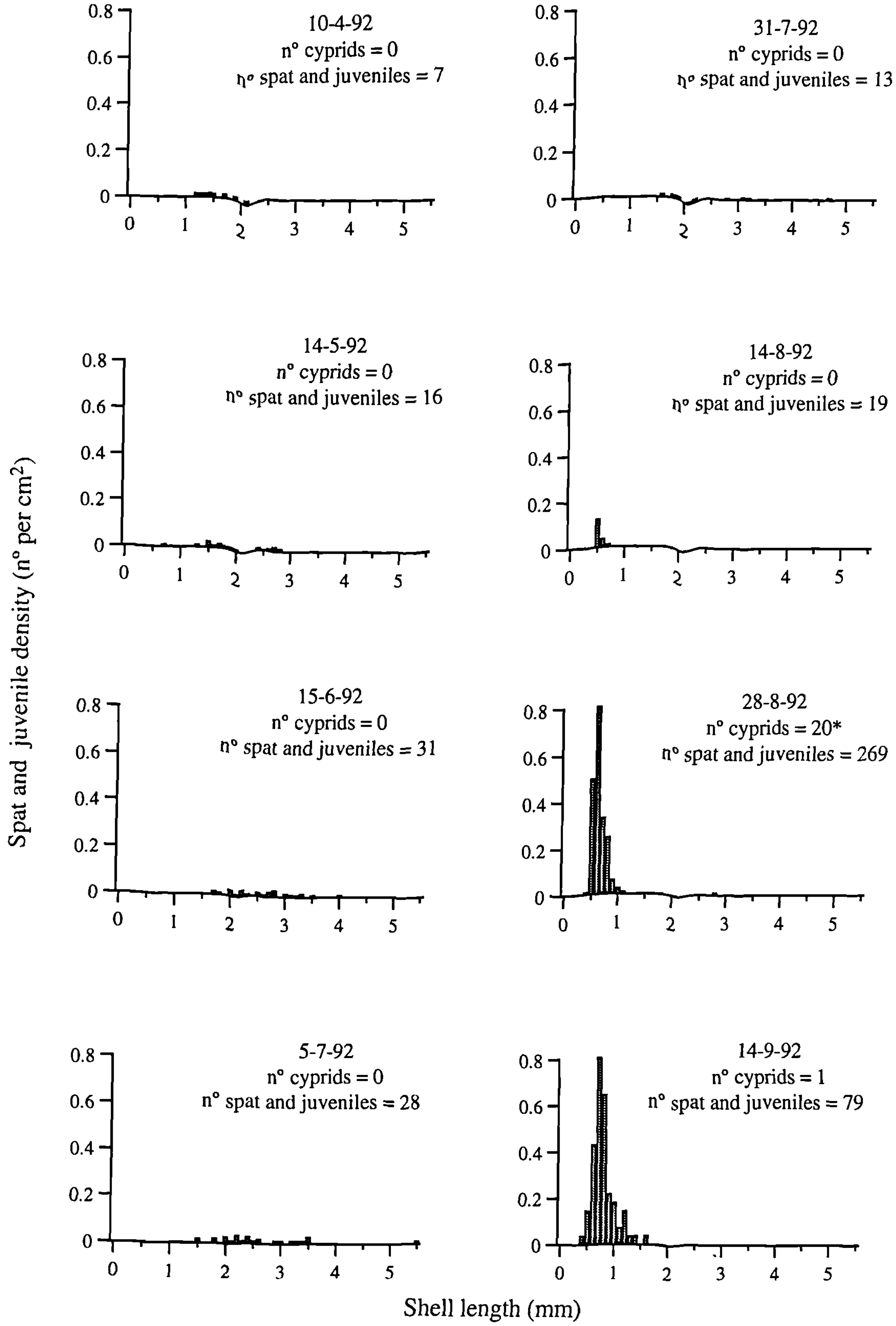


Fig I 3.3.8 (continued): Settlement and recruitment of *Chthamalus* at low shore.
The histograms show changes in density of various size classes of spat and juveniles over time.
The number of animals analysed for this purpose is reported on each graph.
Cyprids are not plotted, but the number of those observed is also reported.
All the data were obtained from the analysis of five rock samples collected on each date at each shore level. The density values are from the five replicates pooled together.

* Some cyprids could be *Elminius*

The size distribution of the newly settled cohort of *Chthamalus* remained much closer than in *S. balanoides*, and a distinct peak of individuals could be discerned at all shore heights. This pattern of size distribution indicated a more uniform rate of growth between individuals and enabled some estimate of early growth rate to be obtained. Over the first six months after settlement the modal size of spat increased from approximately 0.6 mm to 1.1 mm on the high shore and 0.6 to 1.5 mm on the mid shore. This apparent difference between shore heights was not observed later in the year. At the beginning of June barnacles at both shore heights had reached a size of approximately 2 mm. Data on *Chthamalus* from the low shore were limited.

The 1992 settlement season started around mid August simultaneously at all three shore levels. This confirms evidence from the analysis of the photographs. The mid shore main settlement peak coincided with the beginning of the settlement season, while the high shore one took place a fortnight later. No data are available for low shore in mid August. Because the main peak had already moved to 0.6 mm at the end of August, it can be assumed that also low shore settlement started in mid August. A few cyprids were recorded in the 1992 *Chthamalus* settlement season. Due to the simultaneous settlement of *E. modestus*, it is not known how many of these were in reality *Chthamalus* cyprids.

I 3.3.4.3 *E. modestus* (Fig I 3.3.9-10)

From the analysis of the rock samples the settlement season of *E. modestus* appeared to occur mainly during the summer months. A few new settlers were noticed in mid February, but this seemed to be an isolated event in the context of the year of intensive monitoring. The first peak of major settlement was recorded as on the photographs on the 5-7-92. Although less abundant than at low shore, settlers were also found at mid shore. In mid August settlement intensified again especially at mid shore, generating a second peak. With mid September, the number of new settlers started to decrease and eventually disappear. The results obtained for

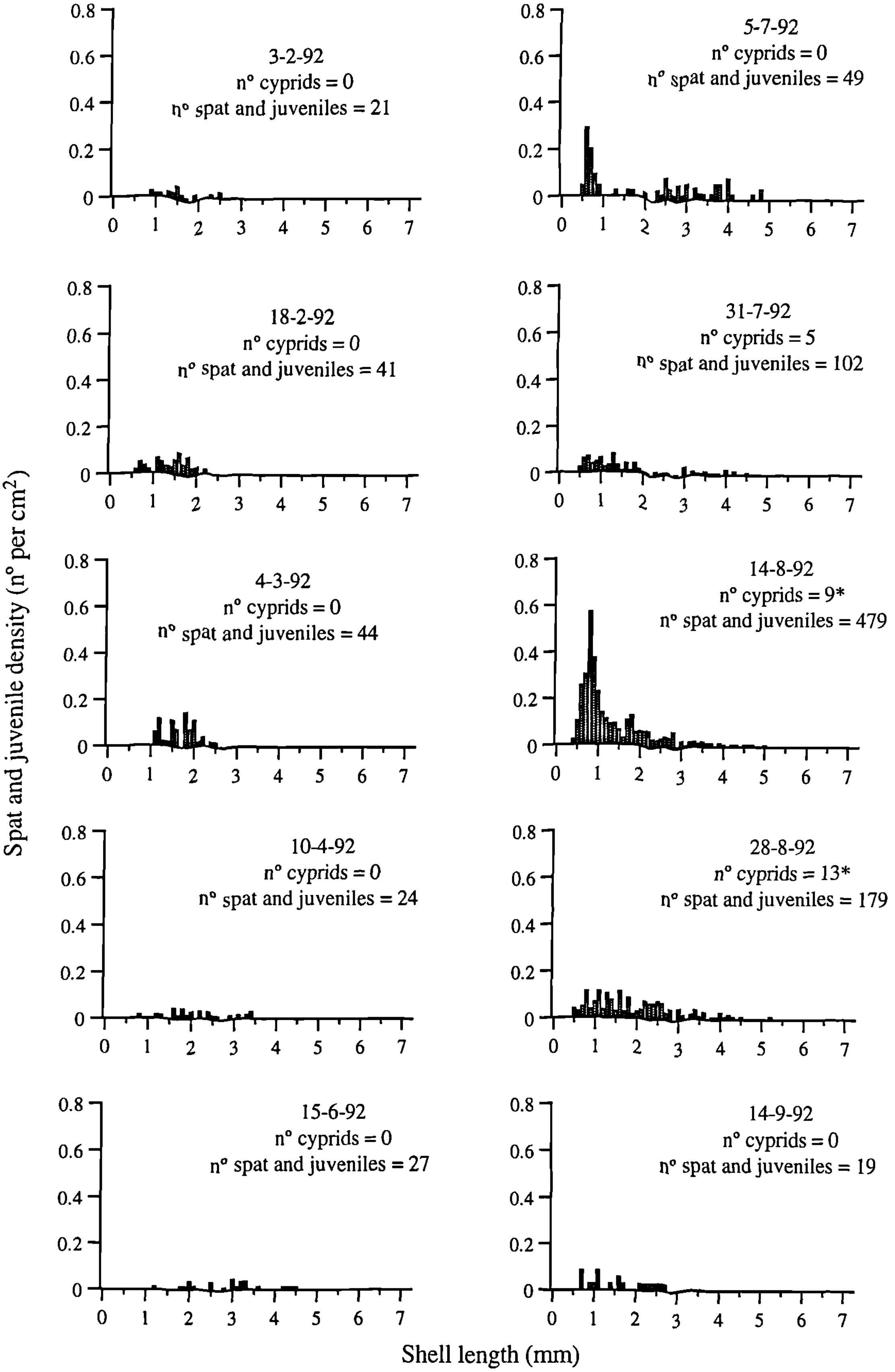


Fig I 3.3.9: Settlement and recruitment of *Elminius modestus* at mid shore.
The histograms show changes in density of various size classes of spat and juveniles over time.
The number of animals analysed for this purpose is reported on each graph.
Cyprids are not plotted, but the number of those observed is also reported.
All the data were obtained from the analysis of five rock samples collected on each date at each shore level. The density values are from the five replicates pooled together.

* Some could be *Chthamalus* cyprids

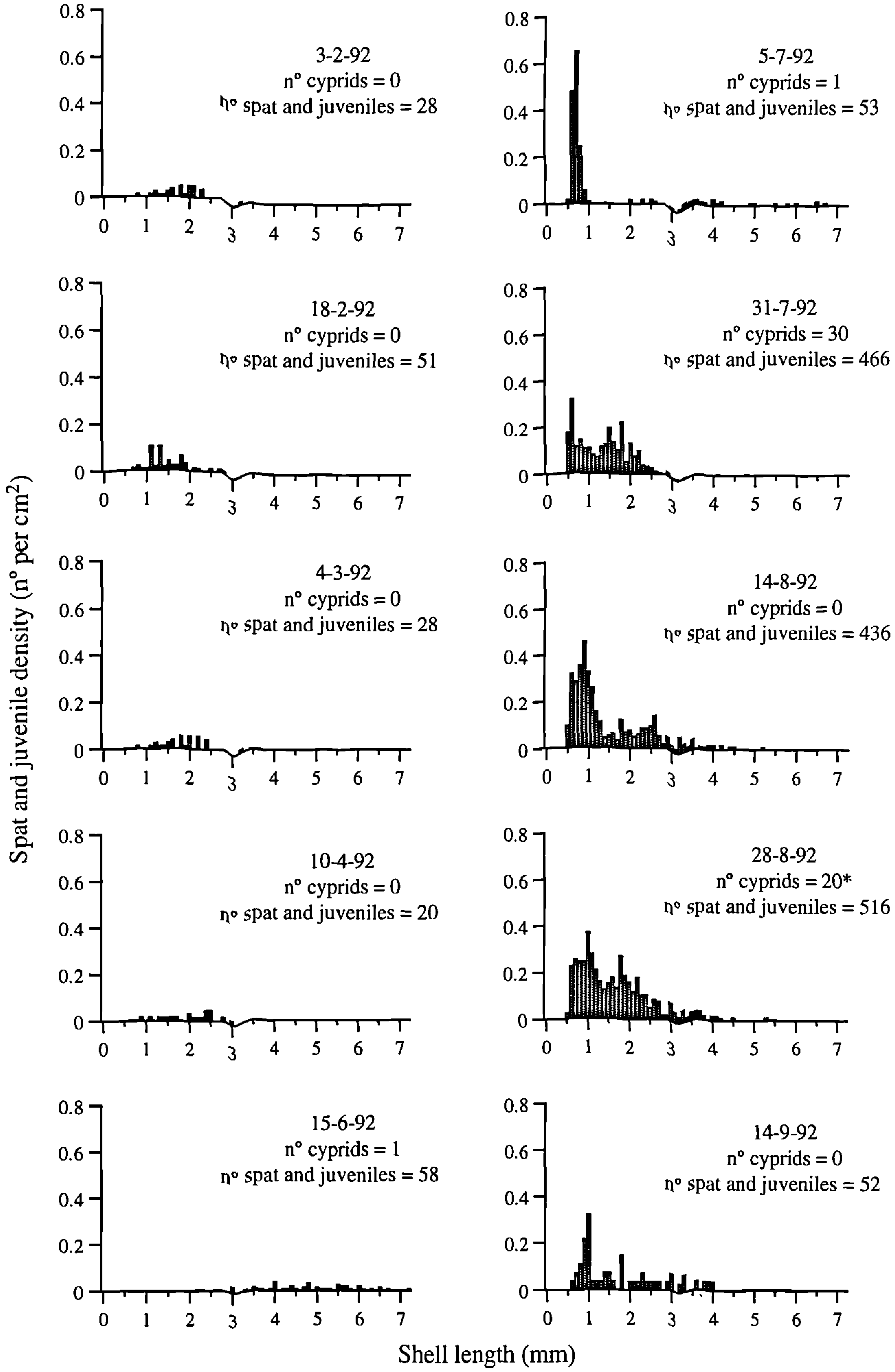


Fig I 3.3.10: Settlement and recruitment of *Elminius modestus* at low shore.
The histograms show changes in density of various size classes of spat and juveniles over time.
The number of animals analysed for this purpose is reported on each graph.
Cyprids are not plotted, but the number of those observed is also reported.
All the data were obtained from the analysis of five rock samples collected on each date at each shore level. The density values are from the five replicates pooled together.

* Some could be *Chthamalus* cyprids

settlement from the rock samples, are in perfect agreement with that observed for *Elminius* settlement on the photographs.

E. modestus early growth appeared to be much faster than that of *Chthamalus,* but slower than *Semibalanus*. In fact, while some of the low shore *Semibalanus* settlers reached a shell length of more than 5 mm in less than three months from settlement, *Elminius* juveniles grew up to only 4 mm in the same amount of time. In contrast, it took at least three months for the largest individuals of *Chthamalus* species to reach a shell length of 2.5 mm. These results are in agreement with that found for growth in class 0+ from the analysis of photographs (see section on growth).

In conclusion, the results obtained for settlement from the analysis of rock samples are in good agreement with that observed from the analysis of photographs. Therefore, it can be suggested that the detailed study of selected quadrats generates information on settlement which is representative of the whole shore.

I 3.4 GROWTH

METHODS

I 3.4.1 Growth of newly settled barnacles over the three year period

The seasonal pattern of growth in the four species was examined by measuring the barnacles on the series of photographs over a period of time. Length of the operculum of the selected barnacles was measured. For all species, measurements of the opercular length were taken every two months over the first year period of intensive study. The barnacles whose growth was going to be followed over time were not randomly chosen, but selected on the basis of the following criteria: barnacles which lived for a year or more were marked as possible candidates for analysis. Thus for *C. montagui*, *C. stellatus* and *E. modestus* which settled in autumn 1991, only those still alive in autumn 1992 were selected. For *S. balanoides* this period of one year occurred from the time of settlement in spring 1992 to spring 1993. At each shore level the marked barnacles were examined and the 30 most long-lived individuals of each species were chosen. The longevity was worked out by checking if the animal in question was still present on photographs taken two/three years later. In some species 30 individuals which lived a minimum of one year could not be found, in which case the maximum possible number was used.

An alternative way to bring the number of selected animals up to thirty was to include some of the barnacles which settled during the second year. This possibility was not used because it was thought that growth rate could change from year to year. If this was the case it would have not been desirable to mix together data on growth of barnacles which settled during two different years. As there were more barnacles of each species settling during the second year than during the first one (see results for settlement), another possibility was to start the study for growth from the barnacles which settled during the second year. This idea was abandoned as it would not have made good use of all the data available and in particular of the detailed information provided by the photographs taken during the first year.

The size of the selected animals was determined every two months during the first year and then on each of the remaining three sampling sessions during the following two years. Two months was thought to be an appropriate interval of time to avoid confusion between real growth and experimental error. The opercular length of each barnacle was measured using vernier callipers on the enlarged photographs. An Image Analyser had also been tried for this purpose but its use was soon abandoned because the image quality was not good enough and the procedure turned out to be too slow for the number of replicates needed.

I 3.4.2 Growth of identified individuals belonging to class 1+ and class 2+ or above over the period of the first year survey

The information obtained from the analysis of growth of newly settled barnacles over a three year period, was used to work out the approximate size of barnacles belonging to class 0+, 1+ and 2+ or above. The size range corresponding to a certain age class, was established for each species and each shore level. With this information available, it was then possible to recognise on the photographs barnacles belonging to different age classes. Together with size, other characters such as colour and degree of erosion of the shell, were used to distinguish between class 0+, 1+ and older barnacles. Thirty barnacles, or as many as possible (see Table I 3.4.1), for each species, age class and shore level were selected and marked on the photographs. No specimens of *E. modestus* (generally considered a low shore species) were found on the high shore. In addition, due to the short life of this species, only a few *Elminius* belonging to the older classes could be selected at mid and low shore.

Chthamalus and *Elminius* barnacles belonging to class 1+ and 2+ or over, were marked on the photographs taken in September 1991. In order to allow the study of growth over a year, only those barnacles which were still alive in September

Table I 3.4.1: Total number of barnacles for each species and each shore level which have been analysed for growth over a one year period.

Class 0+

shore level	*S. balanoides*	*C. montagui*	*C. stellatus*	*E. modestus*
High	10	30	10	-
Middle	29	15	13	10
Low	30	20	30	27

Class 1+

shore level	*S. balanoides*	*C. montagui*	*C. stellatus*	*E. modestus*
High	30	30	30	-
Middle	30	30	27	3
Low	30	27	30	11

Class 2+ and above

shore level	*S. balanoides*	*C. montagui*	*C. stellatus*	*E. modestus*
High	30	30	30	-
Middle	23	30	23	2
Low	19	27	30	4

1992 were chosen. As *S. balanoides* settles during spring, the study of growth for this species had to be started six months later. Hence, growth in *Semibalanus* belonging to class 0+, 1+ and 2+ or above, was measured between April 1992 and April 1993.

RESULTS

I 3.4.3 Growth of identified newly settled barnacles over a three year period

The number of analysed barnacles is summarised in Table I 3.4.1. All the information related to size of barnacles belonging to a certain age class, species and shore level, can be found in Table I 3.4.2. Values in this Table are derived from the growth curves obtained in the following section.

I 3.4.3.1 High shore (Fig I 3.4.1)

Only three species were analysed at this shore level as no specimens of *E. modestus* could be found on the high shore. The growth of *C. montagui* and *C. stellatus* overlapped during the first eight months after settlement. Between the eighth month and the year, *C. montagui* grew slightly faster than *C. stellatus.* After that period, *C. stellatus* accelerated its growth so that by the beginning of the second year, its opercular length was 0.2mm longer than the one of *C. montagui.* From the second year onward, *Chthamalus* growth stabilised and the change in size became very small. After three years and three months monitoring, the opercular length of *C. montagui* was 1.7mm, compared with 1.85mm in *C. stellatus.* At settlement the size of *S. balanoides* was approximately the same as the two *Chthamalus* species, but a high initial growth rate ensured that at the end of the summer of 1992 individuals of *S. balanoides* were larger than those of *Chthamalus* which settled six months earlier. By the end of the study, less than three years after settlement, the opercular size of *S. balanoides* was already up to 2.1 mm.

Table I 3.4.2: Opercular length size (X) in mm for each age class, species and shore level. Values were derived from the growth curves obtained in this section.

Class 0+

shore level	*S. balanoides*	*C. montagui*	*C. stellatus*	*E. modestus*
High	0.5< X <1.7	0.5< X <1.3	0.6< X <1.2	-
Middle	0.6< X <2.2	0.5< X <1.4	0.5< X <1.3	0.8< X <2.2
Low	0.4< X <2.2	0.4< X <1.3	0.6< X <1.5	1.1< X <2.5

Class 1+

shore level	*S. balanoides*	*C. montagui*	*C. stellatus*	*E. modestus*
High	1.7< X <2.1	1.3< X <1.7	1.2< X <1.8	-
Middle	2.2< X <2.5	1.4< X <1.7	1.3< X <1.6	2.2< X <?
Low	2.2< X <?	1.3< X <1.6	1.5< X <1.8	2.5< X <3.2

Class 2+ and above

shore level	*S. balanoides*	*C. montagui*	*C. stellatus*	*E. modestus*
High	X >2.1	X >1.7	X >1.8	-
Middle	X>2.5	X >1.7	X >1.6	-
Low	-	X >1.6	X >1.8	X >3.2

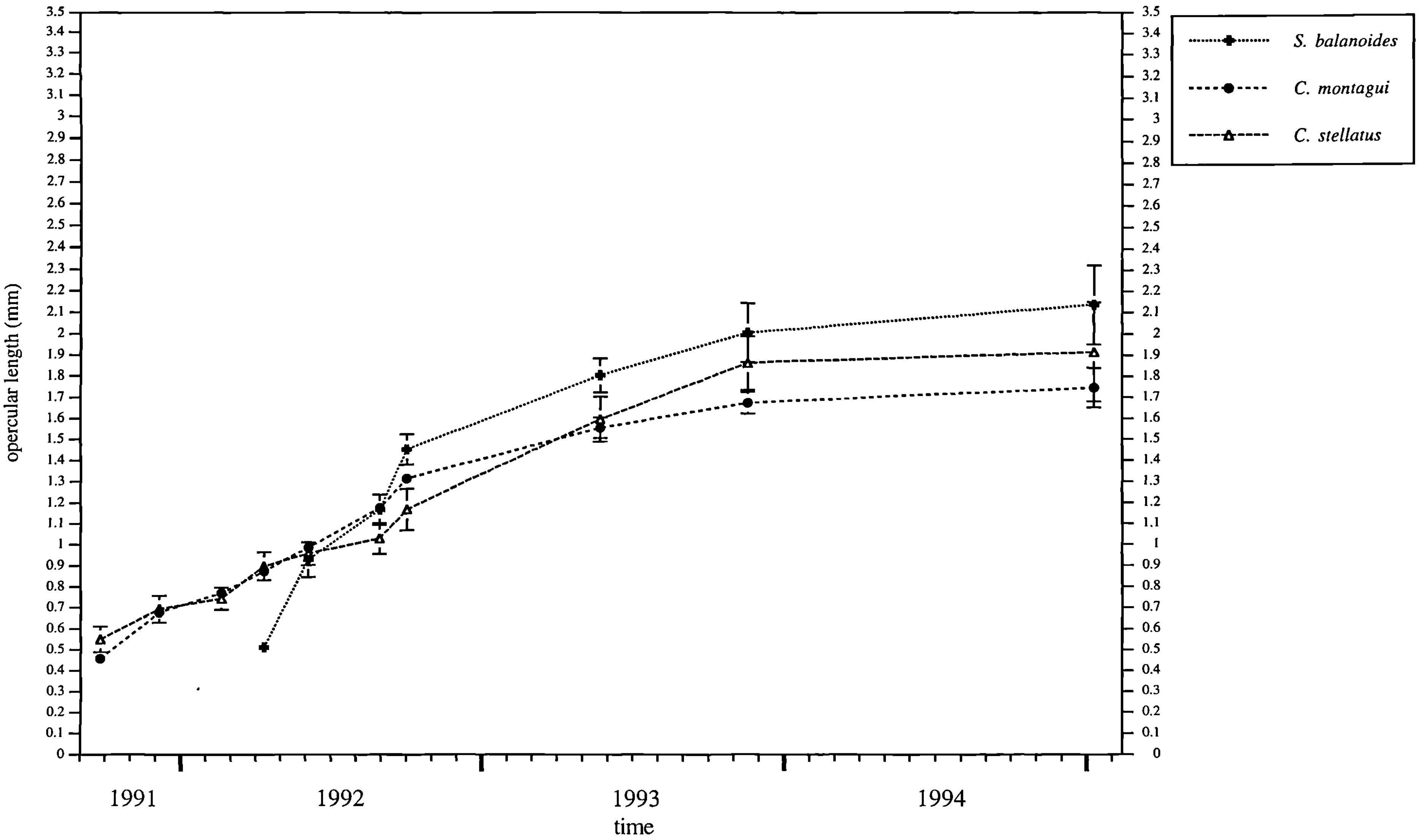


Fig I 3.4.1: High shore. Growth in barnacles over a three year period. No *E. modestus* were found at this level on the shore.

It is interesting to note that the two *Chthamalus* species and *S. balanoides* seemed to grow more or less at the same rate from Autumn 1992 onward.

I 3.4.3.2 Middle shore (Fig I 3.4.2)

The two *Chthamalus* species showed the same growth rate all the way through the study. Also their sizes nearly overlapped most of the time. Three years and three months after settlement, their opercular length reached 2mm in size. A few specimens of *E. modestus*, were found to settle at the same time as *Chthamalus*. Their growth rate during the first six months of life over the winter period was similar to that of the *Chthamalus* species. However during the second six months the growth rate increased dramatically. At the end of September 1992, the mean opercular length of *E. modestus* was 2.2mm compared to 1.35 and 1.25mm in *C. montagui* and *C. stellatus* respectively. *S. balanoides* showed rapid growth immediately after settlement. By the end of the summer six month old *S. balanoides* were larger than one year old individuals of the two *Chthamalus* species At the end of the study (thirty three months after settlement), the mean opercular length of *S. balanoides* at mid-shore was found to be 2.77mm. This value was much greater than the one observed on the high shore.

I 3.4.3.3 Low shore (Fig I 3.4.3)

On the low shore *C. montagui* and *C. stellatus* grew at more or less the same rate, although *C. stellatus* showed a consistently larger size than *C. montagui*. In January 1995 the mean opercular length of *C. montagui* was 2.0mm while that of *C. stellatus* was 2.3mm. The growth curve of *S. balanoides* during the first six months after settlement was even steeper than that observed on the mid-shore. Only two months after settlement, *Semibalanus* had already overtaken eight months old *Chthamalus*. *Semibalanus* growth stayed steady but slowed down considerably between six months and one and a half years. On the selected quadrats at the low shore site, no barnacles of this species which settled in spring 1992, survived until

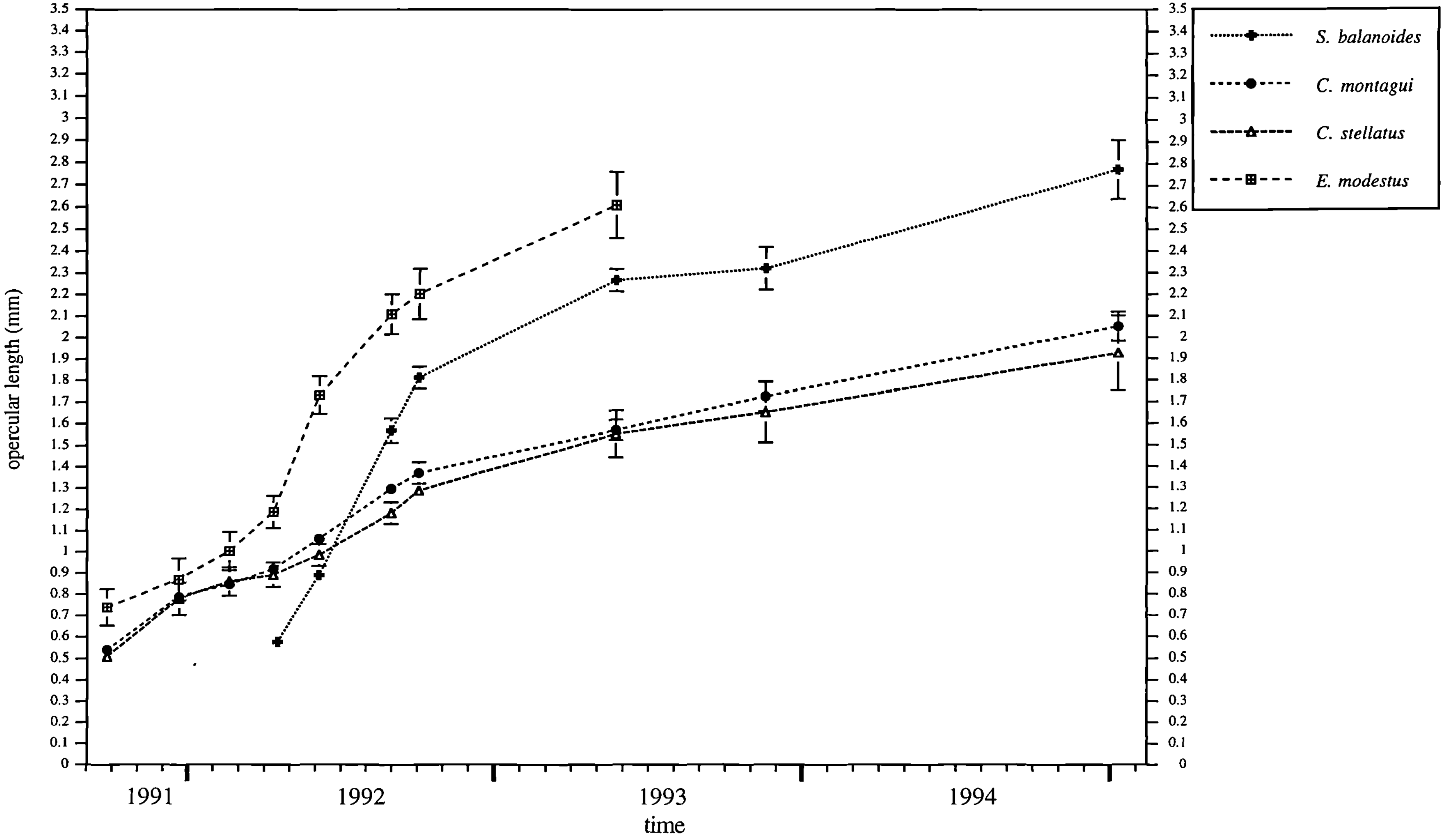


Fig I 3.4.2: Middle shore. Growth in barnacles over a three year period.

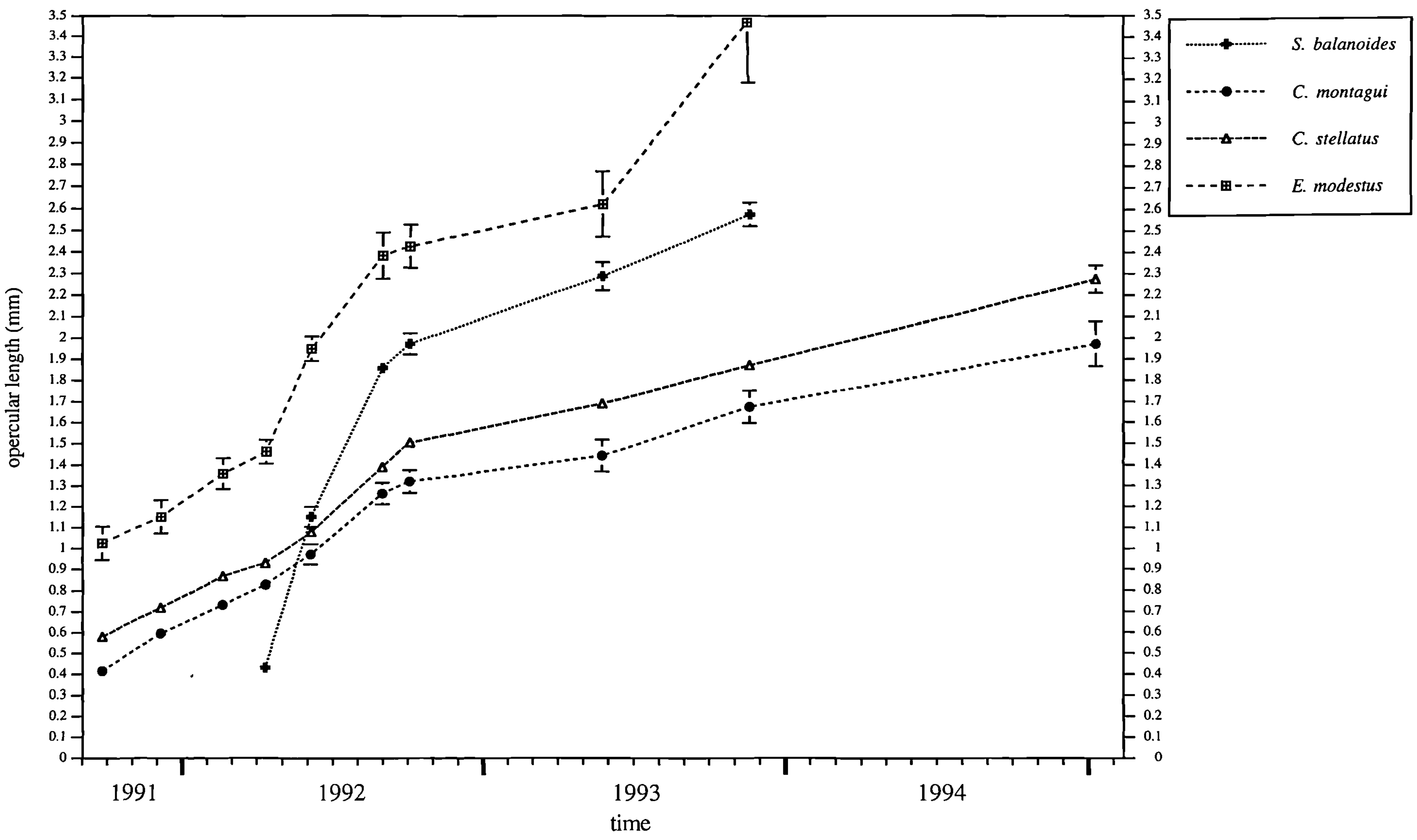


Fig I 3.4.3: Low shore. Growth in barnacles over a three year period.

January 1995. Therefore no information is available on *Semibalanus* growth during the third year. In the case of *E. modestus* the situation resembled quite closely the one observed on the mid-shore: relatively slow growth during the first six months followed by a period of fast growth during the summer period. The same growth pattern was observed during the winter and the summer of the second year. No data are available on growth of *Elminius* during the third year.

To summarise, growth rate increases with decreasing level on the shore. The two *Chthamalus* species showed very similar growth patterns and showed the lowest growth rates. *Semibalanus* showed a marked increase in size, with larger sizes reached at the lower shore levels, during the first six months, but then it slowed down. *Elminius* was not analysed at high shore, but at mid and low shore it was the species which reached the biggest size. Its growth seemed to be seasonal, with a large increase in size during the summer months and a much lower increase during the winter. The results also suggest that the two *Chthamalus* species may show this seasonal pattern of growth during the first year.

I 3.4.4 Comparison of growth of identified individuals belonging to class 0+, 1+ and 2+ or above over one year period of study

The number of analysed barnacles is summarised in Table I 3.4.1.

I 3.4.4.1 Class 0+ (Fig I 3.4.4)

The data for 0+ individuals are described above. Class 0+ data are presented in Fig I 3.4.4 in order to make a comparison with the growth rate of classes 1+ and 2+ (Figs I 3.4.5-6) over the same period.

An interesting point to take from the 0+ growth is the characteristic growth pattern of *S. balanoides*. Class 0+ barnacles of this species seemed to grow during the

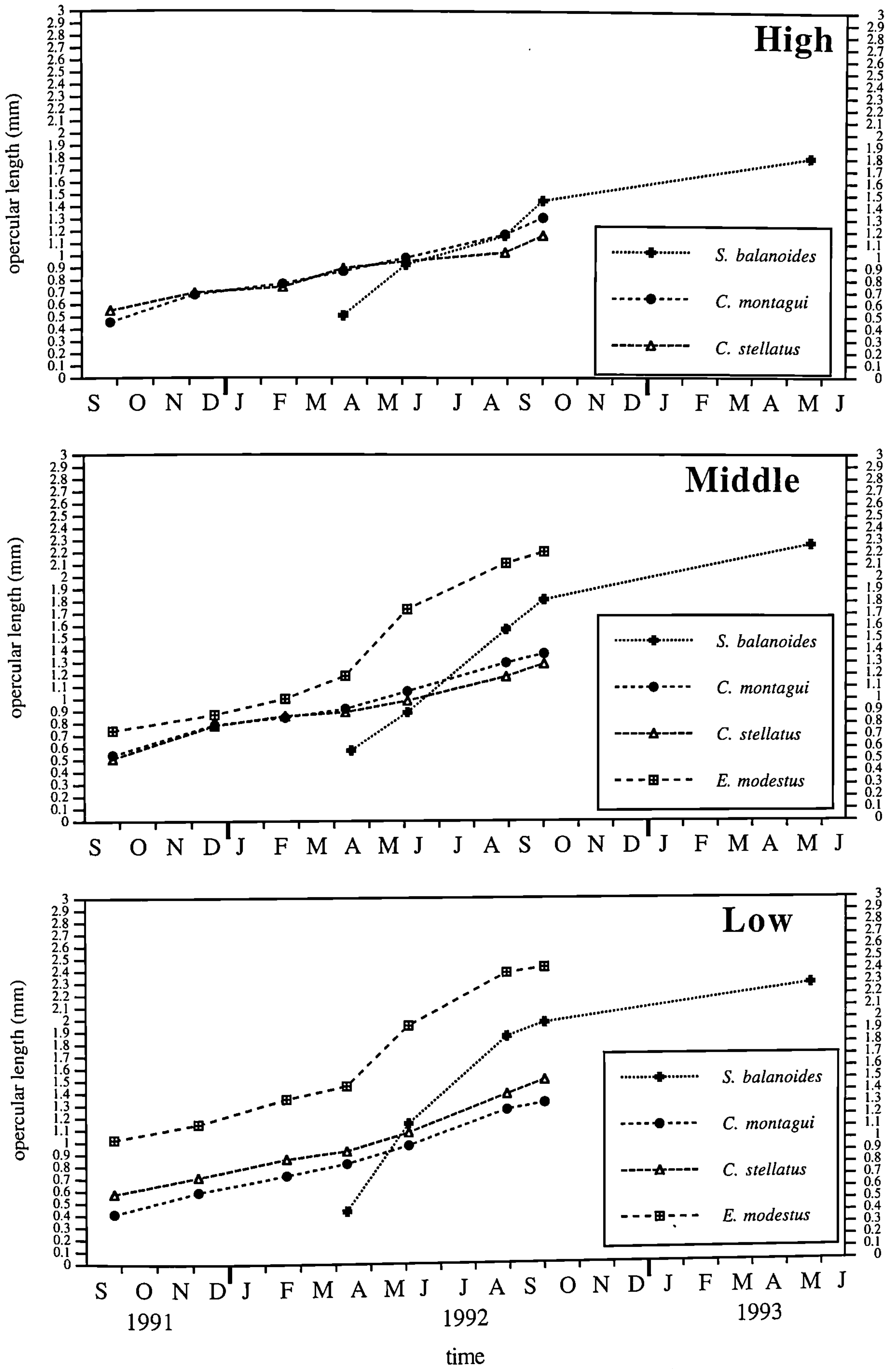


Fig I 3.4.4: Growth in barnacles belonging to class 0+ over one year study. Data points show the mean sizes of 30 animals per each species and shore level. For exceptions to this rule see Table I 3.4.1. Error bars are omitted for clarity.

first six months at different rates on the different shore levels. The differences disappeared almost completely during the second half of the first year, implying that differences in size in relation to shore levels were mainly determined in the first months of life.

I 3.4.4.2 Class 1+ (Fig I 3.4.5)

It is immediately apparent from Fig I 3.4.5 the large reduction in growth rate in the second year of life. Although this is true of all species, it is more obvious in *Elminius* and *Semibalanus.*

High shore The growth rate of class 1+ barnacles on the high shore, was very low with an increase of no more than 0.2mm in the opercular length of the two *Chthamalus* species and less than 0.3mm in *S. balanoides.* The increase in size of *Chthamalus* appeared to occur over the autumn/winter with no increase over the summer.

Mid shore The same pattern as above was observed in *Chthamalus.* The difference in size between the two species became slightly more evident at this level on the shore. Only three specimens of *E. modestus* belonging to class 1+ which survived for at least a year, could be found at mid-shore; their mean size, stayed more or less constant throughout the whole year.

Low shore On the low shore the slightly seasonal pattern of *Chthamalus* growth described above became more evident as did the difference in size between the two species. There was only a very small increase in the growth rate of *Chthamalus* species lower on the shore. *Semibalanus* growth rate was high during spring/summer 1992, but declined during the winter. *E. modestus* showed a quite confusing pattern which could be summarised as a slight increase in size over the one year period.

The slightly seasonal growth pattern of the two *Chthamalus* species, with slower increase during spring/summer and faster increase during autumn/winter, was surprising. It is interesting to note that 0+ individuals showed a reversed pattern.

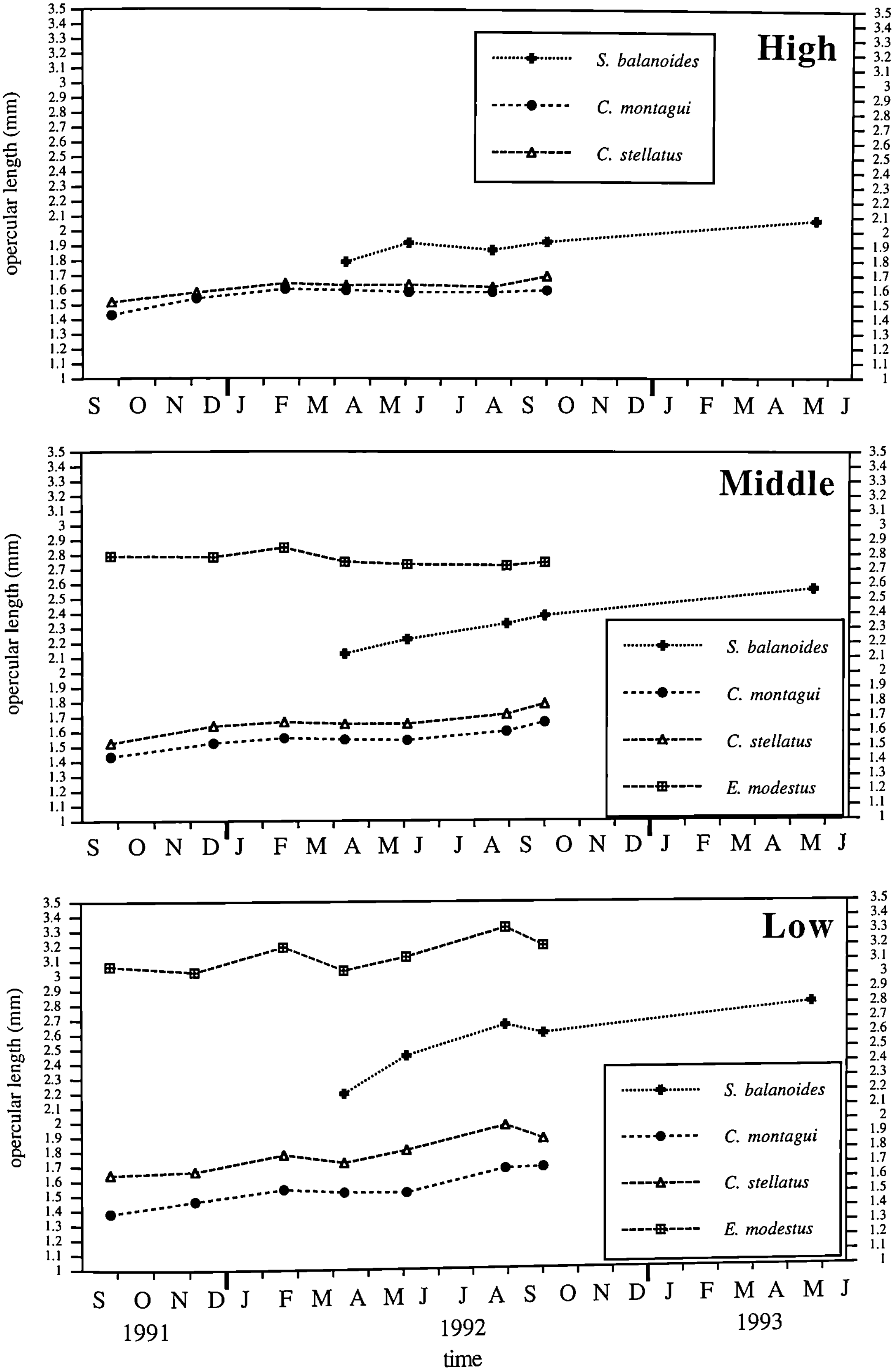


Fig I 3.4.5: Growth in barnacles belonging to class 1+ over one year study. Data points show the mean sizes of 30 animals per each species and shore level. For exceptions to this rule see Table I 3.4.1. Error bars are omitted for clarity.

I 3.4.4.3 Class 2+ and above (Fig I 3.4.6)

High shore Class 2+ *Semibalanus* and *Chthamalus* showed very little increase throughout the year. The growth of *C. montagui* and *C. stellatus* appeared to be limited to the autumn and winter months, in agreement with observations for class 1+ barnacles.

Middle shore The same pattern as above can be seen for *Semibalanus* and *Chthamalus*. *Elminius* growth, although quite limited, took place mainly during spring/summer time.

Low shore At this shore level the difference in size among the four species becomes very evident. *Elminius*, the largest species, showed an opercular length twice as long as that of *C. montagui*. Growth rates, however, did not show any great increase, suggesting that the size distribution of the four species had already been determined in the earlier stages of life. The seasonal pattern of growth on the low shore resembled that observed previously, with *Chthamalus* species growing mostly during autumn/winter and *Semibalanus* and *Elminius* growing in spring/summer.

From the second year after settlement onwards, the growth rate of all four species seemed to slow down consistently. Therefore, it can be suggested that the first two years of life are the most important in defining the size of the animal.

A comparison of growth rate between shore levels using one way ANOVA, reveals significant differences in only one species, *S. balanoides* (Table I 3.4.3). In both 0+ and 1+ age classes, the growth rate of *S. balanoides* increases sharply with decreasing height on the shore. Indeed in class 1+ barnacles the growth rate on the low shore is more than twice that on the high shore. This is clearly shown by the significant results of the ANOVA and Tukey Kramer tests. In age class 2+, *S. balanoides* shows no clear pattern with shore height. In both *Chthamalus* species, no clear pattern emerges, although in *C. stellatus* there is some indication that growth rate increases lower on the shore. However, no significant results were obtained.

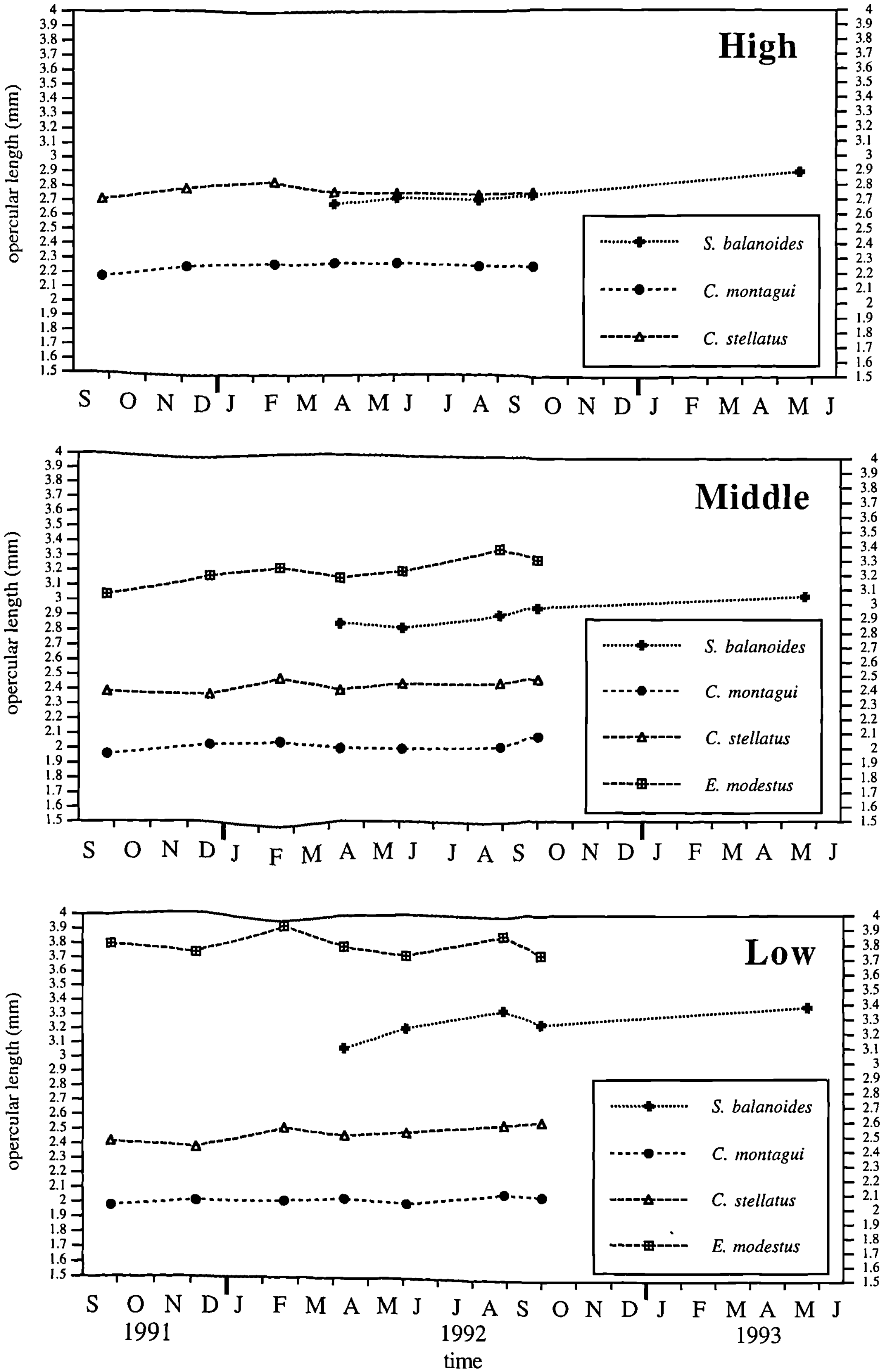


Fig I 3.4.6: Growth in barnacles belonging to class 2+ over one year study. Data points show the mean sizes of 30 animals per each species and shore level. For exceptions to this rule see Table I 3.4.1. Error bars are omitted for clarity.

Table I 3.4.3 One way ANOVA and Tukey Kramer tests to determine differences in growth rates between shore heights.

	Source	df	Mean Square	*F*-Value	*P*-Value	Tukey Kramer Test*
Semibalanus balanoides						
0+	Shore height	2	2.0 x 10-6	8.47	0.007	High ≠ Mid
	Residual	10	2.65 x 10-7			High ≠ Low
1+	Shore height	2	1.0 x 10-6	12.377	0.0006	High ≠ Mid
	Residual	16	8.52 x 10-8			High ≠ Low
						Mid ≠ Low
2+	Shore height	2	4.69 x 10-8	0.344	0.714	NA
	Residual	15	1.36 x 10-7			
Chthamalus montagui						
0+	Shore height	2	1.26 x 10-7	0.964	0.405	NA
	Residual	14	1.30 x 10-7			
1+	Shore height	2	1.29 x 10-7	2.387	0.126	NA
	Residual	15	5.39 x 10-8			
2+	Shore height	2	4.07 x 10-8	0.258	0.776	NA
	Residual	18	1.58 x 10-7			
Chthamalus stellatus						
0+	Shore height	2	1.0 x 10-6	2.709	0.107	NA
	Residual	12	2.9 x 10-7			
1+	Shore height	2	5.38 x 10-8	0.622	0.549	NA
	Residual	16	8.64 x 10-8			
2+	Shore height	2	2.41 x 10-7	3.020	0.081	NA
	Residual	14	7.96 x 10-8			
Elminius modestus						
0+	Shore height	1	7.14 x 10 -8	0.056	0.818	NA
	Residual	9	1.0 x 10-6			

* NA- Not applicable i.e. ANOVA shows no significant difference

≠ indicates significant difference at 5% level between stated shore heights. Experiment wise error rate = 5%

I 3.5 MORTALITY

METHODS

I 3.5.1 Mortality in newly settled barnacles from the analysis of photographs

At intervals throughout the 1991-1992 settlement season of each species, newly settled barnacles were identified on the photographs. The barnacles in each of these subcohorts were marked on the photographs using different coloured labels for each subcohort. Once labelled the number of individuals within each subcohort was assessed at successive sampling dates in the photographic series. In this way it was hoped to determine any difference in mortality rate between early and late settlers. The fate of each subcohort was followed until the end of the study period in January 1995. In this way subcohorts of for example *Chthamalus* were followed for a period of more than three years.

I 3.5.2 Mortality in juveniles and adults from the analysis of photographs

Mortality of individuals belonging to class 0+, 1+ and 2+ or above was monitored for each species from the end of the settlement period in 1991 (*Chthamalus* species) and 1992 (*S. balanoides* and *E. modestus*) until the end of the three year study period in January 1995. Barnacles which settled during the year of intensive monitoring and were still alive at the end of the settlement season were chosen for mortality analysis in class 0+. All individuals of this age class which were present in each replicate were used to determine mortality. Thus in some cases (e.g. low shore *S. balanoides*) up to 100 barnacles in a single replicate were analysed.

In order to identify the age classes 1+ and 2+ or above, barnacles were measured along the opercular length. By using the species-specific growth curves, already available from other data, an estimate of the age was made. At the end of each species' settlement period, sixty individuals were selected for each age class (1+ and 2+) and shore level. In general, ten individuals were randomly chosen from each one of the six replicates.

Although this data provide useful information over a long period, the difference in the settlement seasons between species means different species were monitored over different lengths of time. For this reason mortality rates were calculated over a one year period starting from the end of each settlement period. Sampling dates which were selected for analysis were chosen to allow a calculation of mortality over as close to a one year period as possible. This shorter period also allows the mortality rate to be related to the age class of each cohort.

RESULTS

I 3.5.3 Mortality in newly settled barnacles

The percentage survival of different subcohorts from within a single settlement season are presented in Figs I 3.5.1-3. Replicates for each subcohort have been pooled to provide adequate numbers of individuals to accurately represent percentage survival. Data are therefore not amenable to statistical analysis. However examination of the graphs is sufficient to enable interesting conclusions to be reached as many of the differences are very obvious. Although data are presented for up to a three year period, examination of the trend in mortality over the first two hundred days from the date of the first subcohort provides the most useful information. It is likely that time of settlement will influence survivorship during the first few months of life to a great extent.

In *Chthamalus* species at all shore heights it can clearly be seen that earlier settling subcohorts have higher levels of survival over this period than those barnacles

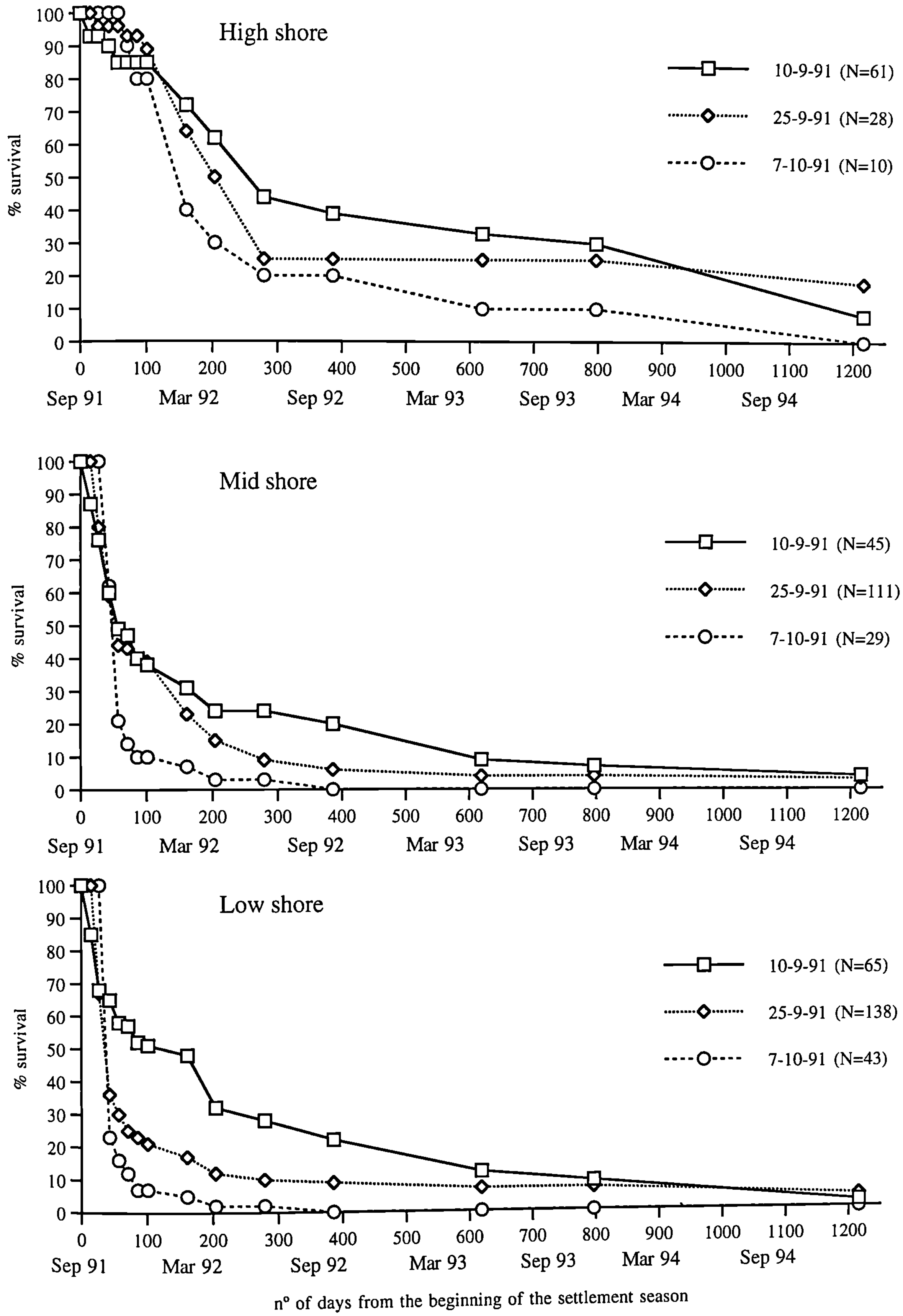


Fig I 3.5.1: Percentage survival of different subcohorts of *Chthamalus* at three shore heights.
Because of the small number of settling barnacles in some of the replicates, all replicates analysed on each date were pooled together.
The legends indicate the date on which each subcohort was identified and the number of individuals monitored.

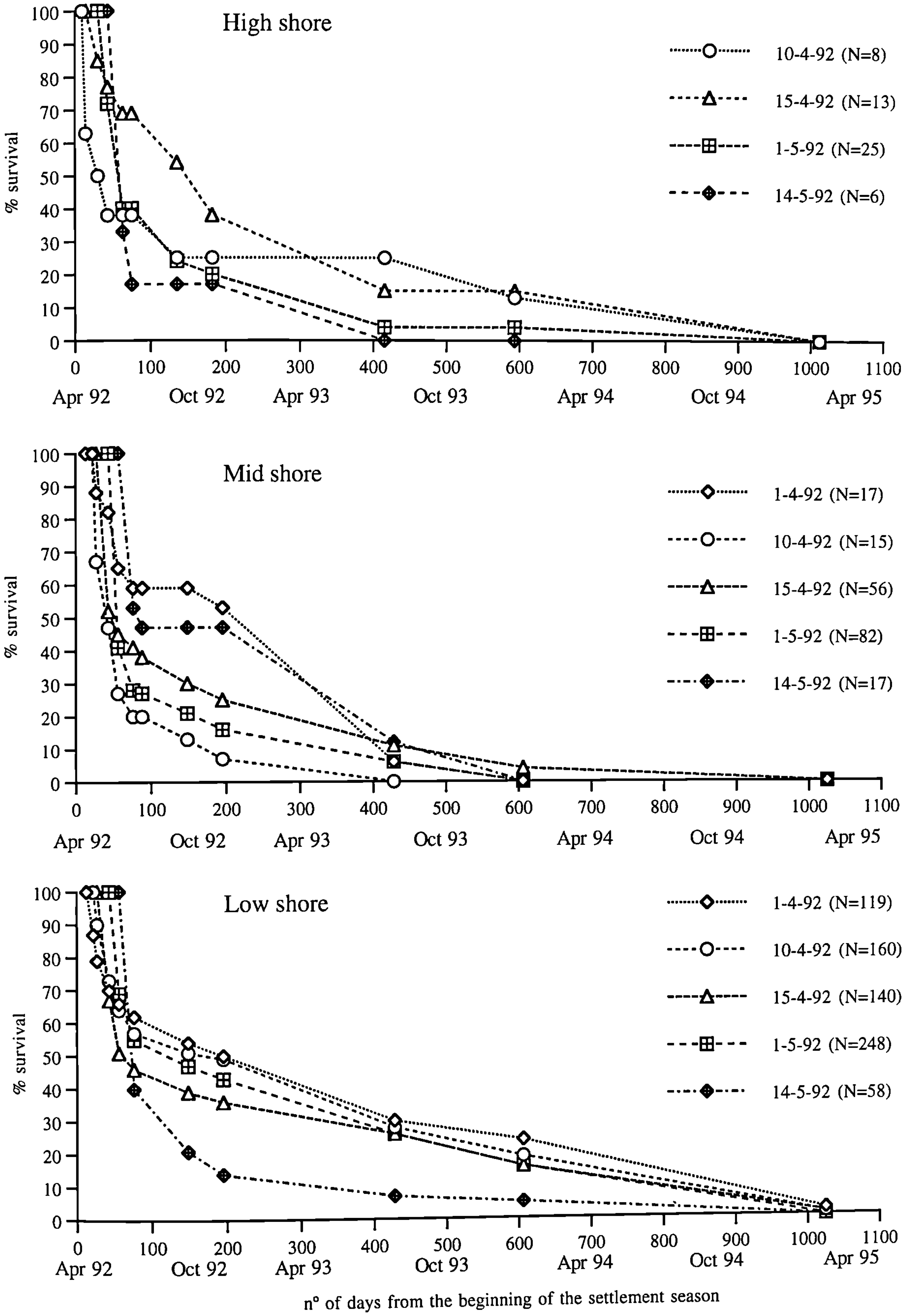


Fig I 3.5.2: Percentage survival of different subcohorts of *Semibalanus balanoides* at three shore heights. Because of the small number of settling barnacles in some of the replicates, all replicates analysed on each date were pooled together. The legends indicate the date on which each subcohort was identified and the number of individuals monitored.

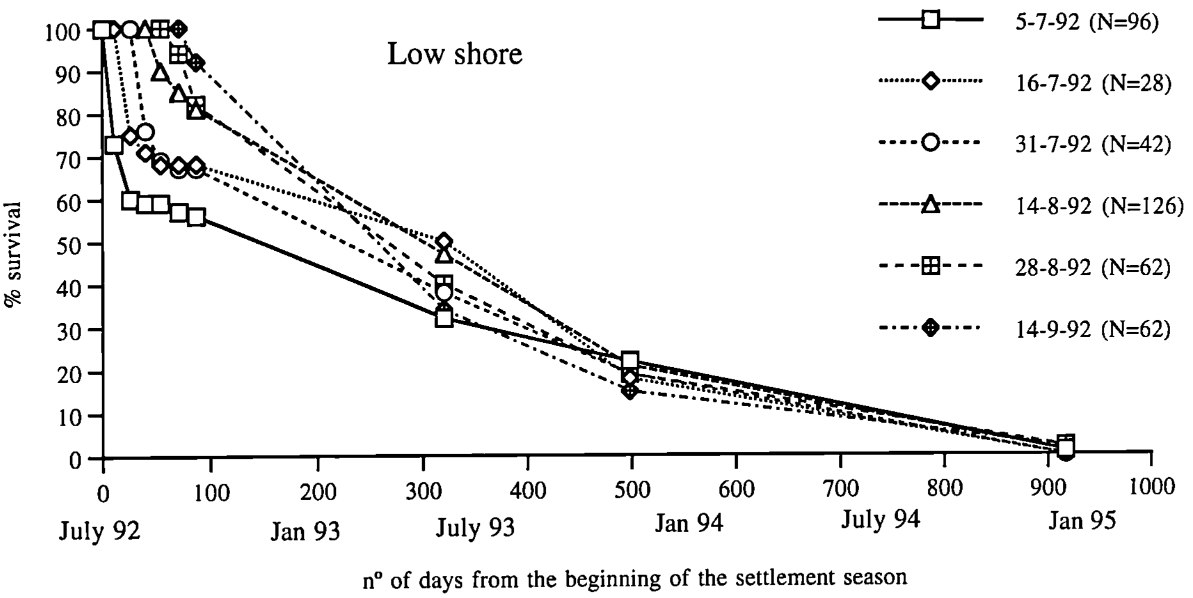


Fig I 3.5.3: Percentage survival of different subcohorts of *Elminius modestus* at three shore heights. Because of the small number of settling barnacles in some of the replicates, all replicates analysed on each date were pooled together. The legends indicate the date on which each subcohort was identified and the number of individuals monitored. No data were collected from high and mid shore due to the scarce presence of the species.

settling later. At the two hundred day sampling point the earliest settlers (the 10/9/91 subcohort) have a higher level of survival than those settling later despite a longer period over which to survive. For example on the low shore the 10/9/91 subcohort had a level of survival of just under 30% compared to 3% in the 7/10/91 subcohort.

In *S. balanoides* mortality in different subcohorts does not show the same consistent pattern as in *Chthamalus* although on the low shore there is evidence to suggest that later settlers suffer increasing levels of mortality (Fig I 3.5.2). For example at the 200 day sampling date the level of survival in the latest settling subcohort was four times less than that in the first subcohort. However this pattern is not displayed at high and mid shore levels.

E. modestus was only monitored on the low shore. This species does not appear to suffer increasing mortality in later settlers. This is probably the result of its capacity to survive under the most various climatic conditions which in fact allow this species to settle all year round.

Because of the long period over which settlers were monitored information on the longevity of different species may be obtained. At the final sampling dates which varied from 910 days after first settlement in *E. modestus* to 1215 days in *Chthamalus* species, levels of survival were very low in all species. Mean percentage survival of separate subcohorts was less than 2% in all species except in *Chthamalus* on the high shore where a mean percentage survival of 9% was observed.

In summary, early settlers of the two *Chthamalus* species, show better survival than later settlers at all three shore levels. The reason for this is unclear. In *Semibalanus* the pattern is not so obvious, although at mid and low shore survivorship seems to be higher in the early subcohorts. For this species, higher mortality in late settlers could be related to the higher desiccation stress that spat undergo towards the end of the settlement season. In contrast to the observations for the other three species, the mortality of *E. modestus* appears to be independent of time of settlement.

In general, mortality at the early stages of life is extremely high, with very few individuals surviving (only 2% of the settling population was found still alive three years later).

I 3.5.4 Mortality in juveniles and adults

I 3.5.4.1 Presentation of data and statistical analysis

The data on mortality of barnacles is presented in Figs I 3.5.4-6 as percentage survival plotted against time. This presentation allows an instant assessment of how barnacle numbers have changed over the whole period of study and gives some indication of longevity. In order to examine and compare mortality rates data are also presented as regression lines through the mean of the natural log of number of individuals plus one (because of zero values) (Figs I 3.5.7-9). These regression lines were calculated for a one year period starting from the end of each species settlement period. In order to statistically compare mortality rates between species, shore heights and age classes, regression coefficients of the natural log of 'number of individuals +1' against time were calculated for each individual replicate in each species, shore height, age class combination. These data are presented in I 3.5.1. Owing to the non-independent nature of the data only comparisons of mortality rates between shore heights could be analysed using Analysis of Variance (ANOVA). Comparisons of mortality rates between species and between age classes using ANOVA were not justified since for example information on two different species was gathered from the same six replicates. To overcome this problem species within a replicate were matched and hence paired t tests were used. The use of multiple t tests in this way does increase the probability of a type I error, that is rejecting the null hypothesis when it is in fact true. This problem is acknowledged and should be borne in mind when examining the results of the tests.

I 3.5.4.2 Percentage survival

In *S. balanoides* the highest level of survival at the final sampling date (after 950

Table I 3.5.1: Regression coefficients of mortality data for individual replicates taken from the regression of (natural log of n° of individuals +1) against time.

		Replicates						
		1	2	3	4	5	6	Mean
Semibalanus balanoides								
0+	High	-0.00424	-0.00274	-0.00192				-0.00297
	Mid	-0.00463	-0.00192	-0.00440	-0.00601	-0.00451		-0.00429
	Low	-0.00168	-0.00432	-0.00212	-0.00142	-0.00221	-0.00172	-0.00224
1+	High	-0.00434	-0.00439	-0.00189	-0.00148	-0.00138	-0.00127	-0.00246
	Mid	-0.00359	-0.00800	-0.00767	-0.00573	-0.00222		-0.00544
	Low	-0.00232	-0.00517	-0.00507	-0.00381	-0.00507	-0.00234	-0.00396
2+	High	-0.00493	-0.00186	-0.00127	-0.00310	-0.00514	-0.00238	-0.00311
	Mid	-0.00125	-0.00571	-0.00456	-0.00798	-0.00732		-0.00536
	Low	-0.00288	-0.00189	-0.00706	-0.00226	-0.00087	-0.00216	-0.00285
Chthamalus species								
0+	High	-0.00529	-0.00166	-0.00083	-0.00259	-0.00517	-0.00216	-0.00295
	Mid	-0.00467	-0.00437	-0.00786	-0.00257	-0.00282	-0.00118	
		-0.00169	-0.00778					-0.00412
	Low	-0.00554	-0.00101	-0.00183	-0.00575	-0.00223		-0.00327
Chthamalus montagui								
1+	High	-0.00292	0.00000	-0.00028	0.00000	0.00000	0.00000	-0.00053
	Mid	-0.00181	-0.00253	-0.00315	-0.00460	-0.00232	-0.00197	-0.00273
	Low	-0.00243	-0.00112	-0.00464	-0.00257	-0.00404		-0.00296
2+	High	-0.00126	0.00000	-0.00028	0.00000	-0.00035	0.00000	-0.00031
	Mid	-0.00366	-0.00113	-0.00316	-0.00150	-0.00084		-0.00206
	Low	-0.00108	-0.00090	-0.00158	-0.00239	-0.00505		-0.00220
Chthamalus stellatus								
1+	High	-0.00474	-0.00106	-0.00106	-0.00040	0.00000		-0.00145
	Mid	-0.00302	-0.00082	-0.00157	-0.00273			-0.00203
	Low	-0.00058	-0.00123	-0.00087	-0.00133	-0.00141		-0.00108
2+	High	-0.00292	0.00000	-0.00038	0.00000	-0.00056	0.00000	-0.00064
	Mid	0.00000	-0.00020	-0.00242	0.00000			-0.00066
	Low	-0.00016	0.00000	-0.00088	-0.00140	-0.00092		-0.00067
Elminius modestus								
0+	Low	-0.00321	-0.00324	-0.00381	-0.00220	-0.00277	-0.00367	-0.00315
1+	Low	-0.00412	-0.00104	-0.00328	-0.00328	-0.00168	-0.00104	-0.00240

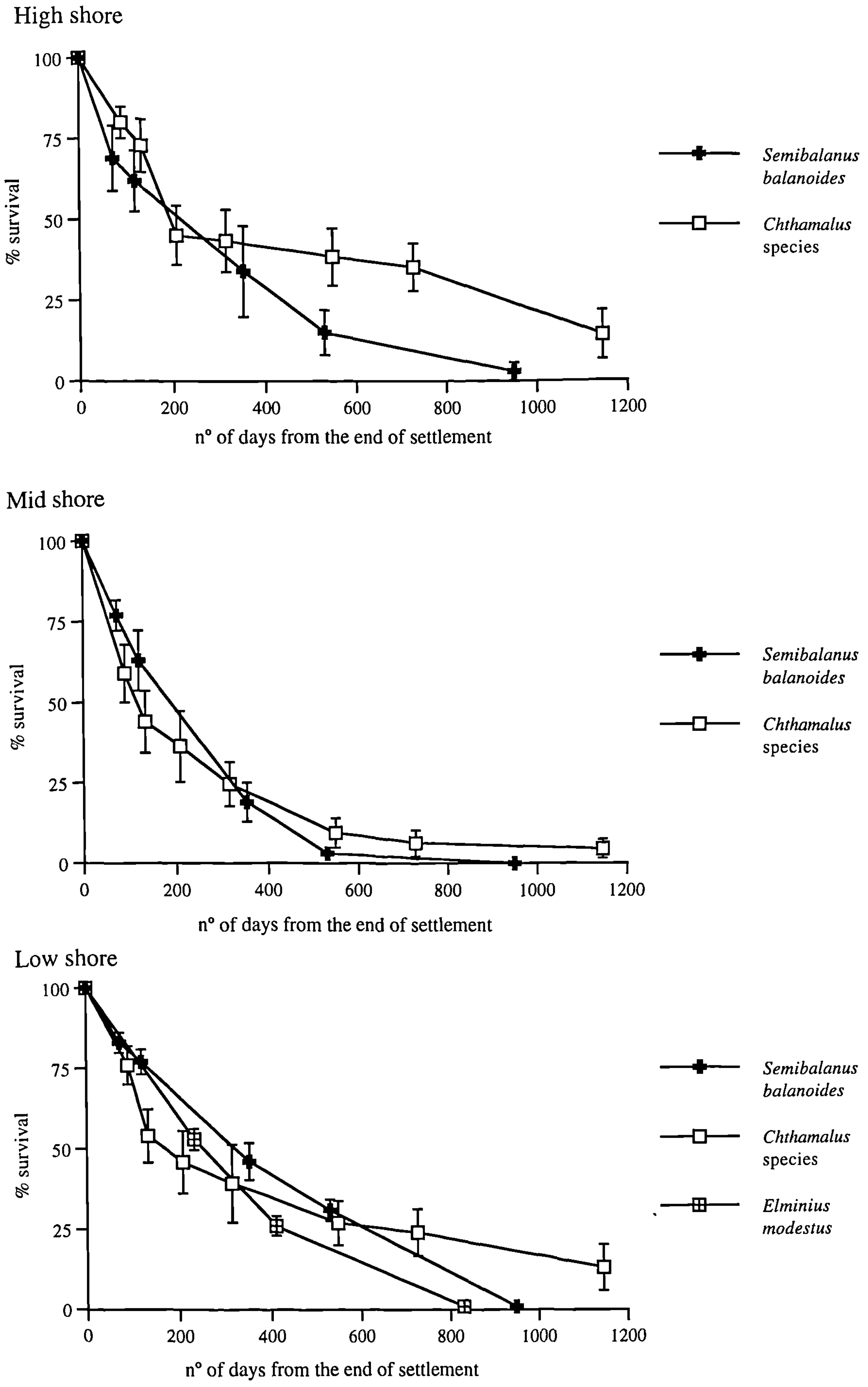
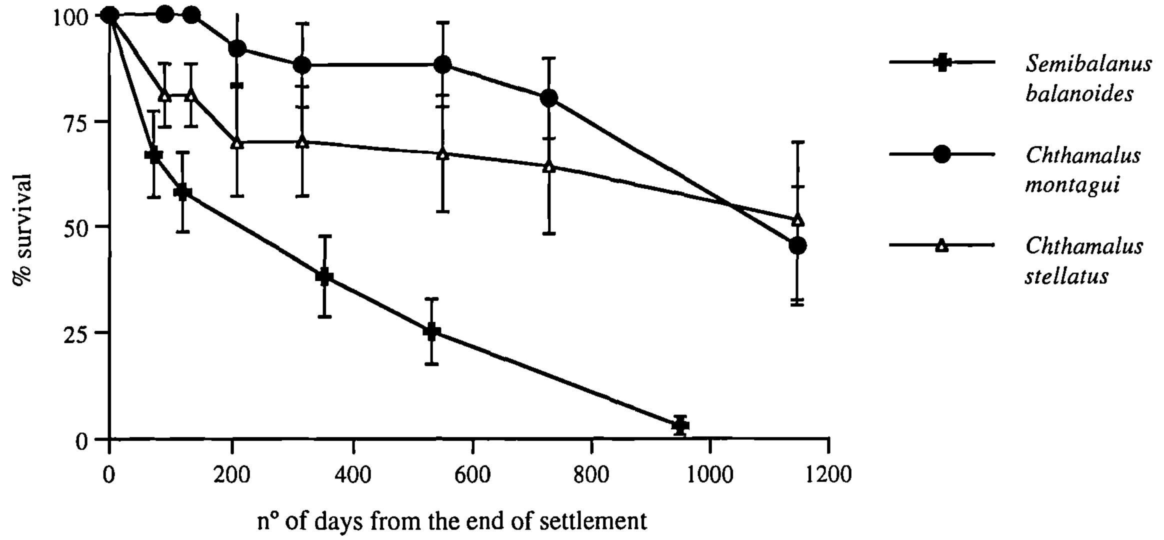
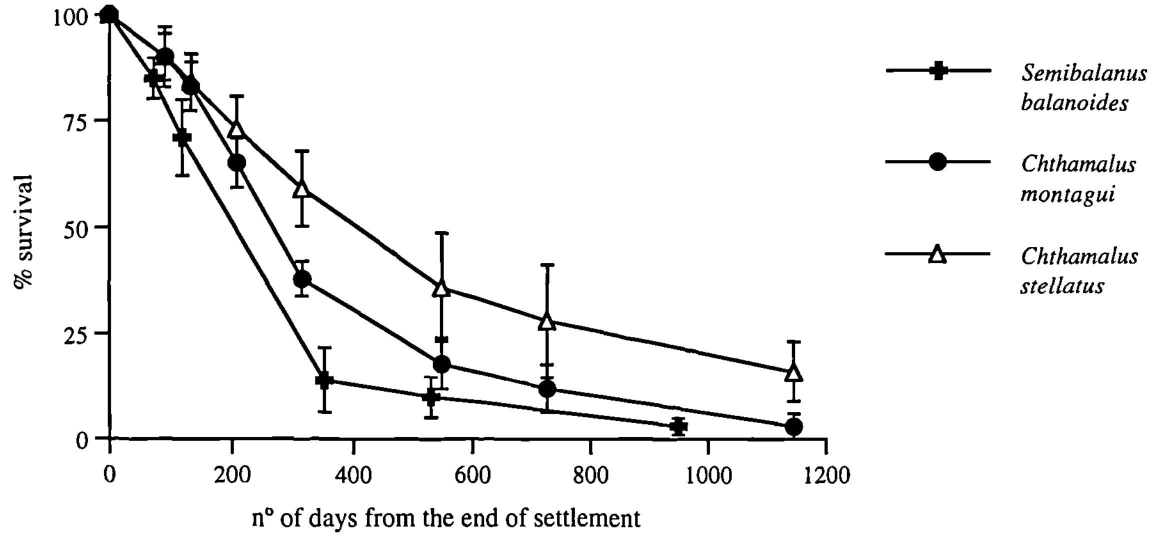


Fig I 3.5.4: Percentage survival of 0+ age class barnacles at Cellar Beach. Error bars = ± 1SE

High shore



Mid shore



Low shore

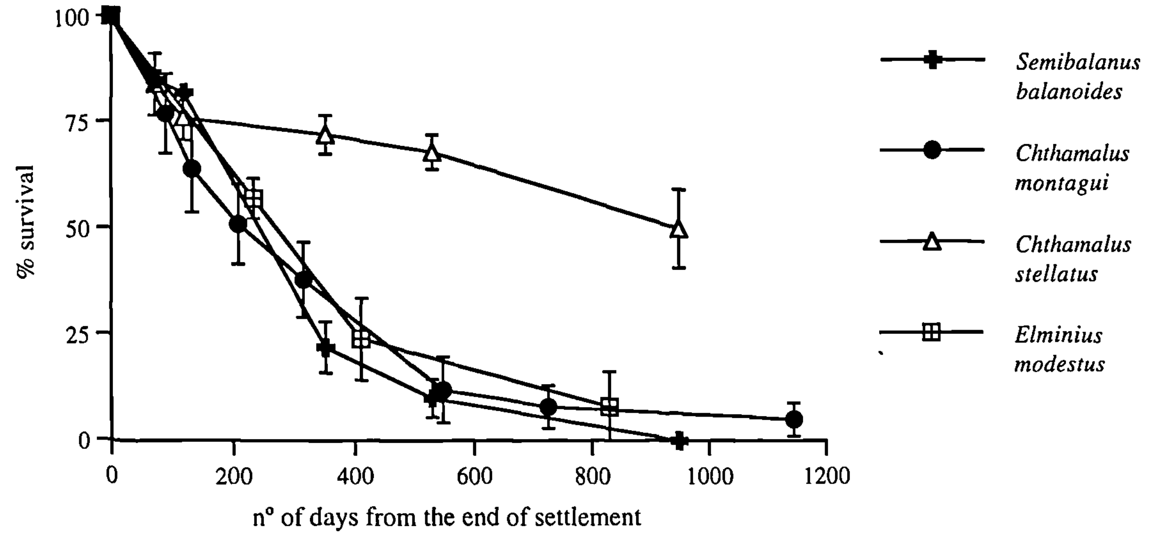
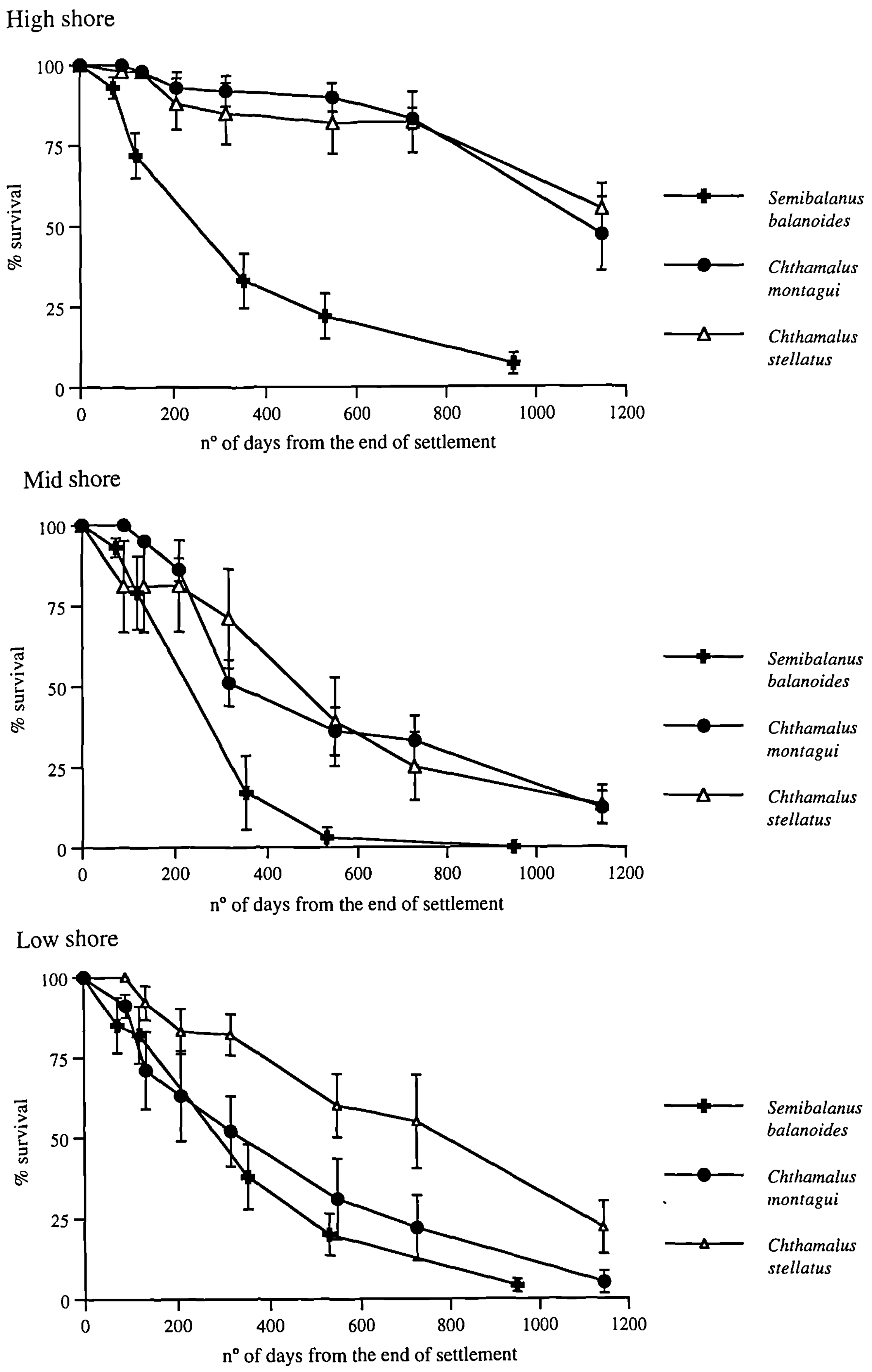


Fig I 3.5.5: Percentage survival of 1+ age class barnacles. Error bars = ± 1 SE



High shore

Mid shore

Low shore

Fig I 3.5.6: Percentage survival of 2+ age class barnacles at Cellar Beach. Error bars = ± 1SE days of monitoring) was only 8% (Fig I 3.5.6a). This was found in the age class 2+ on the high shore. Most other class/height combinations showed much lower levels of survival and in many cases all monitored individuals had died. These results indicate a high level of mortality in *S. balanoides* over three years, but also suggest that this species may survive for up to five years of age at all shore levels.

C. montagui and *C. stellatus* both show very high rates of survival in 1+ and 2+ age classes on the high shore with levels of approximately 50% after 1150 days. Survival decreased down the shore, except for *C. stellatus* 1+ on the low shore. However, in all cases levels of survival were still higher than in *S. balanoides* at the end of the study period (despite a longer period of monitoring). In the age class 0+, *Chthamalus* species showed a level of survival closer to that of *S. balanoides.*

E. modestus showed the lowest percentage survival among class 0+ individuals of the four species. Data from class 1+ show that a few specimens were able to survive for at least three years. These values increase the previously suggested one year life span of this species.

While the rate of mortality is very high in individuals of class 0+, older individuals seem to have a better chance of survival. This is particularly true for the *Chthamalus* species.

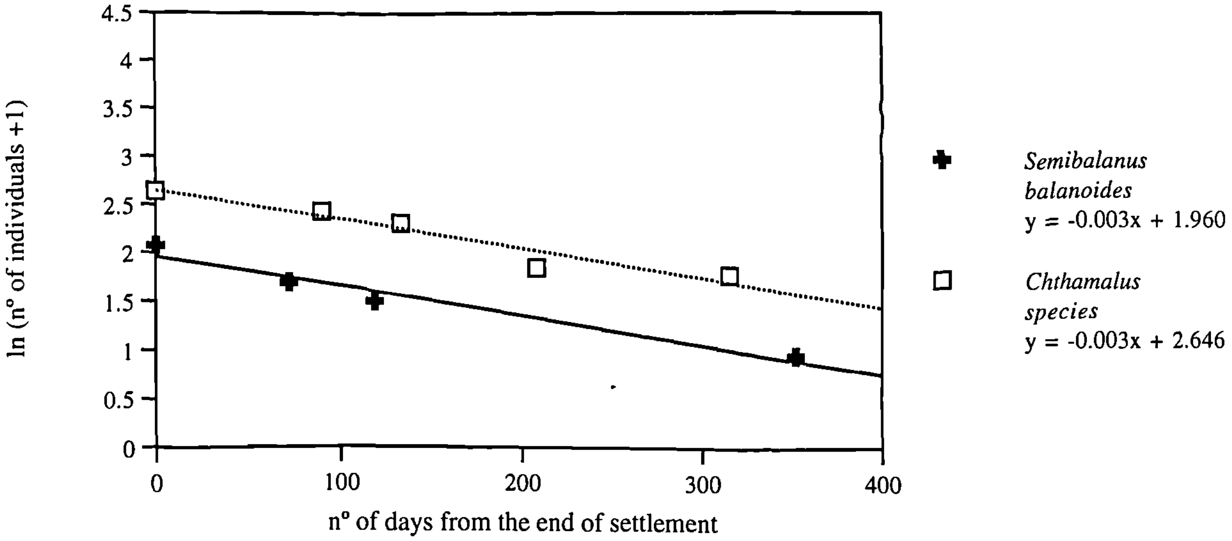
I 3.5.4.3 Mortality rates

Class 0+ In class 0+ individuals, the slopes of the regression lines in Fig I 3.5.7 show little difference between *S. balanoides*, *Chthamalus* species or *E. modestus* at any shore level. No significant differences were found between *S. balanoides* and *Chthamalus* species using matched pairs t tests. Class 0+ individuals of *E. modestus* on the low shore showed no significant difference in mortality rate from *S. balanoides* and *Chthamalus* (matched pairs t test P = 0.102 and 0.854 respectively, Table I 3.5.2). There appears to be little difference in mortality rates between shore levels in *Chthamalus* species of class 0+. It might be expected that individuals in this age class

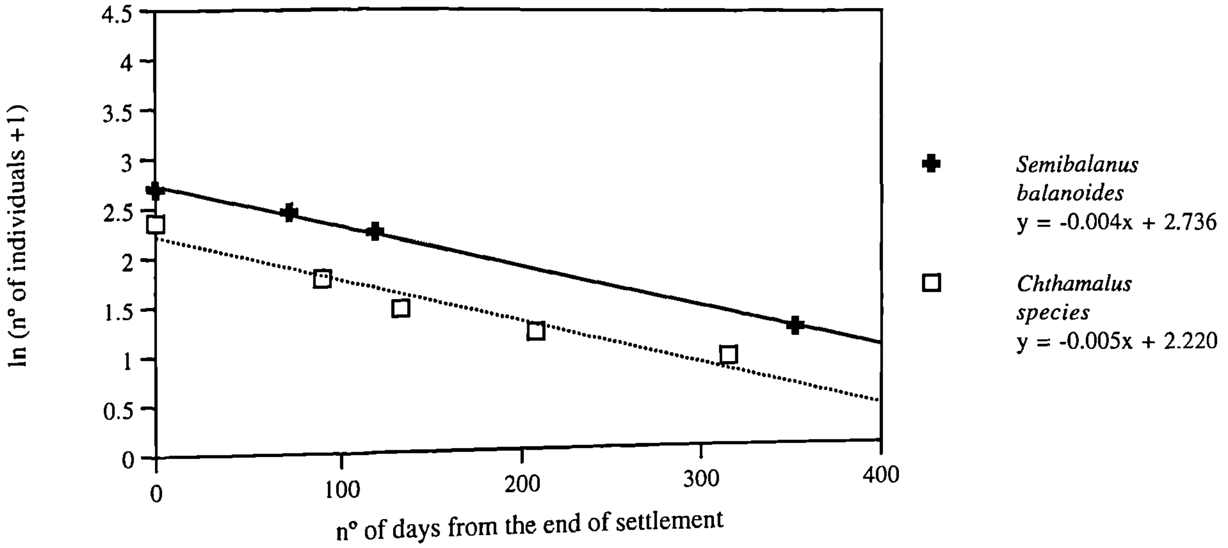
Table I 3.5.2: Matched pairs t tests to determine differences between species in their mortality rate. Tests performed on regression coefficients. Levels of significance: * $P \leq 0.05$, ** $P \leq 0.01$.

		df	t	*P*-value	
Semibalanus balanoides v. *Chthamalus* species					
0+	High	2	0.936	0.448	
	Mid	4	0.343	0.749	
	Low	4	0.771	0.484	
Semibalanus balanoides v. *Chthamalus montagui*					
1+	High	5	3.892	**0.012**	*
	Mid	4	3.437	**0.026**	*
	Low	4	0.716	0.513	
2+	High	5	5.071	**0.004**	**
	Mid	4	1.939	0.125	
	Low	4	0.533	0.622	
Semibalanus balanoides v. *Chthamalus stellatus*					
1+	High	4	1.803	0.146	
	Mid	3	1.724	0.183	
	Low	4	4.592	**0.010**	**
2+	High	5	4.804	**0.005**	**
	Mid	3	3.615	**0.036**	*
	Low	4	2.164	0.096	
Semibalanus balanoides v. *Elminius modestus*					
0+	Low	5	1.997	0.102	
1+	Low	5	1.801	0.132	
Chthamalus montagui v. *Chthamalus stellatus*					
1+	High	4	1.637	0.177	
	Mid	3	0.787	0.489	
	Low	4	2.881	**0.045**	*
2+	High	5	1.219	0.277	
	Mid	3	1.187	0.321	
	Low	4	2.343	0.079	
Elminius modestus v. *Chthamalus* species					
0+	Low	4	0.196	0.854	
Elminius modestus v. *Chthamalus montagui*					
1+	Low	4	0.292	0.784	
Elminius modestus v. *Chthamalus stellatus*					
1+	Low	4	2.249	0.088	

High shore



Mid shore



Low shore

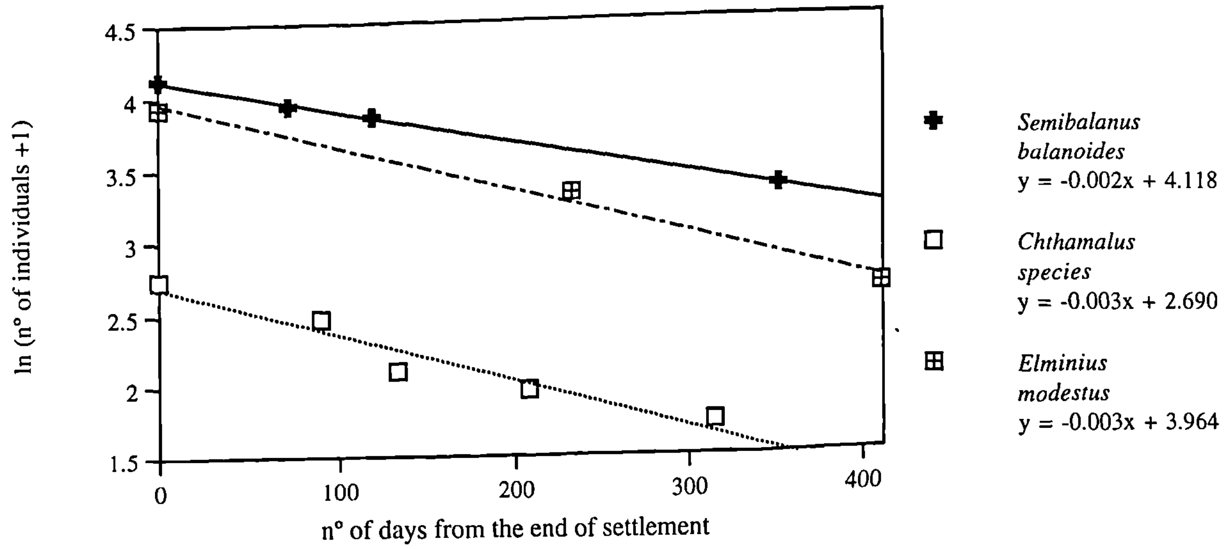


Fig I 3.5.7: Mortality rates of barnacles in the age class 0+ over a one year period. Regression lines have been plotted through the mean of six replicates.

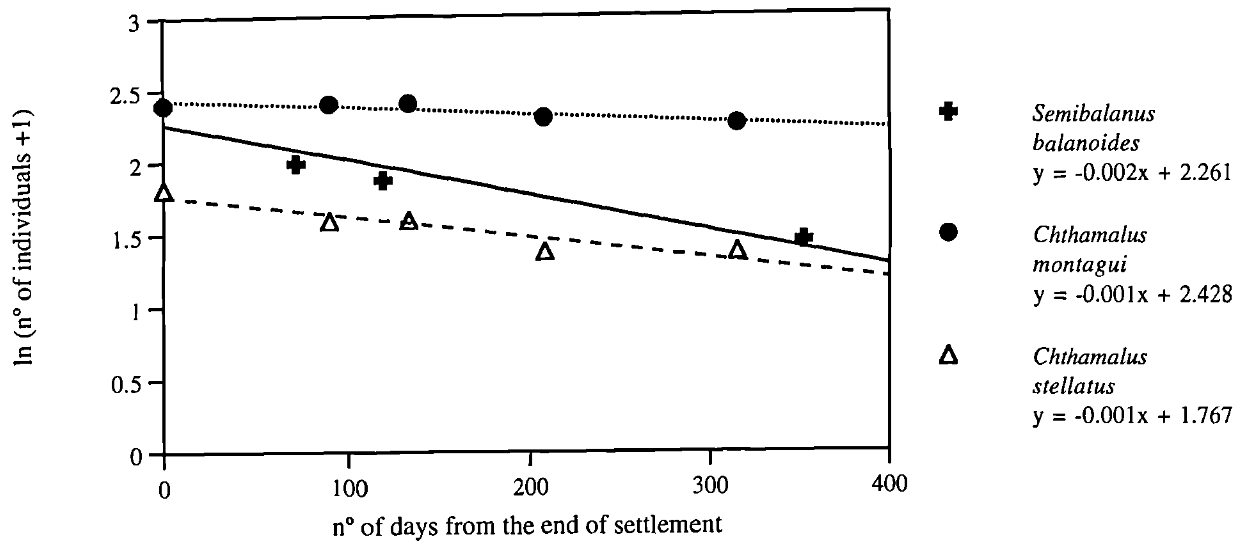
would be especially vulnerable to death from desiccation on the high shore. This does not appear to be the case in *Chthamalus*. For *S. balanoides* mortality is highest in the mid shore. One way ANOVA and Tukey Kramer tests indicate a significant difference between mid and low shore. (Table I 3.5.3).

Class 1+ and 2+ or above In 1+ and 2+ age classes differences between species and shore heights appear to occur. The mortality rate of *S. balanoides* was greater than in either of the two species of *Chthamalus* at all shore levels (Figs I 3.5.8-9). Comparisons of regression coefficients between *S. balanoides* and *C. montagui/stellatus* using matched pairs t tests reveal a number of significant results in these age classes (Table I 3.5.2) The mortality rate of *C. montagui* in both 1+ and 2+ classes on the high shore is very low. Matched pairs t tests show that the mortality rate of *C. montagui* at this shore height is significantly lower than *S. balanoides* in both age classes. Comparisons between *S. balanoides* and *C. stellatus* reveal significantly higher mortality rates in *S. balanoides* in three out of the six tests performed without any apparent pattern between shore height and age classes (Table I 3.5.2).

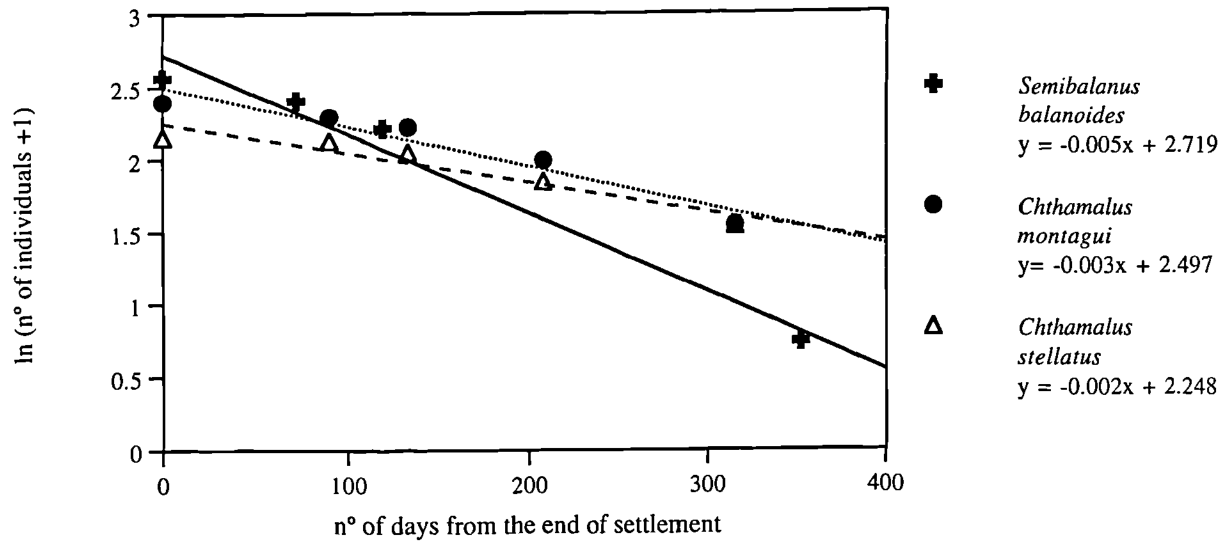
In summary in the year(s) of study, *S. balanoides* appears to show an overall higher rate of mortality than in either of the two *Chthamalus* species in individuals greater than one year old, probably indicating a much shorter life span in this species. For class 0+ individuals, mortality rates are very similar in all four species at any shore level.

Examination of the mortality rate in 1+ and 2+ age classes over the vertical height of the shore reveals some interesting patterns (Figs I 3.5.8-9). A comparison of the two *Chthamalus* species shows mortality was higher for *C. montagui* for both age classes on the low and mid shore. However this pattern is reversed at the high level site where *C. stellatus* shows higher levels of mortality. The drop in mortality rate in *C. montagui* on the high shore is very distinct in both 1+ and 2+ age classes. *C. stellatus* shows no particular pattern over the vertical shore gradient. One way ANOVA shows

High shore



Mid shore



Low shore

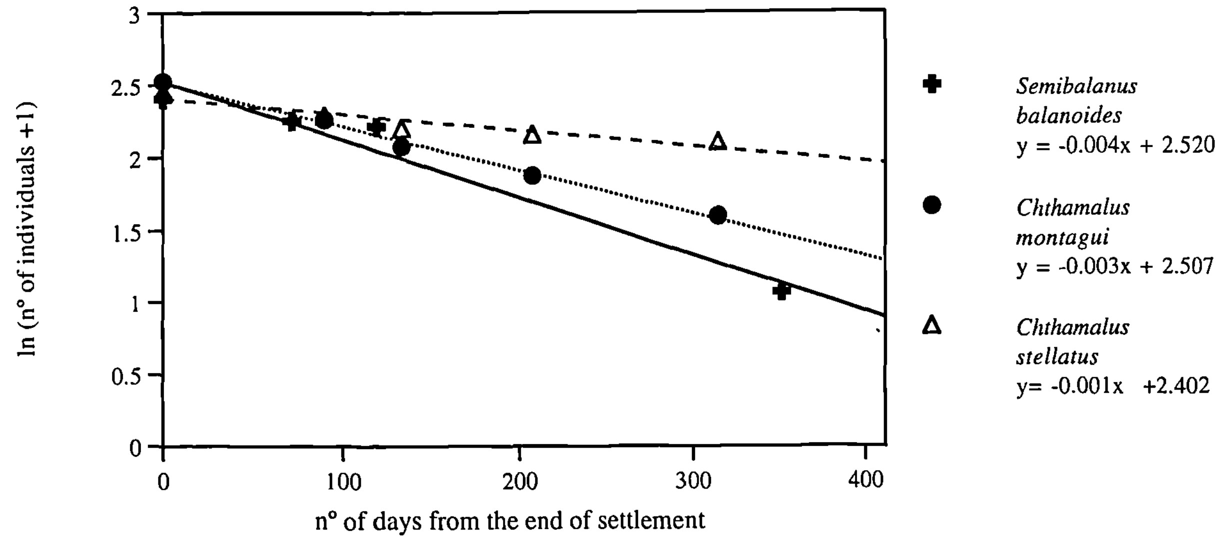
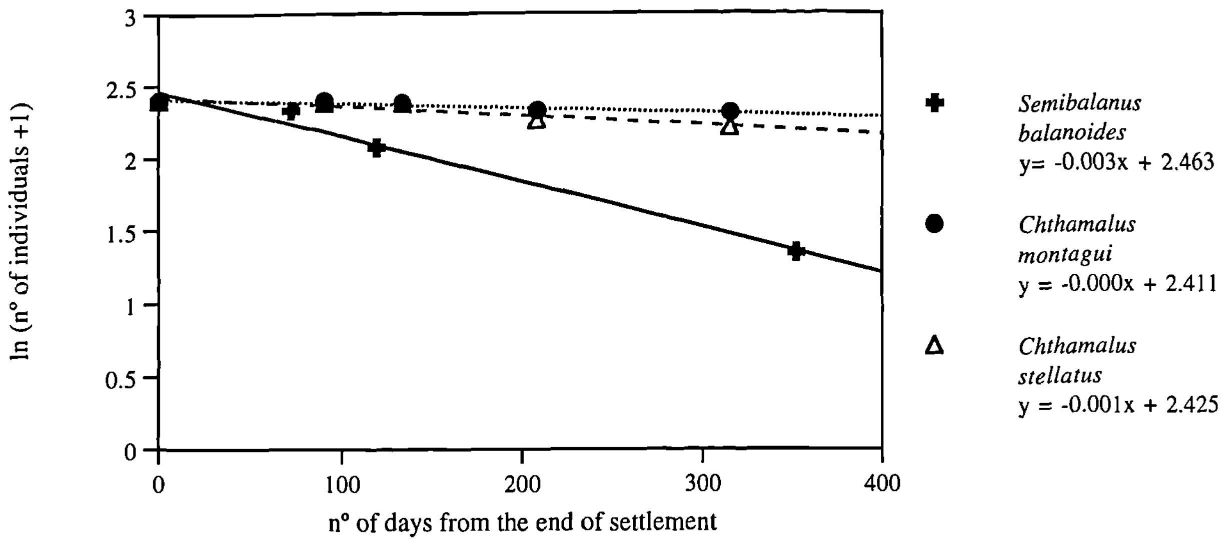
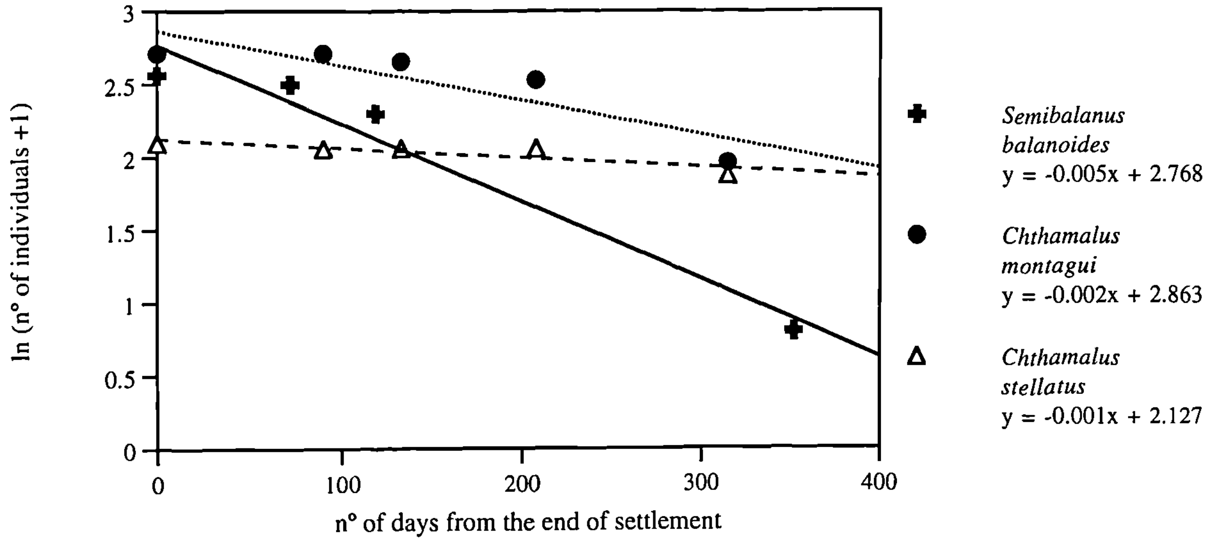


Fig I 3.5.8: Mortality rates of barnacles in the age class 1+ over a one year period. Regression lines have been plotted through the mean of six replicates.

High shore



Mid shore



Low shore

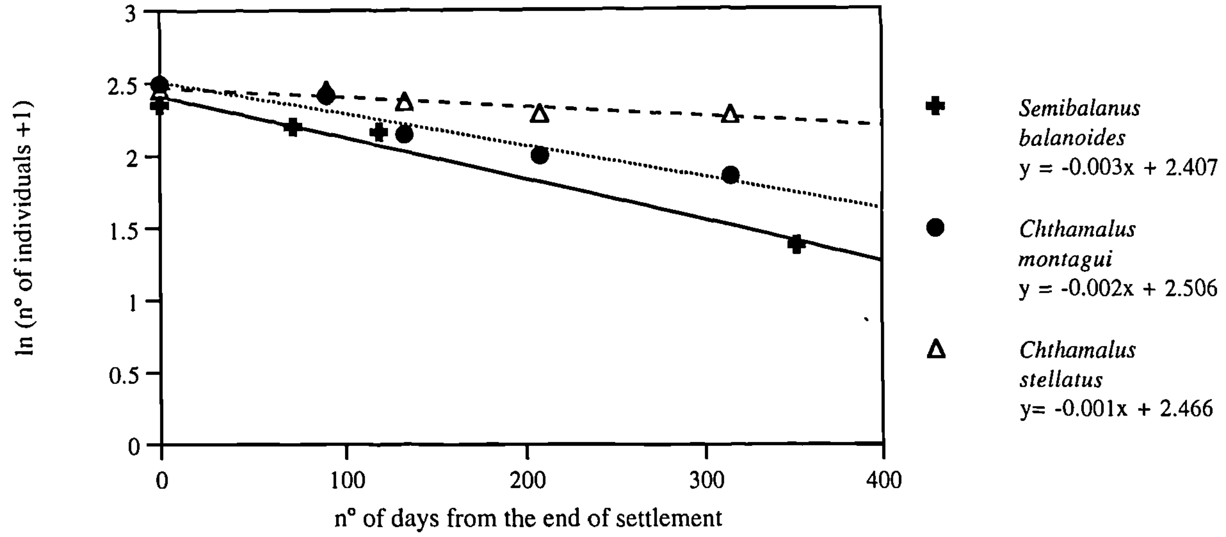


Fig I 3.5.9: Mortality rates of barnacles in the age class 2+ over a one year period. Regression lines have been plotted through the mean of six replicates.

no significant effect of shore height on mortality in *C. stellatus* but a significant effect in *C. montagui*. Tukey Kramer tests applied to *C. montagui* reveal a significantly lower mortality rate on the high shore in age class 1+. The increase in mortality with decreasing height on the shore in *C. montagui* and the relatively constant mortality in *C. stellatus* is demonstrated by results from matched pairs t tests. Mortality is significantly higher in *C. montagui* compared to *C. stellatus* on the low shore (class 1+, P = 0.045; class 2+, P = 0.079). In *S. balanoides* mortality is greatest in the mid shore for all age classes. ANOVA reveals a significant result in classes 0+ and 1+ (Table I 3.5.3).

In summary, *C. montagui* demonstrates its high shore preferences, by showing lower mortality rates at this tide level. *C. stellatus* in contrast, presents a reversed pattern with high mortality rates at high shore. In *S. balanoides*, mid shore proved to be the level at which most of the mortality occurs.

Class 0+, 1+ and 2+ or above A comparison of mortality rates between age classes for a given species at a given shore level reveals that surprisingly there is very little difference between barnacles belonging to the three groups, 0+, 1+ and 2+. Matched pairs t tests reveal only one significant result in sixteen comparisons (Table I 3.5.4). This result shows differences in the mortality rates of *S. balanoides* individuals belonging to class 0+ and 1+ at low shore. Because the likelihood of type 1 errors this result can almost be discounted.

Table I 3.5.3: One way ANOVA and Tukey Kramer tests to determine differences in mortality rates between shore heights. Levels of significance: * $P \leq 0.05$, ** $P \leq 0.01$

	Source	df	Mean Square	F-Value	P-Value		Tukey Kramer Test*
Semibalanus balanoides							
0+	Shore height	2	0.000006	3.701	0.059		Mid ≠ Low
	Residual	11	0.000002				
1+	Shore height	2	0.000012	3.729	**0.050**	*	Mid ≠ High
	Residual	14	0.000003				
2+	Shore height	2	0.000010	2.189	0.149		NA
	Residual	14	0.000005				
Chthamalus montagui							
1+	Shore height	2	0.000010	7.261	**0.007**	**	Low ≠ High Mid ≠ High
	Residual	14	0.000001				
2+	Shore height	2	0.000006	4.213	**0.039**	*	
	Residual	13	0.000001				
Chthamalus stellatus							
1+	Shore height	2	0.000001	0.620	0.556		NA
	Residual	11	0.000002				
2+	Shore height	2	1.2×10^{-9}	0.001	0.999		NA
	Residual	12	0.000001				

* NA: Not applicable i.e. ANOVA shows no significant difference

≠: indicates significant difference at 5% level between stated shore heights.

Experiment wise error rate = 5%

Table I 3.5.4: Matched pairs t tests to determine differences between age classes in their mortality rate. Tests performed on regression coefficients. Levels of significance: * $P \leq 0.05$, ** $P \leq 0.01$.

		df	t	*P*-value
0+ v. 1+				
	Semibalanus balanoides			
	High	2	0.540	0.643
	Mid	4	0.747	0.496
	Low	5	3.717	**0.014** *
	Elminius modestus			
	Low	5	1.182	0.290
1+ v. 2+				
	Semibalanus balanoides			
	High	5	0.754	0.485
	Mid	4	0.051	0.962
	Low	5	1.156	0.300
	Chthamalus montagui			
	High	5	0.744	0.490
	Mid	4	0.996	0.376
	Low	4	1.114	0.328
	Chthamalus stellatus			
	High	4	1.896	0.131
	Mid	3	1.502	0.230
	Low	4	1.759	0.153
0+ v. 2+				
	Semibalanus balanoides			
	High	2	0.184	0.871
	Mid	4	0.849	0.444
	Low	5	0.585	0.584

I 4. DISCUSSION

I 4.1 PROBLEMS with this WORK

A general criticism that can be put forward for this kind of survey is that the monitoring was limited to a single shore and therefore any conclusion drawn from it, cannot be used as a generalisation of what is occurring on a larger scale (Underwood and Denley, 1984; Foster, 1990; Levin, 1992). Field surveys or experiments at a particular site, might not take into account variation in community structuring factors and stochastic events which could differ from one area to the other. This has been shown to be particularly the case in barnacles whose settlement density varies from place to place (e.g. Hawkins and Hartnoll, 1982; Underwood and Denley, 1984; Caffey, 1985). I acknowledge this limitation and I will try to justify the decision of carrying out the study in the chosen way.

Environmental conditions vary in time as well as in space. This particular study was designed to focus more on temporal change rather than spatial variation. A very detailed analysis of changes in the barnacle community over a one year period, was the main aim of the survey. The question can be asked whether it was better to replicate between sites and limit the depth of the investigation, or only examine in detail the barnacle community of Cellar Beach. The answer was that the latter option was the most appropriate. Obviously the benefits of replication between sites were recognised, but time and resources did not allow this type of investigation to be carried out as well (see section on broadening of the spatial scale at the end of the discussion). Care was taken at least, to widen the small scale study which concentrated on barnacles from a particular transect of the shore, to a medium scale investigation representative of the whole shore. This was achieved by comparing the results from the photographs of fixed plots with those obtained from the analysis of rock samples collected from all over Cellar Beach. After allowing for spatial variation of the rock samples, very good agreement was generally found between the results common to the small and mid scale studies. This implied that the investigated transect could be considered representative of the whole shore.

Although limited on a spatial scale, on a temporal basis this study forms part of a very long term survey. Results such as changes in species abundance, can be viewed in a much wider context, thanks to Southward's forty-five year survey (1991) carried out on barnacles from the same transect. Southward's broader scale investigation (Southward *et al.*, 1995) also allows the results to be put into a broader south-west geographical scale.

A second matter of concern comes from the effect of human disturbance on the areas of study. It could be suggested that trampling about on the rocks, even if not actually on the monitored quadrats, by the investigator and other visitors to the shore (e.g. fishermen) could cause damage to the intertidal organisms. Bally and Griffiths (1989) investigated this problem on South African rocky shores and noticed some immediate effects on the community, but no long-term implications. Due to its location, Cellar Beach is not a very frequented shore. Very few visitors were met on the various sampling occasions. It must be said, however, that because sampling was carried out on low tides, while rod and line fishing is mainly done during high tides, probably most of the visiting fishermen were not encountered. On the other hand, as fishing is done at high tides, most of the fishermen will actually stand well above the highest samples and will be unlikely to cause damage to the surveyed quadrats. In conclusion, human disturbance is believed to be very limited in the area investigated. The agreement observed between results from fixed quadrats, which are more likely to reflect changes due to persistent disturbance by the investigator, and those from widespread rock samples, which are in total less likely to be affected by trampling, further support this statement.

Another limitation of this study is related to the incapacity of separating the two *Chthamalus* species at the early stages of life. As already mentioned their morphological features are very similar during the first few months after settlement. Burrows (1988) pointed out that spat of *C. stellatus* can be discerned from the those of *C. montagui* on the basis of the colour of the shell, the former being darker than the latter. Nevertheless separation of the newly settled spat of the two species was found to be impossible on photographs and quite difficult on rock samples. For this reason, detailed studies of the early stages of life of the two separate species could not be carried out.

A final comment must be made on the decision to use opercular length along the rostro-carinal axis to estimate growth rate. I appreciate that length of the base of the shell would be a more meaningful measurement for investigating how different growth rates favour animals in competition for space. In crowded situations, however (as in the case of the individuals I had available for measuring growth), shell growth becomes very irregular due to interactions among barnacles (Stubbings, 1975) and cannot therefore be considered a reliable measure. Opercular length instead, appears to be much less susceptible to distorted growth and also, because the prosoma and thorax are attached to the opercular plates, there is likely to be a high correlation between size of the operculum and biomass of the animal. Burrows (1988) showed high correlation between opercular length and animal biomass in the two *Chthamalus* species for different sites and shore levels. For these reasons this growth parameter was chosen throughout most of the study, with the exception of measurements of spat growth on rock samples where shell length was used instead. At the early stages of life, the effect of crowding on shell growth is normally not detectable.

The relationship between shell and opercular length, was not determined in this work as this analysis should be carried out on free standing individuals which were rarely found on the material collected from Cellar Beach. However, for certain species data on this relationship are already available in literature. For instance, Burrows (1988) suggested that the mean total shell length of a population of either *C.*

montagui or *C. stellatus*, could be roughly obtained by multiplying the mean opercular length by 1.90.

Despite the above considerations, this study allows discussion of certain topics: whether space is limited, what determines the balance of the species and what were the causes of decline in the *S. balanoides* population at Cellar Beach. These are discussed in the rest of this section, before making suggestions for future surveys and further work. A summary of the results, some of which I will refer to in the following pages, is also provided in Tables I 4.1-4.6.

I 4.2 IS SPACE LIMITED?

On a broad geographical scale, it can be said that embayed situations favour barnacle larval retention (Gaines and Bertness, 1992) which leads to saturation of all the limited space available (e.g. Millport and surrounding areas, where Connells' (e.g. 1959; 1961a; 1961b; 1983; 1985) studies were carried out). On open coasts, larval transport is more effective due to strong currents and intense wind action and therefore larvae tend to be more spread out. Turnover and related production of vacant space suitable for settlement is also faster than on sheltered shores as a consequence of the more intense wave action. For these two reasons and because of the large surfaces available, it can be inferred that on open coasts space is always available for settlement.

Cellar Beach, the study site, is at the mouth of a small Ria. Larval supply to this location may be more restricted than at open sites nearby like Church Reef, Renney Rocks and Queener Point (investigated by Burrows, 1988). Nevertheless, the size of this Ria is small enough to suggest that larvae are not retained for long. Hence, Cellar Beach fits better in the open coast category than in the embayed one. In agreement with what has been said about open coasts in the previous paragraph, Cellar Beach showed a certain quantity of space available for settlement all the way

Table I 4.1: Changes in percentage cover of barnacles, bare rock and individual species at three shore levels over the three year survey.

	High shore		**Mid shore**		**Low shore**	
	Sept '91	**Jan '95**	**Sept '91**	**Jan '95**	**Sept '91**	**Jan '95**
tot. barnacles	89%	61%	84%	44%	60-85%	
bare rock	8%	37%	12%	46%	10-36%	
C. montagui	32%	38%	4%	13%	4-9%	
C. stellatus	13%		1%	10%	10-15%	
S. balanoides	44%	7%	77%	16%	55%	18%
E. modestus	0%		1%	8%	5%	24%

Table I 4.2: Breeding - time of larval release in the plankton in 1992

beginning	**High shore**	**Mid shore**	**Low shore**
C. montagui	mid July	mid July	mid July
C. stellatus	no data	mid July	mid July
S. balanoides	end January	January	January
E. modestus	no data	no data	no data
peak			
C. montagui	mid August	July	end July
C. stellatus	no data	mid July/August	end Aug/beg. Sept
S. balanoides	beg. April	mid February	Feb/beg. March
E. modestus	no data	no data	no data
end			
C. montagui	end August	mid August	mid September
C. stellatus	no data	mid September	mid September
S. balanoides	April	mid May	end April
E. modestus	no data	no data	no data

Table I 4.3: Settlement season 1991-1992

beginning	**High shore**	**Mid shore**	**Low shore**
Chthamalus	no data	no data	no data
S. balanoides	beg. April 1992	mid March 1992	mid March 1992
E. modestus	absent	no data	beg. July 1992
peak			
Chthamalus	beg. September 1991	end September 1991	end September 1991
S. balanoides	mid April 1992	beg. May 1992	beg. May 1992
E. modestus	absent	no data	mid August 1992
end			
Chthamalus	beg. November 1991	mid October 1991	end October 1991
S. balanoides	mid May 1992	beg. June 1992	mid May 1992
E. modestus	absent	no data	mid September 1992

Table I 4.4: The increase in opercular length in mm (X) over one year for each species, age class, shore level combination.

class 0+	**High shore**	**Mid shore**	**Low shore**
C. montagui	$0.5 < X < 1.3$	$0.5 < X < 1.4$	$0.4 < X < 1.3$
C. stellatus	$0.6 < X < 1.2$	$0.5 < X < 1.3$	$0.6 < X < 1.5$
S. balanoides	$0.5 < X < 1.7$	$0.6 < X < 2.2$	$0.4 < X < 2.2$
E. modestus	absent	$0.8 < X < 2.2$	$1.1 < X < 2.5$
class 1+			
C. montagui	$1.3 < X < 1.7$	$1.4 < X < 1.7$	$1.3 < X < 1.6$
C. stellatus	$1.2 < X < 1.8$	$1.3 < X < 1.6$	$1.5 < X < 1.8$
S. balanoides	$1.7 < X < 2.1$	$2.2 < X < 2.5$	$2.2 < X < ?$
E. modestus	absent	$2.2 < X < ?$	$2.5 < X < 3.2$
class 2+			
C. montagui	$X > 1.7$	$X > 1.7$	$X > 1.6$
C. stellatus	$X > 1.8$	$X > 1.6$	$X > 1.8$
S. balanoides	$X > 2.1$	$X > 2.5$	no data

Table I 4.5: Instantaneous mortality rates

The values are the slopes of the regression lines which describe the mortality rate

The smaller the value, the steeper the slope and the greater the mortality

(note that these are negative values, therefore -0.00412 is smaller than -0.00295)

class 0+	**High shore**	**Mid shore**	**Low shore**
Chthamalus	-0.00295	-0.00412	-0.00327
S. balanoides	-0.00297	-0.00429	-0.00224
E. modestus	no data	no data	-0.00315
class 1+			
C. montagui	-0.00053	-0.00273	-0.00296
C. stellatus	-0.00145	-0.00203	-0.00108
S. balanoides	-0.00246	-0.00544	-0.0396
E. modestus	no data	no data	-0.0024
class 2+			
C. montagui	-0.00031	-0.00206	-0.0022
C. stellatus	-0.00064	-0.00066	-0.00067
S. balanoides	-0.00311	-0.00536	-0.00285
E. modestus	no data	no data	no data

Table I 4.6: Longevity

	High shore	**Mid shore**	**Low shore**
C. montagui	at least five years	at least five years	at least five years
C. stellatus	at least five years	at least five years	at least five years
S. balanoides	at least five years	approx. five years	at least five years
E. modestus	absent	no data	at least three years

through the study (see Table I 4.1). The amount of bare rock was relatively small (circa 10%) at the beginning, but became increasingly larger (40%) towards the end of the survey. The distribution of the bare rock along the monitored transect was patchy, with dense aggregations of barnacles separated by relatively large areas of bare rock.

Differences in the amount of space available can also be detected between shore levels. In general higher and lower levels on the shore have denser barnacle cover than mid shore. In the Clyde Sea, patches of bare rock are found just below MHWN; high mortality of *S. balanoides* and survival of a few *C. montagui* at this level are considered to be the causes for the existence of such patches (A.J. Southward pers. comm.). Also in the case of Cellar Beach, the mid shore level consistently showed the largest vacant surface available for settlement among the three levels investigated (Table I 4.1). The observed vertical pattern of distribution of space could be due to various causes and probably different factors affect different levels on the shore.

To simplify the matter, I will try to concentrate my attention on deterministic processes, although I acknowledge the importance of stochastic disturbance events in generating large quantities of bare surface from time to time.

When trying to understand what determines space limitation in a barnacle community, several biological factors need to be taken in consideration. Different periods of settlement of the various species will generate a seasonal pattern in the availability of bare rock. Recruitment density and growth rate of the settling species are the factors which determine the immediate changes in the quantity of vacant surface. On a more long-term basis, the rate of turn-over of the predominant species, is also very important for determining the barnacle cover at a given shore level.

At high shore, turn-over is relatively slow. At Cellar Beach, *C. montagui* together with *S. balanoides* are the main colonisers of this shore height (Table I 4.1). In this study it has been shown that *C. montagui* is a long-lived species (Table I 4.6) which survives best higher on the shore (Table I 4.5). *S. balanoides* seems to survive better at high shore too, but the survivorship of the older age classes and the longevity of this species are much lower than in *C. montagui* (Table I 4.5-4.6). Hence, *C. montagui* can be considered the most persistent coloniser of the high shore and mainly responsible for the presence of a dense and constant cover of barnacles at this tide level. Because of the dense barnacle cover, seasonal changes are not normally detected.

At mid shore, *S. balanoides* is definitely the most common species (Table I 4.1). Changes in availability of bare rock can therefore be attributed directly to *Semibalanus*. Mortality rates for this species at this shore level are the highest of the whole shore regardless of age class (Table I 4.5). It follows that turnover is extremely quick and patches of space become available continuously, except perhaps just after the end of *Semibalanus* settlement season at the end of spring/beginning of summer time. The fast growth rate of class 0+ individuals (Table I 4.4), allows drastic changes in the quantity of bare rock available, to occur within a month or two from settlement.

On the low shore the barnacle cover consists of mainly three species: *S. balanoides*, *C. stellatus* and *E. modestus*. At this shore level *S. balanoides* seems to survive relatively well at the early stages of life (Table I 4.5), but also here its life span is quite short (Moore, 1934). *E. modestus* is the least long-lived species of the three, but its great capacity to settle all year round and its very fast growth rate (Crisp and Patel, 1961), make this species an important space occupier low on the shore. *Elminius* could be defined as an 'opportunistic' species which is always ready to settle as soon as some vacant space becomes available. I do not think that at Cellar Beach, *Elminius* can enter in direct competition for space with *Semibalanus*, but rather that the australasian species is capable of filling in much of the space vacated by *S. balanoides*. Between 10 and 15% of the barnacle cover at low shore, is covered by *C. stellatus* (Table I 4.1). This is a very large, slow growing and extremely long lived species. At low shore *C. stellatus* seems to form the most persistent part of the barnacle cover while the other two species alternatively fill in the space left available.

This situation generates a quite dense and consistent cover in spite of the high turnover.

Disturbance caused by predation can also influence the amount of free space available on the shore by increasing the mortality rate of certain species. Before 1985 dog-whelks (*Nucella lapillus*) were common at Cellar Beach (Southward, 1991). Their predation was particularly intense low on the shore and was found to be selective towards certain specimens, particularly larger species of barnacles such as *C. stellatus* (Burrows, 1988). Since 1985 though, dog-whelks disappeared from the study site (in three years of survey I saw none) and mortality due to predation was markedly reduced. It could be suggested that other predators like fish, worms and small crabs, or limpets bulldozing in barnacle cyprids and spat, could also play an important role in structuring the barnacle populations. However, the disturbance caused by these organisms is extremely difficult to quantify and no real estimate of their effect can therefore be made.

I 4.3 WHAT DETERMINES the BALANCE of the SPECIES?

Models have been used to describe the equilibrium between competition, stress and disturbance in the plant kingdom (Grime, 1979). In this context, plants were divided into three major classes: 'competitors', which exploit conditions of low stress and disturbance, 'stress tolerators' (high stress-low disturbance) and 'ruderals' (low stress-high disturbance). These three classes represent the extremes of evolutionary specialisation, but plants adapted to habitats experiencing intermediate intensities of stress and disturbance are probably the most common.

To investigate the balance of the species in the barnacle community of Cellar Beach, a similar classification could be introduced. At Cellar Beach four species of barnacle exist in close contact to each other. Each one of these could be allocated to one of the three classes mentioned above.

S. balanoides could be placed in the competitors class. Its ability to resist desiccation (a type of environmental stress) is quite limited as shown by the high post-settlement mortality of the late spring settlers and of those which settle higher on the shore (Table I 4.5). The reproductive strategy of *Semibalanus*, with production of a single brood per year and settlement over a very short period (Table I 4.3), also prejudices its ability to cope with disturbance. If with disturbance we intend a negative effect (e.g. predation, wave action, competition etc.) that increases the chances of destruction of a certain population, it is easy to understand that the effect of disturbance on *Semibalanus* populations, could potentially have catastrophic consequences. If for instance a population of *S. balanoides* gets damaged just after the end of the settlement season, no recovery will be possible until the next settlement the following year. Summarising, *Semibalanus* is not able to deal very well with life at high shore due to desiccation problems and also its life cycle does not allow it to recover quickly under high disturbance conditions such as those often observed at low shore. These limitations probably make mid shore the safest place for the settlement of this species.

C. montagui could be considered an example of a 'stress tolerator'. Its morphology enables this species to resist high desiccation stress. Thanks to this capacity, *C. montagui* is the only barnacle species able to survive well at high shore (Connell, 1961b; 1971a; Foster, 1971b). Burrows (1988) developed this observation even further by suggesting that *C. montagui* particularly favours settlement at high shore because only there it finds suitable conditions for hardening its shell plates. The higher rates of mortality observed in my study at mid and low shore (Table I 4.5), support the argument put forward by Burrows. The limited settlement season (Table I 4.3) combined with the slow growth rate (Table I 4.4) of this species, does not make *C. montagui* a good coloniser of very disturbed areas. Hence, the high desiccation tolerance is a very important feature for the success of this species since it allows *C. montagui* to find a niche at high shore where conditions of disturbance are probably lower.

Trying to fit *C. stellatus* in one of the three above categories is a difficult task. At Cellar Beach this species is not very abundant, probably because the relatively low grade of wave exposure of this shore. From previous studies (Burrows, 1988; O'Riordan, 1992) *C. stellatus* has been shown to be susceptible to desiccation. As in the case of *C. montagui*, the limited period of settlement and the slow growth speed do not favour this species in disturbed conditions. From these observations, this species does not appear to be particularly able to cope with stress nor with disturbance, but its real advantage on the other species is that once settled it is much more persistent (my results showed very low mortality rates in older individuals at all shore levels, Table I 4.5). For these reasons *C. stellatus* could fit in the competitor class as it seems to do well in conditions of low stress and disturbance.

E. modestus is the typical example of an organism that succeeds in conditions of low stress and high disturbance, hence its place is in the ruderals class. This species is very rarely found at high shore because of its low capacity to stand desiccation stress. On the other hand, the continuous production of broods through the year and its extremely fast growth rate, make this species a very powerful coloniser in conditions of high disturbance, as for instance at low shore.

At Cellar Beach an equilibrium seems to be reached as each species favours a certain niche, although considerable overlap occurs. The biological characteristics of each one of these lead to the selection of a favoured area on the shore. In this way inter-specific competition for space within barnacle aggregations is probably reduced. However competition occurs at all levels of the shore where dense barnacle aggregations are present, and certainly plays an important role in influencing the dynamics of the populations and in determining the species distribution (see section on studies of interactions for future work on this topic). Having said that it is worth pointing out that competition among barnacles does not seem to take place in a direct way. While analysing photographs and rock samples in fact, I hardly ever observed competing barnacles damaging each other. Competition among barnacles probably works in a more subtle way, for example by limiting access to food resources. Furthermore, fluctuations in recruitment probably help to maintain coexistence (see Figs I 4.1a-4.3a).

I 4.4 CAUSES of DECLINE in the *SEMIBALANUS BALANOIDES* POPULATION

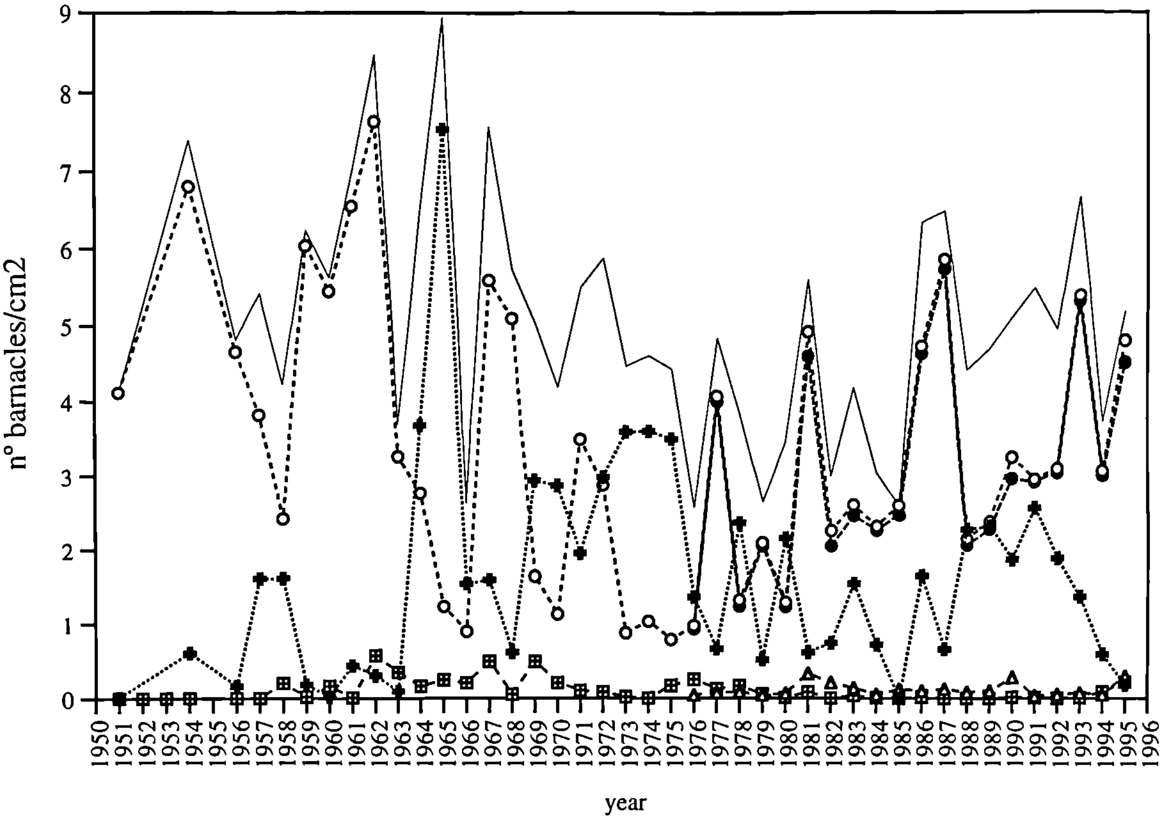
A general decrease in barnacle cover and density, mainly caused by a great reduction at all shore levels of the *S. balanoides* population, constitutes the most important finding of this section (Table I 4.1). The beginning of the decrease of this species, has been observed by Southward in his long term survey (Figs I 4.1a-4.3a). By comparing the long and the short term studies (Figs I 4.1-4.3), it becomes evident that the trend of decrease of *S. balanoides* coincided with the start of the intensive survey in 1991.

The reason for the decline is probably quite complex. There are a number of factors which should be taken into consideration while trying to find an explanation for this phenomenon.

Inter- and intraspecific competition for space is one of these. The decline of the *Semibalanus* population generated large quantities of bare rock at all tide levels. Only a relatively small amount of this surface actually became colonised by the other species during the following years. This consideration leads to the conclusion that competition for space was probably not the cause of the observed decline.

Predation is also to be excluded. Since 1984 the main *Semibalanus* predator, the dog-whelk *Nucella lapillus*, has completely disappeared from the transect and from everywhere else nearby (Bryan *et al.*, 1986; Gibbs and Bryan, 1986; Southward, 1991). No other predators can be thought to be responsible for such a reduction in the *Semibalanus* population. While analysing specimens for breeding, only a few *S. balanoides* were shown to be affected by the parasite *Hemioniscus balani*, hence also this possibility can be counted out.

a) Long term changes



b) short term changes

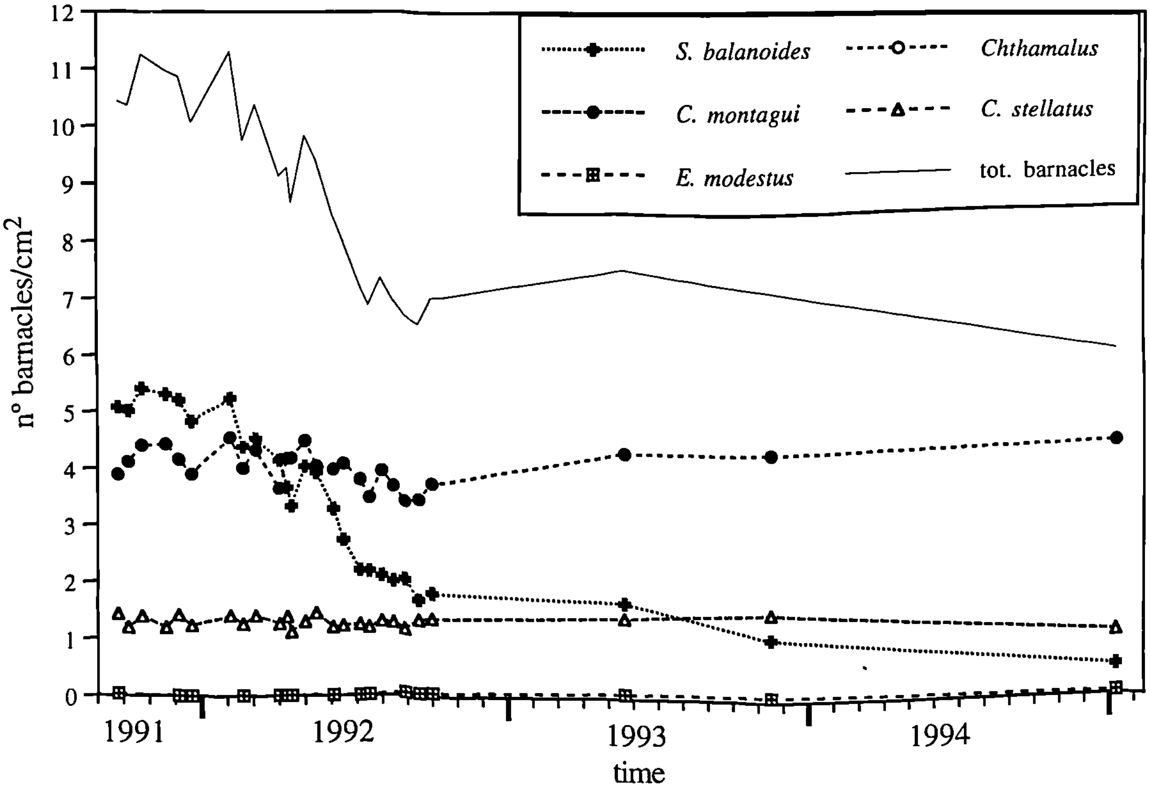
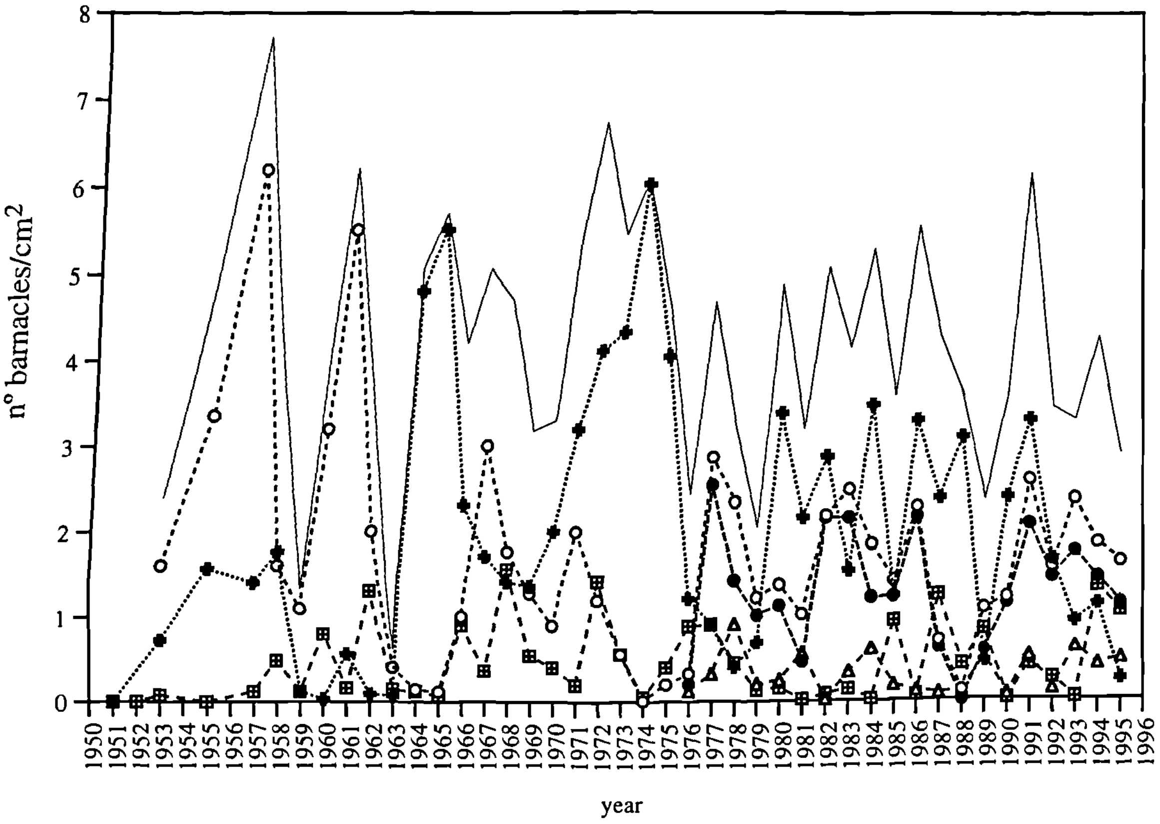


Fig I 4.1: High shore. Changes in barnacle species composition on the same transect at Cellar Beach.
a) Changes in species composition over a period of forty years (1951-1995).
Data collected by A.J. Southward.
b) Changes in species composition over a period of three years (1991-1995).
Data obtained from the analysis of photographs.

a) Long term changes



b) short term changes

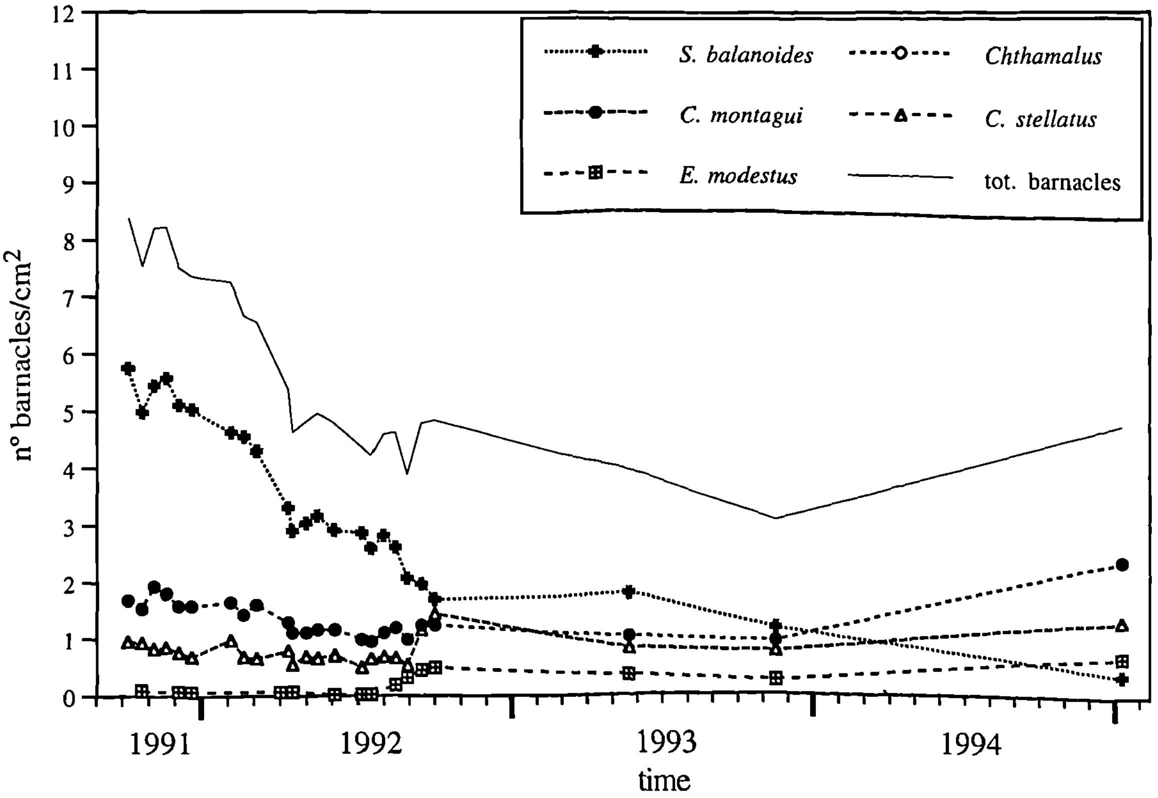
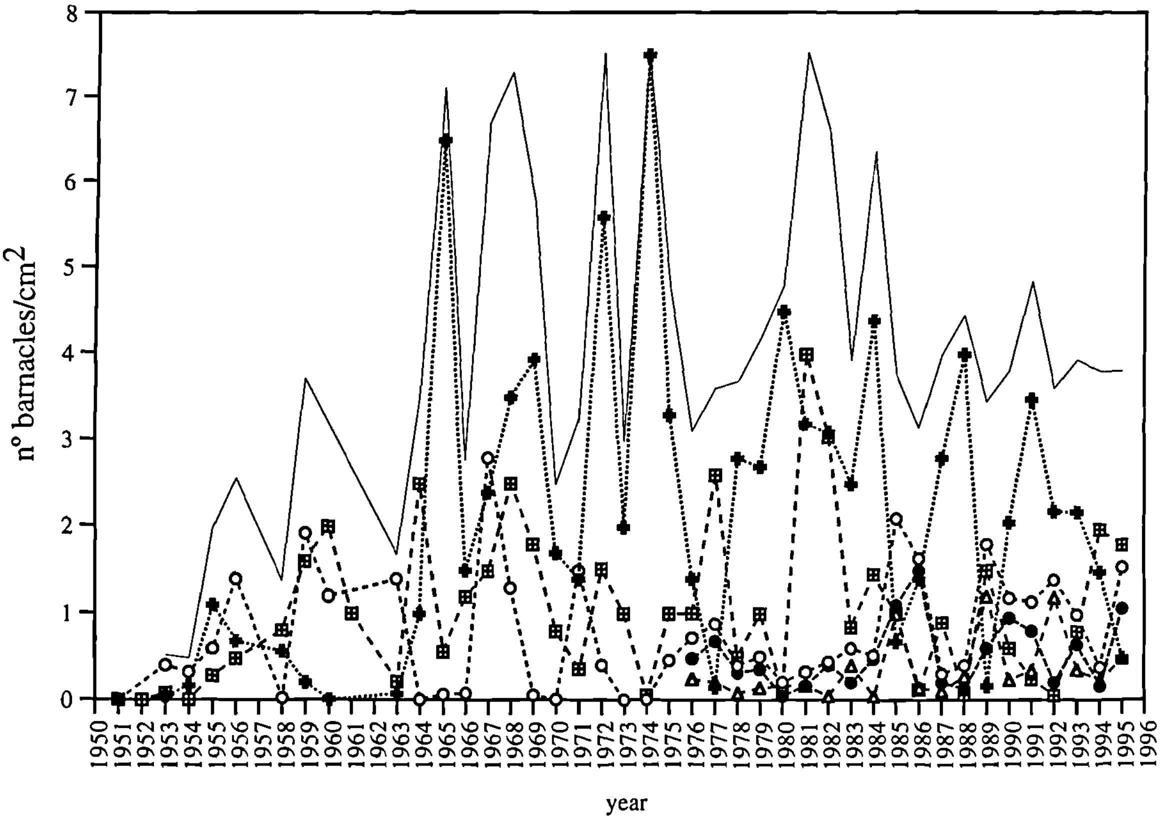


Fig I 4.2: Mid shore. Changes in barnacle species composition on the same transect at Cellar Beach.
a) Changes in species composition over a period of forty years (1951-1995).
Data collected by A.J. Southward.
b) Changes in species composition over a period of three years (1991-1995).
Data obtained from the analysis of photographs.

a) Long term changes



b) short term changes

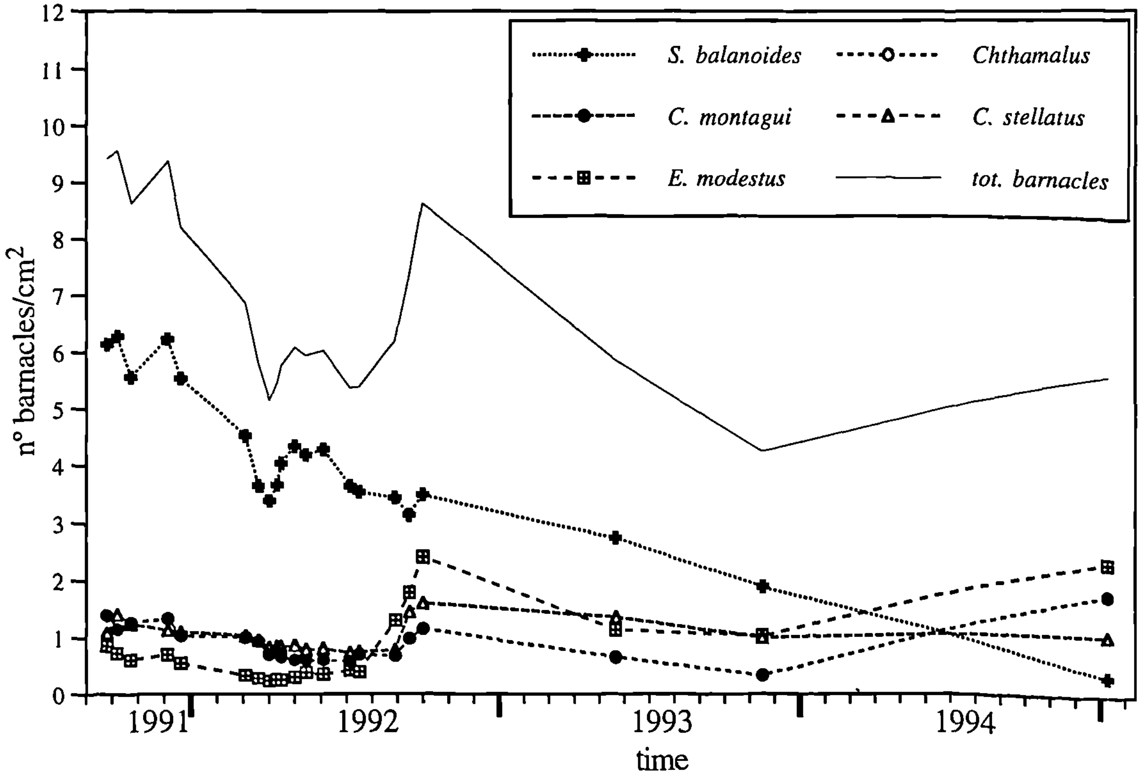


Fig I 4.3: Low shore. Changes in barnacle species composition on the same transect at Cellar Beach.
a) Changes in species composition over a period of forty years (1951-1995).
Data collected by A.J. Southward.
b) Changes in species composition over a period of three years (1991-1995).
Data obtained from the analysis of photographs.

Wave action could be another possible factor. At one particular quadrat at low shore for instance, most of the *S. balanoides* population was removed during a storm in November 1991 (see Plate I 2.). Stochastic events, like the one just mentioned, are very unlikely to generate trends like the one observed in *Semibalanus.* Furthermore, the decrease of this species was observed at all shore levels while normally wave action affects different areas of the shore in different ways. Also, if wave action was the main cause, it should have affected not only *Semibalanus* but also the other neighbouring species (although maybe not with the same intensity). Instead, no consistent decrease was observed in the other three species.

Figs I 4.1a-4.3a show forty-five years of fluctuations in the abundance of *Semibalanus* along the surveyed transect, the present situation seems to correspond to the descending slope of the most recent cycle. Natural fluctuations in the recruitment of the *Semibalanus* population, could possibly explain the observed pattern. A large quantity of literature (e.g. Hawkins and Hartnoll, 1982; Caffey, 1985; Gaines *et al.*, 1985; Kendall *et al.*, 1985; Wethey, 1985b; Raimondi, 1990 and see section on settlement and recruitment in introduction for other references), supports the idea that recruitment varies spatially and temporally. Recruitment has been observed to vary in relation to larval supply (e.g. Gaines *et al.*, 1985; Kendall *et al.*, 1985; Sutherland, 1987; Bertness *et al.*, 1992), development (e.g. Wethey, 1984), transport (e.g. Hawkins and Hartnoll, 1982; Shanks, 1986) and behaviour (e.g. Knight-Jones and Stevenson, 1950; Knight-Jones, 1953; Barnett and Crisp, 1979; Wethey, 1984; Hui and Moyse, 1987; Minchinton and Scheibling, 1993a) and also post-settlement mortality (e.g. Foster, 1971a; 1971b; Grosberg, 1982; Burrows, 1988). In my search of the literature, however, I have never come across a paper in which an author described the above factors as capable of directly inducing regular fluctuations in the abundance of *S. balanoides* over time. Hence, these factors are probably under the influence of a more general agent which imparts the cyclic nature.

It has been suggested (Southward and Crisp, 1952; 1954; 1956; Southward, 1967; Southward *et al.*, 1975; Southward, 1991; Southward *et al.*, 1995) that fluctuations in barnacle species abundance reflect changes in climate (see section on barnacle as bioindicators of long-term climate changes in introduction). Up to the seventies, fluctuations in global temperatures and consequently in barnacle data, were phased with curves of annual mean sunspot numbers. After this time, radical changes in the weather pattern (global warming) led to destabilisation of the regular temperature cycles and promoted an increased short-term climate variability. As a direct consequence of this, the barnacle data also started to diverge from the solar cycle and to show less ample and regular fluctuations and more fit with the mean inshore sea temperatures (Connell, 1961a; Southward, 1980). The intensive survey falls into a period of such variability. As mentioned above, the decline of the *S. balanoides* population, appears to correspond to the descending slope of the most recent cycle. Climate almost certainly played an important role in the decrease of this population. As mentioned in the introduction, increases in air and seawater temperatures produce a negative effect on the recruitment of *S. balanoides*. Warm weather for instance could stimulate the larval release at a time of the year when the phytoplankton bloom is not taking place yet, so endangering the survivorship of the planktonic larvae (Southward and Crisp, 1956). In addition, warm temperatures can affect post-settlement mortality by increasing the risk of mortality caused by desiccation. Since 1988, in the South West district the mean differences from the average air temperature were consistently positive, implying that the observed mean annual temperatures were always higher than those expected from the 1951-80 averages (The Meteorological Office, 1988-1993; Royal Meteorological Society, 1993-1994). Before 1988 (between 1985 and 1987) average temperatures showed the opposite trend, with negative departures from the 1951-80 mean temperature. The second half of the eighties was characterised by an increase in the *S. balanoides* density. Since 1991 the trend reversed and the *S. balanoides* population started to decline (Figs I 4.1a-4.3a). If the two-year lag between changes in temperature and changes in barnacle species composition is taken into account (see section on long-term climate changes in introduction for details), an agreement between temperature and density of *S. balanoides* can be observed. In the last ten years, colder weather led to an increase in density, warmer weather to a decrease.

Possible changes in the larval supply to the area of study, could also be responsible for the reduction of the surveyed *Semibalanus* population. Due to its small size, the barnacle population of Cellar Beach is believed to rely on larval input from populations elsewhere. In the fifties and sixties, areas east of Plymouth were the main suppliers of *Semibalanus* larvae (A.J. Southward pers. comm.). After 1970, the populations of Whitsand Bay and Looe, both west of Plymouth, increased in size; since then, the western shores probably became the largest contributors towards larval supply to the area of study (A.J. Southward pers. comm.). Possibly after 1988 the western populations started to decline, maybe as a consequence of climate changes, the number of larvae produced decreased and so did the larval supply to Cellar Beach from the west. Due to changes in wind direction, also the larval supply from the east diminished. In recent years, during the months of February and March (when *Semibalanus* larvae are found in the plankton), the prevailing winds in the Plymouth area have been coming mainly from south, south-west and west (The Meteorological Office, 1988-1993). As wind direction determines the direction of flow of the superficial currents and barnacle larvae are mainly found in the superficial layers of water (Le Fèvre and Bourget, 1991), it follows that the direction of the larval transport is highly dependent on that of the wind. Hence, if the prevailing winds during the settlement season were not coming from the east anymore, larvae from the eastern region could not reach Cellar Beach. In addition, Hawkins and Hartnoll (1982) observed that on the Isle of Man winds from the west tend to break down the phytoplankton bloom, and this may occur in the English Channel as well (S.J. Hawkins pers. comm.). Any damage to the phytoplankton bloom can inhibit barnacle larval release and cause starvation and eventually lead to mortality in those larvae already released. The combination of all the above factors probably caused a decrease in the larval supply to Cellar Beach.

It could be suggested then, that the influence of the slightly increasing temperatures on larval supply and post-settlement mortality, combined with changes in wind direction, could be among the major factors which caused the decrease of the *Semibalanus* population at Cellar Beach.

The decline of the *Semibalanus* population at Cellar Beach created large quantities of space free for colonisation. This space was only partly filled in by the other three species.

In the case of *Chthamalus*, this could be explained through low rates of recruitment and growth typical of these species. These characteristics prolong the time required to fill in vacant surfaces. Throughout the three year study both *Chthamalus* species maintained constant densities, although a slight upward trend was noticed towards the end of the survey (Figs I 4.1b-4.3b). Data from Southward's long term survey, show small fluctuations in *Chthamalus* density since 1991 (Figs I 4.1a-4.3a). These fluctuations can probably be attributed to spatial variation among the different areas sampled on different years. Overall the trend from Southward's data is going upwards, in agreement with that observed on the photographs. *C. montagui* and *C. stellatus* are probably benefiting from rising temperatures, although as a consequence of their biology, their response to these favourable conditions appears to be slow.

To the decrease of *S. balanoides,* corresponded also an increase in the *E. modestus* population at both mid and low shore (Figs I 4.2b-4.3b). The fast growth rate of *Elminius*, allowed it to occupy quickly quite a large quantity of the bare rock available. The *Elminius* population of Cellar Beach is considered to be ephemeral (Southward, 1991) and liable to extreme interannual fluctuations. According to Southward, it probably relies on recruitment from denser populations up the Yealm estuary and is generally renewed from one year to the other. Given *Elminius'* short survivorship, I agree with Southward, that most of the *E. modestus* cover is formed by recently settled individuals. From the analysis of my quadrats though, a certain component of older individuals was also found to be present. The years 1992 and

1994 seemed to be very successful for the settlement of this species (Figs I 4.2b-4.3b). The forty-five year survey also indicated a large increase in the number of *Elminius* at mid and low shore from 1992 onwards (Figs I 4.2a-4.3a). The reduced competition for space against *S. balanoides* at low shore combined with availability of bare rock and warm temperatures are probably responsible for the success of this species.

The algal overgrowth observed on some of the replicates analysed at mid shore, also deserves some attention. *Laurencia pinnatifida* was the alga responsible for this invasion. On average (over all ten replicates analysed at mid shore) the algal cover expanded from 9 to 50%, but in a particular case, a quadrat was totally overgrown. The peak in the increase in *Laurencia* cover was noticed during 1993, after this year the cover started to decrease again. Changes in algal cover could be related to variation of the microclimatic conditions (e.g. changes in shade, humidity, temperature and grazing pressure) or to natural fluctuations of the species in question. However, it is very difficult to find specific causes which could generate the observed pattern. The presence of this alga on the transect was recorded by Southward (1991) since the beginning of the long-term survey. He observed patches of varying extent and position on the steep shaded faces between MHWN and MLWN. Over the forty-five year survey he noticed changes in *Laurencia* cover with values varying between 5 and 50%. According to him no evident trend could be detected from these fluctuations.

I 4.5 SUGGESTIONS FOR FUTURE PHOTOGRAPHIC SURVEYS

I 4.5.1 What would I change if I was going to repeat the survey?

Due to practical reasons, the investigations could not be started at any other time but September 1991. If I was going to repeat the one year intensive survey at the same location, I would try to start the monitoring during the winter period. At this time of the year very little happens on the shore and none of the barnacle species settle. A similar starting time would allow observations on the whole settling season of each one of the four species without any interruption in between. January would therefore be a suitable starting month, but if the breeding season of *S. balanoides* needs also to be monitored, November would then be a better time.

During this study I also realised the importance of taking care of small practical details in order to make the analysis of the photographs much easier and faster later on. For instance, having a scale directly on the photograph which is going to be analysed is certainly a point of primary importance. The indirect method used in this study (of taking a photograph with and one without a coin and then work out the scale from the former one and relate it to the latter one) has the great advantage of not causing any loss of information provided by the photographs. On the other hand, the indirect estimation of the scale is a very long job and the results produced can sometimes be not very accurate. Having a scale on the photograph also gives the advantage of saving time while taking photographs and reduce the quantity of film used. In future I think I would adopt the method of placing a small piece of acetate paper with marks on, always in the same position on a flat surface at a side of the photographed area. Alternatively two screws could be fixed in the quadrat and the distance between them measured at the beginning of the study; the danger in this case would be that the screws could get lost.

To speed up the analysis of the prints, I should also have enlarged all photographs to exactly the same size (this was attempted but small differences in scale were still present). This trick would have enabled individuals to be marked on a piece of acetate paper which could have been placed over all photographs of the series. As in my case the enlargements were not all of the same size, each individual had to be labelled separately on each photograph.

The analysis would have also been facilitated by improving the quality of some of the photographs. Wherever feasible, photographs should always be taken in the driest possible conditions (a situation difficult to achieve during the winter months) and quadrats selected on flat surfaces.

To facilitate statistical analysis, a balanced design with an equal number of replicates at each shore level should have also been favoured. In addition, replicates should have given a very homogeneous species composition in order to allow individuals belonging to different species and age classes to be selected in the same number from each quadrat. To overcome this problem in the patchy community of Cellar Beach, distinct squares rather than true random quadrats were selected. This strategy partly succeeded in creating homogeneity, but replicates from mid shore still showed slight differences among each other.

I 4.5.2 Sampling frequency

The ideal frequency of sampling is entirely dependent on the aims of the study. Investigations on breeding, settlement and recruitment are obviously better done as frequently as possible during the breeding/settlement season of the species object of interest. Long sampling intervals will lead to underestimates of settlement, because post-settlement mortality will affect some of the newly settled spat before the following sampling session. According to Minchinton and Scheibling (1993b) an accurate estimate of settlement may be attained when increases in sampling frequencies do not result in any significant increase in estimates of recruitment. The same authors demonstrated that *S. balanoides* settlement on the east coast of Canada was better estimated when sampling was carried out daily. They also suggested that data collected on every tidal cycle could probably demonstrate that even daily sampling is too infrequent for an accurate estimation of settlement. Sampling for settlement and recruitment is then better done as frequently as possible, especially for species such as *S. balanoides* that settles in large numbers over a relatively short period of time.

For studies of growth, the ideal frequency of monitoring should be decided in relation to the species and age class investigated. Class 0+ individuals grow faster than older ones and therefore require more frequent sampling. In species such as *S. balanoides* and *E. modestus* changes in size can be detected fortnightly. Slow growing species like *Chthamalus* will instead require bi-monthly intervals between sampling sessions in order to observe any growth. Older classes in general can be sampled every three months; more frequent monitoring would lead to confusion between real increases in size and experimental error. If, as in this study, comparisons of growth and mortality rates among age classes and species are required, the sampling interval suitable for the slowest growing individuals should be adopted for all classes and species.

Studies of mortality also vary with age class and species. Young barnacles should be sampled as often as possible especially during the first three months after settlement (see above for suggested sampling frequency of recruitment). For older classes three months interval seems to be a suitable time to detect mortality.

I 4.6 FURTHER WORK

I 4.6.1 Broadening the spatial scale of monitoring

Broadening the spatial scale of this study, by adding extra sites along the south-west coast of England, would lead to results which can probably be considered representative of the whole region. Southward's long-term study was not limited to investigations at Cellar Beach but was designed as a wide-ranging survey of the south-west of England (Southward, 1967; Southward *et al.*, 1995). Sites on the coasts of south Devon and both north and south Cornwall were monitored annually for changes in barnacle species composition. Hence, his observations on fluctuations of the barnacle populations as a consequence of long-term climate changes, should be considered a general phenomenon for the south-west of England.

Thanks to Southward's studies, the changes in community structure observed in this work could be related to the more general survey carried out for the whole of the south-west area. However, for generalising the detailed study of the biological characteristics of the four species, at least two other sites should have been selected elsewhere in the south-west region on areas with similar barnacle cover to the one found at Cellar Beach. Ideally the three sites (including Cellar Beach) should be found within approximately 100 km from each other on both south and north coasts of the Devon/Cornwall peninsula. The problem with such an experimental design is the amount of travelling involved and the time required to analyse the large number of photographs generated. Unless more than one person were involved I doubt that such a study could be practically carried out. A reduction in the sampling frequency and in the number of biological parameters investigated could make such a project more feasible.

I 4.6.2 Inter and intra-specific interactions

Although areas of bare rock were available all the way through the study, their distribution was patchy. For this reason, barnacles were often found to be organised in dense aggregates where competitive interactions for space could easily take place. A detailed study of inter and intra-specific competition among the four species of barnacles living at Cellar Beach was going to be undertaken, but due to lack of time could not be carried out. In the immediate future, it is hoped to perform this type of analysis on the existing photographs as part of the Eurorock Mast III programme.

The main aim of such a study is to investigate the effect of competition on the growth and mortality rates of selected individuals. As a first approach, the number of interactions among barnacles of the four species should be estimated. This involves choosing random individuals of a certain species and counting the number of conspecific and non conspecific barnacles which come in direct contact with them. The preliminary investigation is aimed to identify the most common interactions among the four species. These interactions are the ones that are going to be studied in further detail. Random individuals involved in the most common interactions will then be identified on the photographs and followed until the end of the survey or until death. The growth and mortality rates of individuals of the same age class and species which are involved in different interactions will then be compared (e.g. is *S. balanoides* more likely to grow / die faster when surrounded by conspecifics / mixture of conspecifics and non conspecifics / all non conspecifics?). The potential for such a study is great and the number of possible combinations very large indeed especially if surrounding species are identified separately (e.g. instead of scoring as *S. balanoides* surrounded by non conspecifics, one specifies *S. balanoides* surrounded by four *C. montagui* and two *C. stellatus*). Because the large number of combinations, selected questions should be addressed and the number of interactions analysed kept to the minimum.

I 4.6.3 Genetic factors including selection after settlement

On every sampling session of the first year survey, additional samples of recently settled larvae and growing juveniles were collected from each one of the three shore levels. On arrival at the laboratory, these samples were immediately frozen in a biological freezer (-70°C) and kept for future genetic analysis. The aim of this sample collection was to investigate genetic selection after settlement in each species and at different shore levels. In other words, to investigate if a certain genotype favoured survival better than another one. Biochemical techniques, such as electrophoresis, are probably not appropriate for this type of investigation as they do not provide sufficient resolution to enable detection of tiny changes in the genotypes of individuals belonging to the same population. This statement is supported by the relatively great genetic homogeneity observed in specimens of *Chthamalus* (Pannacciulli *et al.*, 1995), *S. balanoides* (Flowerdew and Crisp, 1975; 1976; Flowerdew, 1983a; 1983b) and *E. modestus* (Flowerdew, 1984) from all over their distribution range, when studies were carried out by using such electrophoretic techniques. Molecular techniques such as Random Amplified Polymorphic DNA (RAPD) would certainly be more appropriate as they offer a greater potential resolution; this better resolution is achieved at a greater financial cost and investment of time per individual analysed.

The accomplishment of a similar study would certainly advance greatly the knowledge we have of genetic selection in barnacles at the early stages of life and will parallel nicely the study of intraspecific competition described above. Also in this case the barnacles are already available and it is hoped in the future to find the financial support and the time to carry out the investigation.

I 4.6.4 Effect of vertical transplantation on breeding and survival

Vertical transplantation experiments could be easily carried out by removing rock chips carrying barnacles at one shore level and cementing them down at a different shore height. Alternatively, settlement could be induced on plates and as soon as the settlement season finishes, plates could be swapped among tide levels.

These types of experiments would allow one to test for differences in breeding and survival of barnacles of the same species but from different shore levels and determine if these differences are genotypic or phenotypic. Transplantation experiments to test for differences in breeding, had already been carried out by Crisp on a very large geographical scale. In his studies Crisp (1964b; 1968) transplanted specimens of *S. balanoides* from Menai Bridge, North Wales to the east coast of north America and *vice versa.* American specimens were found to differ from the British ones for the earlier onset of breeding in identical habitats, the larger size of the eggs and the more rapid development of the embryo. These differences were maintained after transplantation, implying that genotypic differences were responsible for the different behaviour.

SECTION II

POPULATION GENETIC STUCTURE OF TWO SPECIES OF *CHTHAMALUS* IN THE NORTH-EAST ATLANTIC AND MEDITERRANEAN

II 1. INTRODUCTION

The thoracican barnacle *Chthamalus stellatus* (Poli) was accorded a panglobal distribution by Darwin (1854). Subsequently various distinct species have been recognised within this taxon in different regions (Newman and Ross, 1976; Southward, 1983). In the North East Atlantic and Mediterranean Sea, *Chthamalus montagui* Southward was distinguished from *C. stellatus* relatively recently (Southward, 1976). Nevertheless, the degree of genetic divergence between the two species, taken together with their morphological differences, would place them in separate subgroups (Dando and Southward, 1980).

Both species occur intertidally and they are often found together on the shore, although *C. stellatus* is more abundant on very exposed shores while *C. montagui* prefers more sheltered areas (Southward, 1976). Their planktonic larvae lead a pelagic life for some weeks, but it is thought that larvae of *C. stellatus* live longer and disperse further offshore than those of *C. montagui* (Burrows, 1988). This strategy appears to allow *C. stellatus* to colonise offshore islands and headlands (Crisp *et al.*, 1981) where *C. montagui* is less abundant or absent. The geographical range of the two species covers the North East Atlantic coasts, the Mediterranean Sea and the Black Sea (Crisp *et al.*, 1981; Pannacciulli *et al.*, unpublished) (Figs II 1.1 and 1.2). In most cases their distributions overlap but on offshore islands like the Açores and Madeira, *C. stellatus* is the only species found.

Dando and Southward (1981) suggested the existence of "Atlantic" and "Mediterranean" forms of *C. montagui* on the basis of electrophoretic analyses. No division of this kind has been noted for *C. stellatus.* In their paper Dando and Southward also stated : "... it is clear that further comparison of the Atlantic and Mediterranean warm-temperate faunas by modern methods, including enzyme electrophoresis, would be very rewarding ...".

A recent review (Fredj *et al.*, 1992) showed that endemic species constitute 28% of the Mediterranean fauna. In the case of littoral barnacles, for instance,

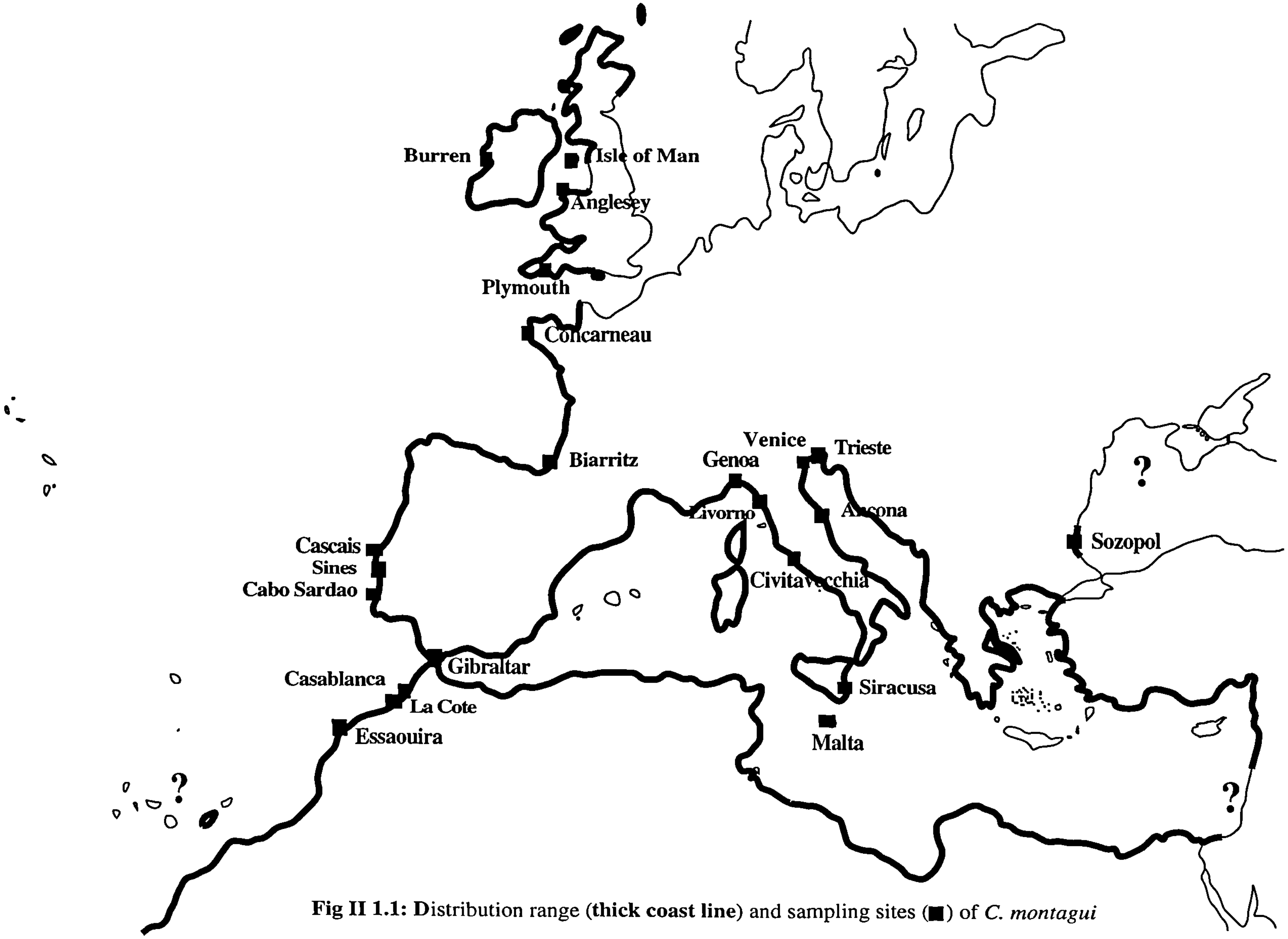


Fig II 1.1: Distribution range (**thick coast line**) and sampling sites (■) of *C. montagui*

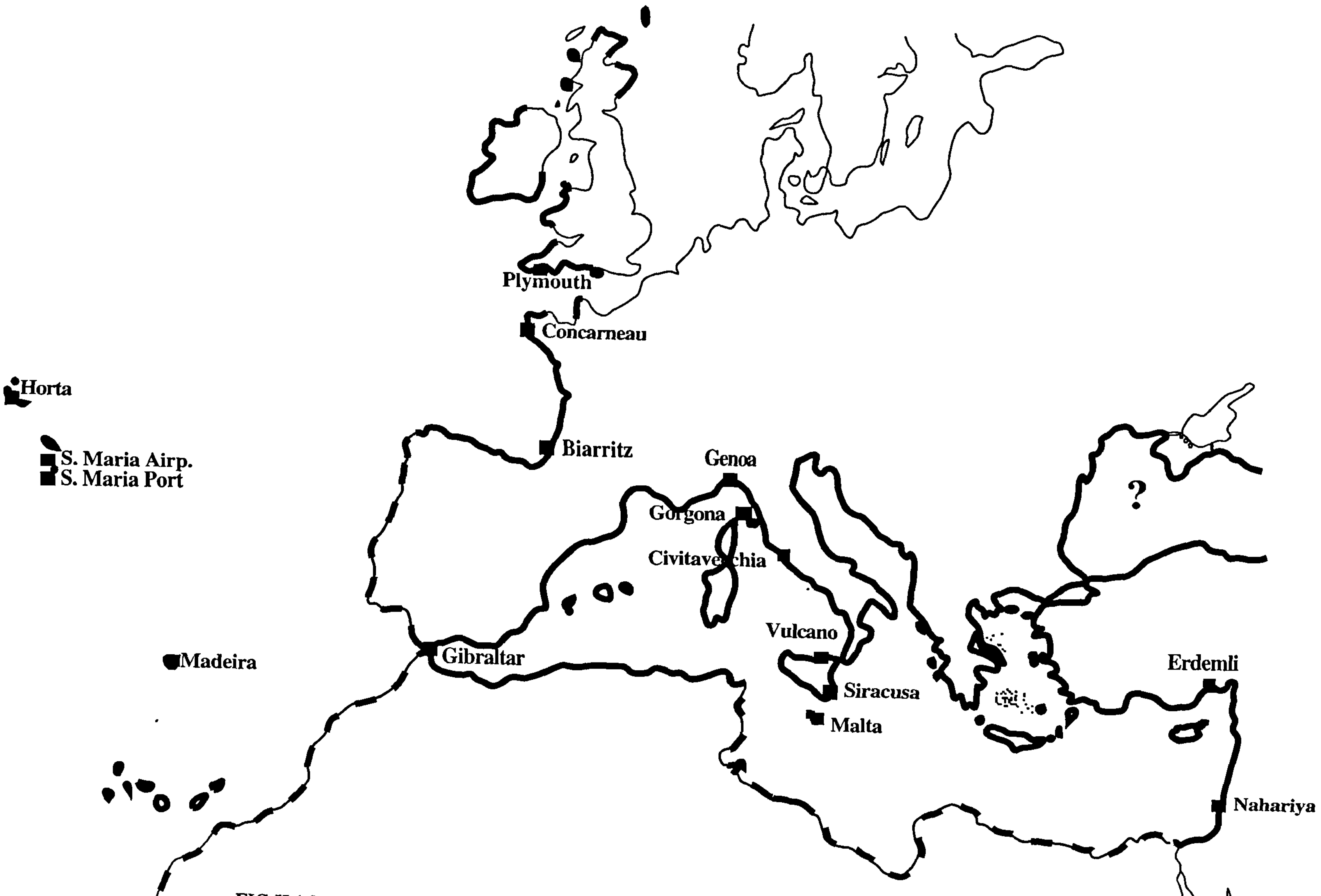


FIG II 1.2: distribution range (━━ line), occasional presence (━ ━ line) and sampling sites (■) of *C. stellatus*

Euraphia depressa, one of the three species of intertidal barnacles found in this basin, is limited to the Mediterranean (Kensler *et al.*, 1965; Crisp *et al.*, 1981). To cite another example, about 44% of the 453 species of Demosponges found in this basin are endemic (Vacelet, 1981). Tortonese (1981; 1985) suggested that 5 out of 31 species of Asteroidea, 60 out of 550 species of fish and 35 out of the 144 species of echinoderms were endemic to the Mediterranean. However, Fredj *et al.* (1985) observed that the endemicity of the Mediterranean fauna seems to decrease with increasing depth.

Tortonese (1981), with reference to his work on Asteroidea, pointed out that there are problems deciding whether populations living in the Mediterranean and Atlantic are conspecific or represent congeneric species pairs. The literature provides many instances, in different marine species, of intraspecific genetic differentiation between the Mediterranean and North East Atlantic area. There are numerous marine organisms for which, on the basis of genetic analysis, intraspecific genetic differentiation between the two regions has been recorded. These include: the oyster *Ostrea edulis* (Jaziri *et al.*, 1987; Saavedra *et al.*, 1993), the spionid polychaete *Malacocerus fuliginosus* (Guerin and Kerambrun, 1984), a large number of species of fish such as the flounder *Platichthys flesus* (Galleguillos and Ward, 1982; Berrebi *et al.*, 1985), the iberian toothcarp *Aphanius iberus* (Garcia-Marin *et al.*, 1990), the hake *Merluccius merluccius* (Pla *et al.*, 1991), the brown trout *Salmo trutta* (Guyomard, 1991; Estoup *et al.*, 1993), the blue whiting *Micromesistius poutassou* (Mork and Giaever, 1993), the bib *Trisopterus luscus* (Tirard *et al.*, 1993), the swordfish *Xiphias gladius* (Magoulas *et al.*, 1993) and the grey mullet *Mugil cephalus* (Crosetti *et al.*, 1993). Also on the basis of pure morphological analysis it has been possible to separate Atlantic and Mediterranean forms of organisms such as: the ascidian *Pyura microcosmus* (Nishikawa and Turon, 1992), some micronektonic crustaceans (Casanova, 1981), the copepod *Calanus helgolandicus* (Fleminger and Hulsemann, 1987), various species of fish including *Benthosema glaciale* (Badcock, 1981), *Sardina pilchardus* (Freon and Stequert, 1982), *Chromis chromis* (Re and Gomes, 1982), and historical samples of monk seals (Bree and Ronald, 1984).

In other cases, the process of differentiation appears to have proceeded to full speciation. Some examples of species pairs which replace each other in the Mediterranean and Atlantic basins are: the Mediterranean sea-anemone *Epizoanthus arenaceus* and *E. conchii* in the North East Atlantic, the corals *Caryophyllia clavus* and *C. smithii*, the limpets *Patella caerulea* and *P. depressa*, the topshells *Gibbula divaricata* and *G. cineraria*, plus *Monodonta turbinata* and *M. lineata*, the scallops *Pecten jacobeus* and *P. maximus*, the crabs *Xantho rivulosus* and *X. incisus*, the feather stars *Antedon mediterranea* and *A. bifida* and the fish *Blennius pavo* and *B. pholis*. All these species pairs may have originated from the divergence of a common ancestor. While these examples show end-products of speciation, in the case of the two "forms" of *Chthamalus montagui* we might be witnessing speciation in progress, as hypothesised by Dando and Southward (1981). In the case of limpets for instance, their relatively limited larval dispersal (Dodd, 1957) might have played an important role in speciation (Côrte-Real *et al.*, in press).

All these kinds of speciation events are likely to be common in the North East Atlantic/Mediterranean area. This is a very young system that has been subjected to rapid changes in configuration and climate over the last six million years (Pérès, 1967; Blanc, 1968). The combination of events such as the opening and closing of the Strait of Gibraltar, advances and contractions of glaciation, and changes in current patterns, quite certainly contributed to make the North East Atlantic/Mediterranean area a notable generator of diversity.

Besides Dando and Southward's (1981) work on *Chthamalus*, intraspecific genetic differentiation has also been detected in other barnacle species. Flowerdew (1983a; 1983b) compared the pan boreal species *Semibalanus balanoides* populations from the Pacific coast of Alaska and from the North Atlantic Ocean and found a possible taxonomic split at subspecies level between barnacles from the two oceans. Physiological differences between the two subspecies had already been noted by

Southward (1964) who, using the relationship between cirral activity and temperature, was able to show slight but consistent differences in the cirral activity. Crisp (1964b; 1968) detected variation also in the reproductive phenology of *Semibalanus* from the two sites; transplantation experiments indicated that the differences in the reproductive mode had a genetic basis and were not merely an ecophenotypic response to environmental differences (Flowerdew and Crisp, 1975). Seasonal variation of allele frequencies at the Esterase locus in British *Semibalanus balanoides* was noticed by Flowerdew and Crisp (1976). They suggested that the variation of the frequencies of putative alleles was dependent on some environmental agent such as temperature or day length. According to Holm and Bourget (1994) temperature also affected the genetic structure of populations of *Semibalanus balanoides* along Northwest Atlantic coasts. Thermal selection seemed to be responsible for variation in allozyme frequencies and size of *Balanus amphitrite* living in the canals of the cooling system of an electrical plant (Nevo *et al.*, 1977). The relationship between seasonality, latitude and trophic resource stability as factors regulating genetic variability had also been investigated by Hedgecock (1979) in tropical species of *Chthamalus*. He found that the correlation between average heterozygosity and latitude was positive but not significant.

By comparing the life history of *Semibalanus* with that of *Mytilus edulis* Flowerdew (1983a) found that although the dispersal potential is greater in the mussel, intraspecific genetic differentiation events seemed to be more common in *Mytilus* than in *Semibalanus*. This consideration led him to the conclusion that for marine invertebrate species with a prolonged larval stage and able to settle in a variety of environments over a wide geographical range, genetic differentiation between populations is not a simple function of dispersal potential (Flowerdew, 1983a). Of the same opinion was Burton (1983) who said: "... the genetic structure of natural populations of marine invertebrates cannot be reliably inferred from their apparent dispersal capacities". However from his work on the colonisation of the North East Atlantic coasts by *Elminius modestus*, Flowerdew (1984) concluded that very effective dipersal, as in this species, can suppress genetic differentiation between populations over a wide geographical area. The effects of selection and/or genetic drift are still undetected in these newly established populations.

Various authors have assessed the possible influence of selection, acting at different stages of the life history, upon the net effects of gene flow mediated by larval dispersal. Where there are strong selective pressures on larval or juvenile stages (e.g. Johnson and Black, 1984 working on siphonarian limpets) it has been argued that the importance of gene flow through planktonic larval dispersal is not that genetic divergence cannot occur, rather, divergence cannot accumulate but must be renewed each generation. Considering the persistence of immigrant genes in the adult population, Hedgecock (1986) observed that differentiation of marine invertebrates having pelagic larvae can arise from either physical or biological barriers to larval dispersal or from differential survival or fecundity of immigrants. From this he concluded that gene flow depends not only on the rate of exchange of migrants, but perhaps more importantly upon migrant fitness. Exchange of migrants seems to be facilitated in fouling barnacles which can rely on remote dispersal on ships (Furman *et al.*, 1989). As regards migrant fitness, in his study on *Balanus glandula* Hedgecock (1982) discovered that populations with statistically heterogeneous gene frequencies at the adult stages could arise from genetically homogeneous recruits. From this, he concluded that differential mortality after settlement is the main determinant of the genotypic composition of adult populations of *B. glandula*.

With this study I attempt to enlarge Dando and Southward's electrophoretic work by analysing samples of *C. montagui* from its entire geographical range and also by extending the previous analyses of *C. stellatus*. I will try to assess if physical, geographic, hydrographic factors and the history of the Atlantic/Mediterranean basins may have interacted with present-day ecological factors to produce the observed genetic pattern. Comparisons of the biology of the two species in terms of their different distribution on the shore, exposure to wave action and different larval dispersal will also be taken in account when trying to explain this genetic differentiation.

II 2. METHODS

II 2.1 SAMPLES

Samples of *C. montagui* and *C. stellatus* were collected from sites along the North East Atlantic, Mediterranean and Black Sea coasts (Figs II 1.1 and 1.2). Sample collection took place over a period of a year and a half starting from February 1993.

Chips of rocks bearing barnacles were collected using a hammer and chisel. The rock chips were then dampened in sea water, wrapped loosely in unsealed plastic bags to keep them aerated and moist, and kept cool (ideally in a cool box). If kept in this way both the species survived well for at least a week out of water and could easily be transported. At Port Erin the samples were maintained in the laboratory sea water circulation. The animals stayed alive for more than 18 months, although their enzymatic activity decreased slightly a few months after collection. Maintenance of living specimens allowed direct comparisons between samples collected at different times throughout the study.

A total of 22 samples of *C. montagui* and 16 of *C. stellatus* were collected (Figs II 1.1 and 1.2); it was not always possible to find both the species at the same sampling site. No *C. montagui* were found in the Açores and Madeira. About 60 animals from each sample (see Appendix II 1 and 2) were analysed using standard horizontal starch gel electrophoresis of allozymes.

II 2.2 EXTRACTION

Live adults were removed from the rock and developing egg masses (if any) removed from the mantle cavity. The whole animal attached to the operculum was pushed out from the shell complete with the adductor and depressor muscles. Very small barnacles were used whole. The prepared animal was then placed in an

Eppendorf vial and soluble proteins were extracted without homogenization for 2 hours at 2° C in 25 μl of extraction buffer. This buffer was slightly modified from the recipe of Dando and Southward (1979): instead of 2 % 2-phenoxyethanol, 2 % 1-phenoxy-2-propanol was added; the original recipe was also tried on both species but did not give good results. At the end of the extraction period the animal was removed from the vial and put in a tube with its shell and 1ml 70% ethanol; the plates and the remains of the animal were preserved in case a further check on species identification was needed. The vial with the extraction buffer and the extracted enzymes was then stored in liquid nitrogen or in a -75° C freezer until required for electrophoresis. Freezing at very low temperature seemed to increase the enzyme activity.

II 2.3 ELECTROPHORESIS

Twenty-five enzyme systems, and nine buffer recipes, were investigated using horizontal electrophoresis on starch gel and on cellulose acetate plates. Unfortunately the N-(3-aminopropyl)-diethanol-amine-citric acid buffer used by Dando and Southward (1979) to resolve certain enzymes was no longer available from the suppliers. With the available buffers, only six loci for *C. montagui* and four for *C. stellatus* were found to give scorable results (Table II 2.1). These loci were all polymorphic apart from *SOD*—scored only for *C. montagui*—which showed a monomorphic pattern. All other enzymes investigated either showed no activity or were uninterpretable. The scorable loci were equally well resolved on both starch gel and cellulose acetate plates but for practical reasons the former technique was used. Atlantic and Mediterranean samples were run together to allow direct comparison.

The buffer system used was Tris-Borate-EDTA (Dando *et al.*, 1979), pH 8.7. Gels were 6 mm thick and contained starch (SIGMA Chemical Co.) at a concentration of 10 %. They were run at 400 V, 50 mA for approximately 4.5 hours, the time required for the reference protein, horse-spleen ferritin (SIGMA Chemical Co.), to run 4.5 cm. The gels were stained for the enzymes in Table II 2.1. Staining recipes were

Table II 2.1: enzymes examined (* not scorable for *C. stellatus*)

ENZYME	ABBREVIATION	E.C. number	QUAT. STRUCT.
Phosphoglucose isomerase	PGI	5.3.1.9.	dimeric
Phosphoglucomutase	PGM	5.4.2.2.	monomeric
Glutamate-oxalacetate transaminase	GOT	2.6.1.1.	dimeric
Mannose phosphate isomerase	MPI	5.3.1.8.	monomeric
Peptidase	PEP*	3.4.11-13	dimeric
Superoxide dismutase	SOD*	1.15.1.1	monomeric

all based on Harris and Hopkinson (1977) apart from that for GOT which was taken from Jeremiah *et al.* (1982). Since the barnacles were too small to provide enough tissue for two electrophoresis runs, gels were photographed and preserved to allow comparisons of allelic mobility between all the samples and to permit re-interpretation in case of doubts on relative allelic mobilities.

II 2.4 DATA ANALYSES

Unless otherwise stated, all computations were carried out using the computer package Biosys-1, Release 1.7 (Swofford and Selander, 1989) .

II 2.4.1 Heterozygosity

The measure of heterozygosity based on Hardy-Weinberg expectations and referred to as H_{bias} in Biosys-1 was used (Table II 3.2). This index was chosen because it was thought to be a better measure of genetic variation in a sample than direct-count heterozygosity. In fact, unlike the direct-count estimate, H_{bias} is independent of sample size, natural selection and inbreeding (Nei and Roychoudhury, 1974).

As Biosys does not provide standard deviation values of H_{bias} per locus and per sample, this was calculated as the square root of the binomial sampling variance: $\sigma = \sqrt{\text{HET} \times \text{HOM} \div \text{N}})$ with HET = proportion of heterozygotes, HOM = proportion of homozygotes and N = n° of individuals analysed.

II 2.4.2 Number of alleles

Although heterozygosity is the usual measure of genetic diversity within a population, this depends on both the number of alleles at a locus and their frequency. I also wished to compare directly the number of different alleles present in the

Atlantic and Mediterranean populations, without the influence of allele frequencies. However, the observed number of alleles per locus is highly dependent on sample size. Comparisons of allele diversity amongst samples is not biologically meaningful unless sample size is more or less the same. In practice, common sample size is very difficult to achieve and in the case where the largest sample also has the highest number of alleles it is difficult to decide if this difference is due only to sample size. To overcome this problem I adapted the species richness (rarefaction) equation of Hurlbert (1971) to the study of allele diversity in order to estimate the expected number of alleles in a sample of given size. Hurlbert's formula for sampling with replacement was used, with the notation adapted for alleles instead of species:

$$E(A_n') = \sum_{i=1}^{A}\left[1-(1-p_i)^n\right]$$

where $E(A_n')$ = expected n° of different alleles in a sample of n genes, where:
p_i= frequency of the i-th allele, n = sample size, A = number of alleles in total sample.

I appreciate that the alternative formula, for sampling without replacement, would have been more suitable for this study since each gene was sampled only once, but the computational difficulties of this made me opt for the stated version. In this study the unit of sampling was two genes, because I was dealing with diploid organisms, but the generally observed conformity to Hardy-Weinberg expectations (see below) indicated that alleles within genotypes were not strongly correlated and could therefore be treated as independent entities.

Hurlbert's formula was used to compare the number of alleles present in the Atlantic and Mediterranean basins by calculating the expected number of alleles in samples of the same size. For this purpose all samples from each basin were pooled (Sozopol in the Black Sea joining the Mediterranean) to create a Mediterranean and an Atlantic population. Note that the observed heterogeneity of allele frequencies within these samples (Table II 3.5) implied that a degree of differentiation existed within each basin, calling the strict validity of this pooling process into question.

II 2.4.3 Hardy-Weinberg equilibrium

The calculated expected genotype frequencies were often very small even after pooling of rare alleles, and therefore did not fulfill the requirements for the use of goodness-of-fit Chi square tests. Exact tests were then the only valid alternative to test departure from Hardy-Weinberg equilibrium (Lessios, 1992) and were therefore used throughout. Exact tests were carried out on three classes of pooled genotypes: 1) homozygotes for the most common allele, 2) heterozygotes between the most common allele and other alleles, 3) other homozygotes and heterozygotes. To avoid Type-I error the significance level of 0.05 was adjusted for the number of tests carried out by using the Hochberg method of sequential comparisons (Hochberg, 1988).

II 2.4.4 F statistics

Wright's F statistics (Wright, 1965; 1978; Nei, 1977) were calculated by Biosys. Unbiased estimates of these were obtained using the computer program FSTAT (Goudet, 1994) for calculation of Weir and Cockerham's unbiased estimators of F-statistics. Individual multi-locus genotypes were required by FSTAT while Biosys accepted genotype frequencies.

To detect levels of genetic structuring between regions, grouping procedures were applied to the samples (Goudet *et al.*, 1994). Weir and Cockerham (W-C) F_{IS} (Weir, 1990) was calculated over all loci and all samples at different levels of grouping. At stage 1 all samples were kept separate; at stage 2 some geographically close samples were pooled together, data reprocessed and a new estimate of F_{IS} obtained. The procedure was repeated until all samples were pooled and the last estimate obtained (Tables II 3.7-3.8). To visualise structuring, the different estimates were plotted on a graph with the pooling stages on the X-axis and the W-C F_{IS} values on the Y-axis (Fig II 3.4). Error bars were obtained by bootstrapping over loci to estimate the 95% confidence interval.

The grouping procedure generates a great heterogeneity in the sample sizes. Unless unbiased estimators are used, such as Weir and Cockerham's F_{IS} which is weighed for sample size, the levels of structuring may stay hidden (Goudet *et al.*, 1994).

II 2.4.5 Genetic distance

Nei's measure of unbiased genetic distance (D) among the various samples was used. Cluster analysis was performed on these measures using the unweighted pair-group method with arithmetic averaging (UPGMA) as described by Sneath and Sokal (1973).

II 3. RESULTS

II 3.1 ALLELE FREQUENCIES

According to Wright's F_{st} values (Table II 3.1), *PGM* in *C. montagui* and *GOT* in *C. stellatus* were the loci with the greatest overall genetic differentiation. At both loci the allele given a relative mobility of 100 had the greatest value of F_{st} and was therefore the most variable in frequency between samples. The allele PGM^{100} of *C. montagui* was found to be very common in the Atlantic and at Gibraltar but its frequency dropped sharply in the Mediterranean samples. It also decreased going from west to east along the Mediterranean coasts and into the Black Sea (Fig II 3.1). *C. stellatus* in contrast showed a steadily descending cline in the frequency of the allele GOT^{100} going from the Atlantic to the Mediterranean, with further reduction in the Eastern Mediterranean (Fig. II 3.2).

II 3.2 HETEROZYGOSITY

In both species the values of mean heterozygosity per locus were higher in Mediterranean samples than in Atlantic ones (Table II 3.2) (Mann-Whitney U test P = 0.0001 for *C. montagui* and P = 0.0019 for *C. stellatus*).

Variation of the heterozygosity for each locus and in each sample is shown in Fig II 3.3. The samples from Gibraltar were grouped with those from the Atlantic in agreement with the results obtained from the UPGMA dendrograms (see below). Heterozygosity of the loci *PGI* and *GOT* in *C. stellatus* and all the polymorphic loci in *C. montagui* differed significantly between the Atlantic and the Mediterranean basins (Table II 3.3). The Mediterranean heterozygosity values were always higher than the Atlantic ones in *C. montagui*, with the exception of *PEP* which showed the opposite trend (see Fig II 3.3). The relatively indistinct pattern at this locus, with several

Table II 3.1: Values of Wright's F_{st} per locus over all samples

locus	*C. montagui*	*C. stellatus*
PGI	0.025	0.016
MPI	0.017	0.034
GOT	0.040	0.094
PGM	0.244	0.032
PEP	0.071	-

mean	0.126	0.069

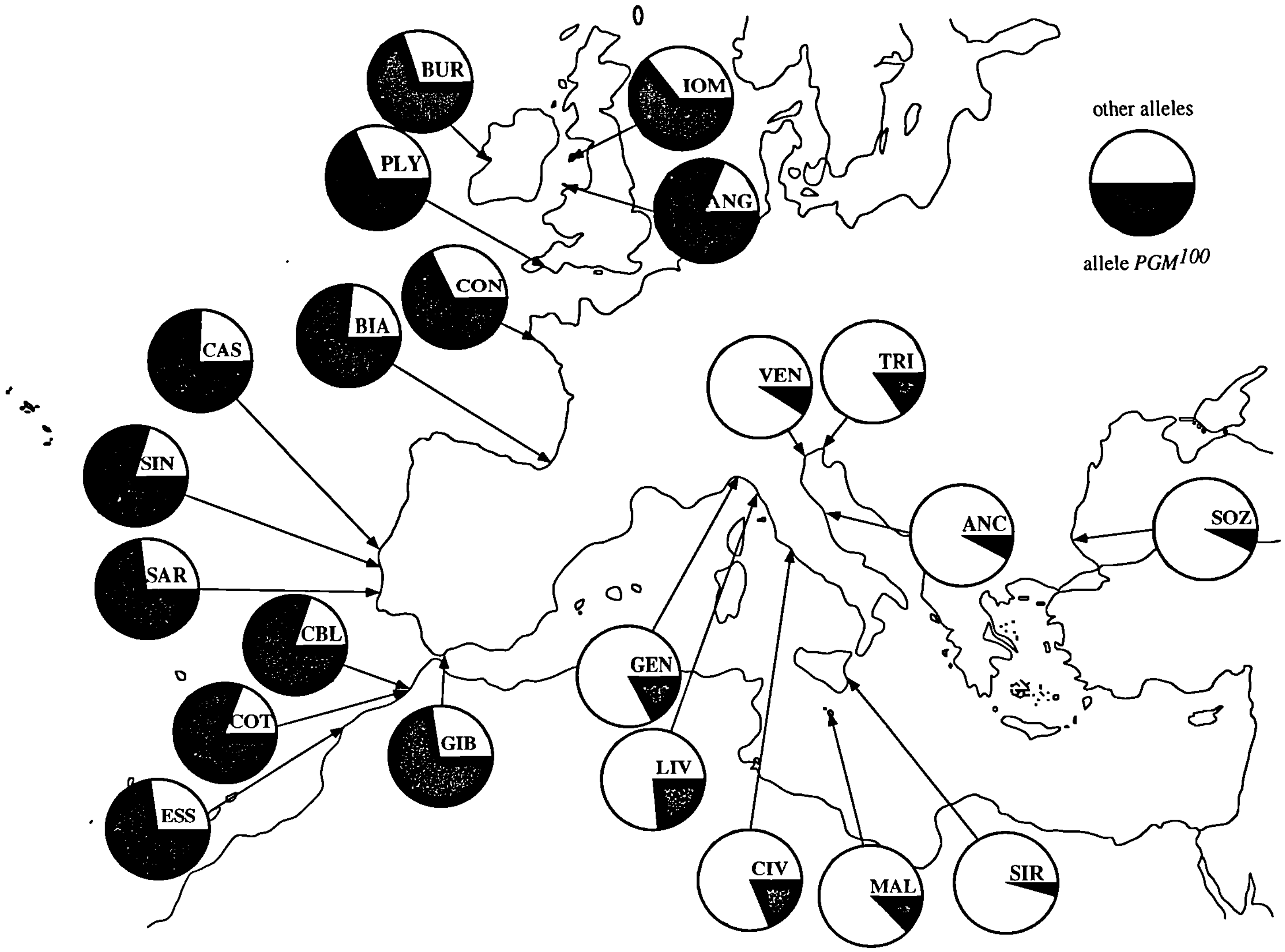


FIG II 3.1: *Chthamalus montagui,* variation of allele frequencies at the *PGM* locus
(ANC = Ancona, ANG = Anglesey, BIA = Biarritz, BUR = Burren, CAS = Cascais, CBL = Casablanca, CIV =Civitavecchia, CON = Concarneau, COT = La Cote, ESS = Essaouira, GEN = Genoa, GIB = Gibraltar, IOM = Isle of Man, LIV = Livorno, MAL = Malta, PLY = Plymouth, SAR = Cabo Sardao, SIN = Sines, SIR = Siracusa, SOZ = Sozopol, TRI = Trieste, VEN = Venice)

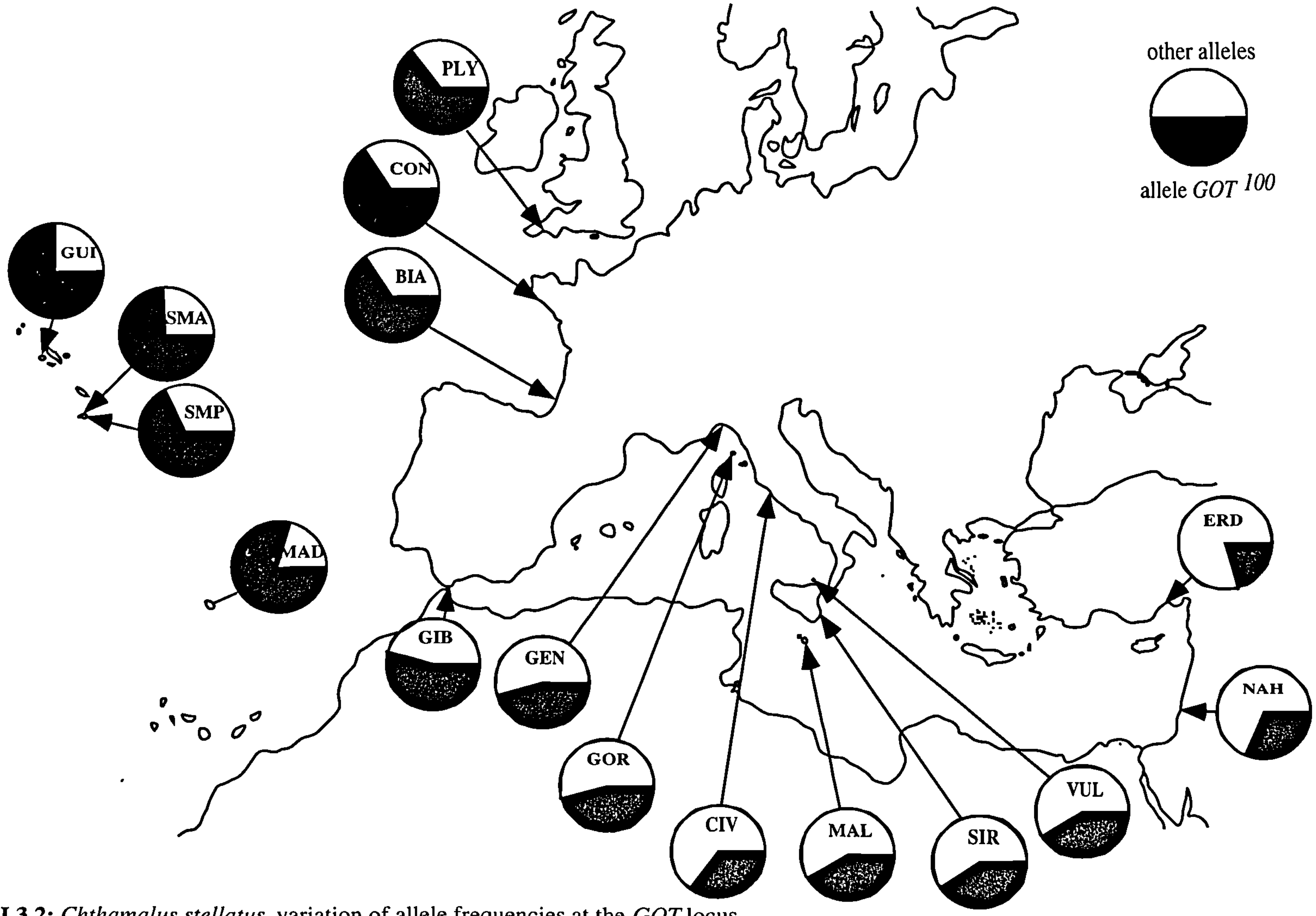


Fig II 3.2: *Chthamalus stellatus*, variation of allele frequencies at the *GOT* locus
(BIA = Biarritz, CIV = Civitavecchia, CON = Concarneau, ERD = Erdemli, GEN = Genoa, GIB = Gibraltar, GOR = Gorgona, HOR = Horta, MAD = Madeira, MAL = Malta, NAH = Nahariya, PLY = Plymouth, SIR = Siracusa, SMA = S. Maria airport, SMP = S. Maria port, VUL = Vulcano)

Table II 3.2: Mean per - locus heterozygosity H_{bias} (with standard error)

C. montagui

Atlantic			Mediterranean		
Sample	H_{bias}	S.E.	Sample	H_{bias}	S.E.
BURREN	0.179	0.065	GENOA	0.266	0.094
ISLE OF MAN	0.223	0.072	LIVORNO	0.298	0.097
ANGLESEY	0.182	0.062	CIVITAVECCHIA	0.297	0.090
PLYMOUTH	0.201	0.066	SIRACUSA	0.236	0.083
CONCARNEAU	0.202	0.068	MALTA	0.272	0.106
BIARRITZ	0.145	0.060	ANCONA	0.249	0.094
CASCAIS	0.239	0.068	VENICE	0.251	0.099
SINES	0.135	0.055	TRIESTE	0.286	0.101
CABO SARDAO	0.131	0.066	SOZOPOL	0.248	0.100
CASABLANCA	0.187	0.052			
LA COTE	0.157	0.047			
ESSAOUIRA	0.211	0.065			
GIBRALTAR	0.190	0.068			

C. stellatus

Atlantic			Mediterranean		
Sample	H_{bias}	S.E.	Sample	H_{bias}	S.E.
PLYMOUTH	0.192	0.100	GENOA	0.262	0.108
CONCARNEAU	0.210	0.113	GORGONA	0.206	0.118
BIARRITZ	0.226	0.131	CIVITAVECCHIA	0.268	0.117
HORTA	0.187	0.076	VULCANO	0.288	0.085
S. MARIA air	0.156	0.082	SIRACUSA	0.232	0.128
S. MARIA port	0.151	0.105	MALTA	0.228	0.121
MADEIRA	0.134	0.074	ERDEMLI	0.259	0.101
GIBRALTAR	0.195	0.121	NAHARIYA	0.239	0.101

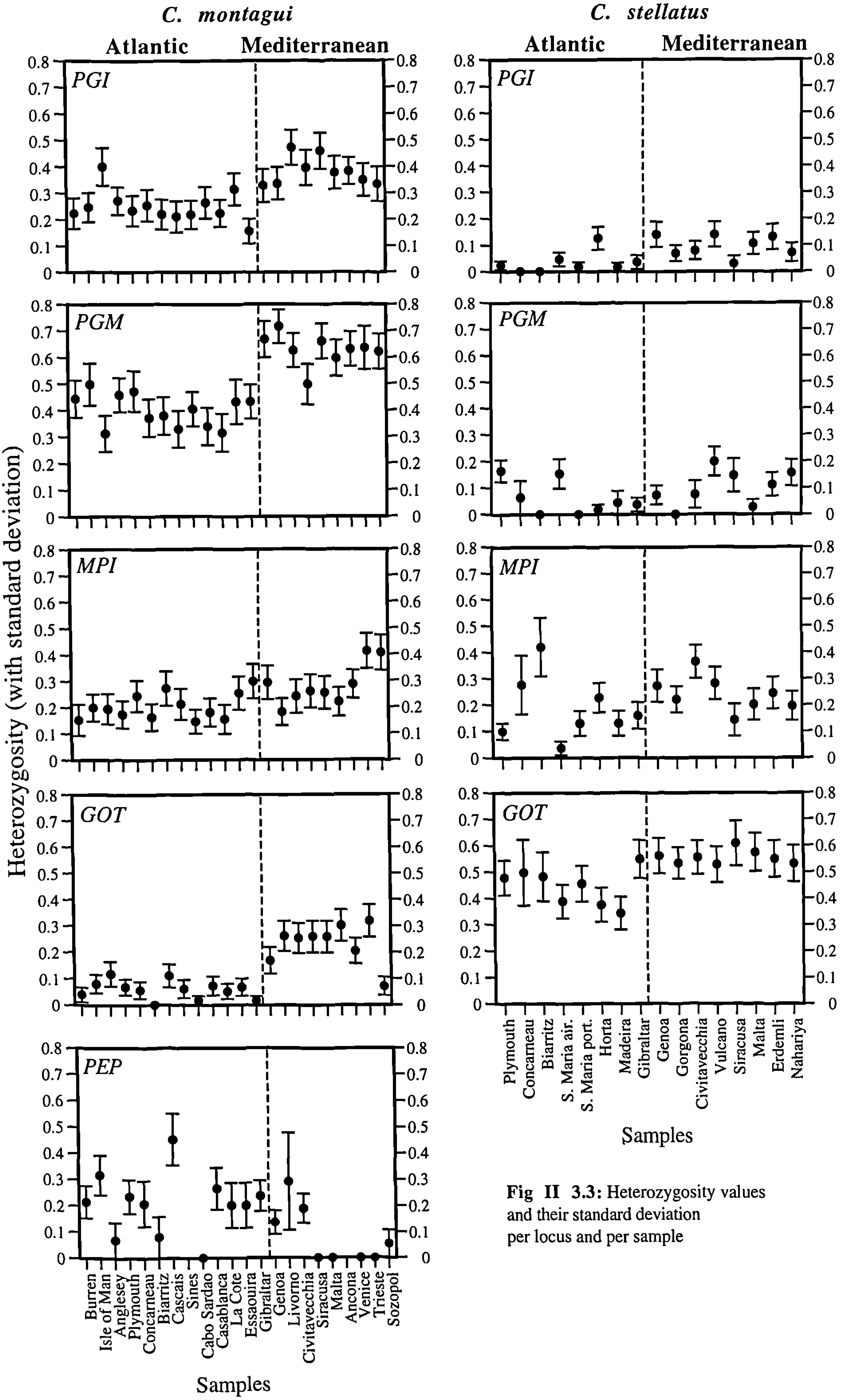


Fig II 3.3: Heterozygosity values and their standard deviation per locus and per sample

Table II 3.3: Probability values obtained from the Mann-Whitney non-parametric test testing the null hypothesis: heterozygosity Mediterranean = heterozygosity Atlantic. (the computer programme Minitab was used to calculate the *P* values). Significant values in bold.

C. montagui

	Atlantic		Mediterranean			
locus	N	Median het	N	Median het	*W*	*P*
PGI	13	0.2330	9	0.3770	98	**0.0007**
PGM	13	0.4040	9	0.6310	91.5	**0.0001**
MPI	13	0.1940	9	0.2600	112	**0.0135**
GOT	13	0.0610	9	0.2580	95	**0.0003**
PEP	13	0.2080	9	0.0000	182	**0.0326**

C. stellatus

	Atlantic		Mediterranean			
locus	N	Median het	N	Median het	*W*	*P*
PGI	8	0.0210	8	0.0935	43	**0.0101**
PGM	8	0.0405	8	0.0935	57	0.2701
MPI	8	0.1445	8	0.2330	55	0.1893
GOT	8	0.4665	8	0.5515	39	**0.0028**

monomorphic samples, is probably a consequence of small sample sizes and possibly of difficulties in scoring the gels.

II 3.3 NUMBER OF ALLELES

Despite the smaller number of individuals analysed, the Mediterranean population of *C. montagui* showed (Table II 3.4) a greater range of alleles than the Atlantic one. As would be anticipated, this difference persisted at all loci when the expected number of alleles was recalculated for equal sample sizes. In *C. stellatus*, the use of Hurlbert's equation was essential to enable comparisons of allele counts because for this species the population with the larger sample size (the Mediterranean one) also had the higher number of observed alleles. Thus a direct comparison could not be made unless the number of alleles was re-estimated for equal sample sizes. As for *C. montagui*, for equal sample sizes *C. stellatus* showed a higher expected number of alleles in the Mediterranean population at all loci.

Unfortunately, given the low number of loci analysed, no statistical analysis could be carried out on the data for single species. For instance the Wilcoxon's signed-ranks test requires at least six differences (in this context, loci). The only way I could obtain an adequate number of differences was by pooling the data from the two species. If the expected numbers were used to test the hypothesis that, in the two species together, the Mediterranean population had a larger range of alleles than the Atlantic one, a highly significant result was obtained (Wilcoxon's signed-ranks test, *P* (n° alleles Med = n° alleles Atl) < 0.0039). A qualitative analysis led to the same conclusions: in the uncorrected data 3 loci out of 5 in *C. montagui* and 4 out of 4 in *C. stellatus* showed a greater number of alleles in the Mediterranean population. The same was true for all loci in both species if the expected values were for equal sample sizes.

Table II 3.4: Observed and expected number of alleles per locus in the Atlantic and in the Mediterranean basin

C. montagui

	ATLANTIC observed		MEDITERRANEAN observed		ATLANTIC expected		MEDITERRANEAN expected	
Locus	n° of alleles	n° genes analysed	n° of alleles	n° genes analysed	n° of alleles	n° genes analysed	n° of alleles	n° genes analysed
PGI	**10**	1472	**17**	1088	**9.036**	1088	**14.774**	1088
MPI	**10**	1290	**11**	974	**8.800**	974	**9.703**	974
PGM	**7**	1266	**8**	902	**6.902**	902	**7.496**	902
GOT	**9**	1425	**9**	1042	**7.946**	1042	**8.313**	1042
PEP	**5**	664	**5**	446	**4.417**	446	**4.576**	446

C. stellatus

	ATLANTIC observed		MEDITERRANEAN observed		ATLANTIC expected		MEDITERRANEAN expected	
Locus	n° of alleles	n° genes analysed	n° of alleles	n° genes analysed	n° of alleles	n° genes analysed	n° of alleles	n° genes analysed
PGI	**7**	752	**8**	862	**6.075**	752	**7.936**	752
MPI	**6**	700	**7**	830	**5.448**	700	**6.444**	700
PGM	**4**	580	**5**	702	**3.864**	580	**4.371**	580
GOT	**6**	724	**11**	848	**5.130**	724	**10.009**	724

II 3.4 CONFORMITY TO HARDY-WEINBERG EXPECTATIONS

There was little evidence from any of the loci examined of departure from Hardy-Weinberg equilibrium. Of the 102 simultaneous exact tests of fit to Hardy-Weinberg expectations carried out on *C. montagui* only 3 were significant ($P < 0.05$), all showing a deficit of heterozygotes at the *MPI* locus. Fifty-nine tests were performed on *C. stellatus,* and also in this species three tests showed significant departure. Unlike *C. montagui*, *C. stellatus* had no consistent trend regarding which loci were out of Hardy-Weinberg equilibrium and the direction of the deviation. After the rejection level of 0.05 was corrected for the number of tests conducted (Hochberg, 1988), none of the loci in any of the samples of either species departed significantly from Hardy-Weinberg equilibrium.

Contingency Chi square tests for homogeneity of allele frequencies between samples within basins gave significant probability values at most loci (Table II 3.5), implying that heterogeneity existed within each basin. Nevertheless, if all samples were pooled together within each basin and exact tests for conformity to Hardy-Weinberg equilibrium carried out on the resulting populations, no significant departure from Hardy-Weinberg equilibrium was observed in *C. stellatus* (Table II 3.6). This may in part reflect the well-known weakness of the Chi square test in detecting departures from Hardy-Weinberg expectations in samples of the size I studied (Ward and Sing, 1970; Fairbairn and Roff, 1980; Valenzuela, 1985; Lessios, 1992). In contrast, *C. montagui* showed significant departure, after Hochberg correction, at the locus *PGM* of the Mediterranean population and at the locus *GOT* of the Atlantic one.

II 3.5 POPULATION STRUCTURE

Genetic structuring between samples was analysed by changes in the estimates of F_{IS} calculated for different levels of pooling of sites (Tables II 3.7-3.8, Fig II 3.4).

Table II 3.5: Contingency Chi square tests for homogeneity of allele frequencies between samples within basins. Significant values in bold.

Chthamalus montagui

Locus	ATLANTIC	MEDITERRANEAN
PGI	**P<0.001**	**P<0.001**
MPI	**P<0.001**	**P<0.001**
PGM	0.05<P<0.1	**P<0.001**
GOT	**0.01<P<0.05**	**P<0.001**
PEP	0.05<P<0.1	**P<0.001**

Chthamalus stellatus

Locus	ATLANTIC	MEDITERRANEAN
PGI	0.1<P<0.25	0.1<P<0.25
MPI	**P<0.001**	0.1<P<0.25
PGM	**P<0.001**	**0.001<P<0.005**
GOT	**0.025<P<0.05**	**P<0.001**

Table II 3.6: Probability values from exact tests for departure from Hardy-Weinberg equilibrium within pooled samples from the Atlantic and the Mediterranean basins. Significant values in bold.

C. montagui	*PGI*	*MPI*	*PGM*	*GOT*	*PEP*
Mediterranean	0.219	0.315	**0.003**	0.849	0.271
Atlantic	0.283	0.691	0.078	**0.003**	0.170

C. stellatus	*PGI*	*MPI*	*GOT*	*PGM*	*PEP*
Mediterranean	1.000	0.409	0.696	0.286	-
Atlantic	1.000	0.730	0.622	1.000	-

Table II 3.7: *C. montagui*: pooling strategy for calculation of F_{IS} (Mediterranean localities underlined)

Pooling	Populations																					
1st stage	BUR	IOM	ANG	PLY	CON	BIA	CAS	SIN	SAR	CBL	COT	ESS	GIB	GEN	LIV	CIV	SIR	MAL	ANC	VEN	TRI	SOZ
2nd stage	{BUR-IOM-ANG}			PLY	CON	BIA	CAS	SIN	SAR	CBL	COT	ESS	GIB	GEN	LIV	CIV	SIR	MAL	ANC	VEN	TRI	SOZ
3rd stage	{BUR-IOM-ANG-PLY}				CON	BIA	CAS	SIN	SAR	CBL	COT	ESS	GIB	GEN	LIV	CIV	SIR	MAL	ANC	VEN	TRI	SOZ
4th stage	{BUR-IOM-ANG-PLY-CON}					BIA	CAS	SIN	SAR	CBL	COT	ESS	GIB	GEN	LIV	CIV	SIR	MAL	ANC	VEN	TRI	SOZ
5th stage	{BUR-IOM-ANG-PLY-CON-BIA}						CAS	SIN	SAR	CBL	COT	ESS	GIB	GEN	LIV	CIV	SIR	MAL	ANC	VEN	TRI	SOZ
6th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR}									CBL	COT	ESS	GIB	GEN	LIV	CIV	SIR	MAL	ANC	VEN	TRI	SOZ
7th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR-CBL-COT}											*ESS*	*GIB*	*GEN*	*LIV*	*CIV*	*SIR*	MAL	ANC	VEN	TRI	SOZ
8th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR-CBL-COT-ESS}												GIB	GEN	LIV	CIV	SIR	MAL	ANC	VEN	TRI	SOZ
9th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR-CBL-COT-ESS-GIB}													GEN	LIV	CIV	SIR	MAL	ANC	VEN	TRI	SOZ
10th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR-CBL-COT-ESS-GIB-GEN-LIV}															CIV	SIR	MAL	ANC	VEN	TRI	SOZ
11th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR-CBL-COT-ESS-GIB-GEN-LIV-CIV}																SIR	MAL	ANC	VEN	TRI	SOZ
12th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR-CBL-COT-ESS-GIB-GEN-LIV-CIV-SIR}																	MAL	ANC	VEN	TRI	SOZ
13th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR-CBL-COT-ESS-GIB-GEN-LIV-CIV-SIR-MAL}																		ANC	VEN	TRI	SOZ
14th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR-CBL-COT-ESS-GIB-GEN-LIV-CIV-SIR-MAL-ANC}																			VEN	TRI	SOZ
15th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR-CBL-COT-ESS-GIB-GEN-LIV-CIV-SIR-MAL-ANC-VEN-TRI}																					SOZ
16th stage	{BUR-IOM-ANG-PLY-CON-BIA-CAS-SIN-SAR-CBL-COT-ESS-GIB-GEN-LIV-CIV-SIR-MAL-ANC-VEN-TRI-SOZ}																					

Key

ANC = Ancona, ANG = Anglesey, BIA = Biarritz, BUR = Burren, CAS = Cascais, CBL = Casablanca, CIV = Civitavecchia, CON = Concarneau, COT = La Cote, ESS = Essaouira, GEN = Genoa, GIB = Gibraltar, IOM = Isle of Man, LIV = Livorno, MAL = Malta, PLY = Plymouth, SAR = Cabo Sardao, SIN = Sines, SIR = Siracusa, SOZ = Sozopol, TRI = Trieste, VEN = Venice.

Table II 3.8: *C. stellatus*: pooling strategy for calculation of F_{IS} (Mediterranean localities underlined)

Pooling	Populations
1st stage	PLY CON BIA HOR SMA SMP MAD GIB GEN GOR CIV VUL SIR MAL ERD NAH
2nd stage	{PLY-CON} BIA HOR SMA SMP MAD GIB GEN GOR CIV VUL SIR MAL ERD NAH
3rd stage	{PLY-CON-BIA} HOR SMA SMP MAD GIB GEN GOR CIV VUL SIR MAL ERD NAH
4th stage	{PLY-CON-BIA-HOR-SMA-SMP} MAD GIB GEN GOR CIV VUL SIR MAL ERD NAH
5th stage	{PLY-CON-BIA-HOR-SMA-SMP-MAD} GIB GEN GOR CIV VUL SIR MAL ERD NAH
6th stage	{PLY-CON-BIA-HOR-SMA-SMP-MAD-GIB} GEN GOR CIV VUL SIR MAL ERD NAH
7th stage	{PLY-CON-BIA-HOR-SMA-SMP-MAD-GIB-GEN-GOR-CIV} VUL SIR MAL ERD NAH
8th stage	{PLY-CON-BIA-HOR-SMA-SMP-MAD-GIB-GEN-GOR-CIV-VUL-SIR} MAL ERD NAH
9th stage	{PLY-CON-BIA-HOR-SMA-SMP-MAD-GIB-GEN-GOR-CIV-VUL-SIR-MAL} ERD NAH
10th stage	{PLY-CON-BIA-HOR-SMA-SMP-MAD-GIB-GEN-GOR-CIV-VUL-SIR-MAL-ERD} NAH
11th stage	{PLY-CON-BIA-HOR-SMA-SMP-MAD-GIB-GEN-GOR-CIV-VUL-SIR-MAL-ERD-NAH}

Key

BIA = Biarritz, CIV = Civitavecchia, CON = Concarneau, ERD = Erdemli, GEN = Genoa, GIB = Gibraltar, GOR = Gorgona, HOR = Horta, MAD = Madeira, MAL = Malta, NAH = Nahariya, PLY = Plymouth, SIR = Siracusa, SMA = S. Maria airport, SMP = S. Maria port, VUL = Vulcano.

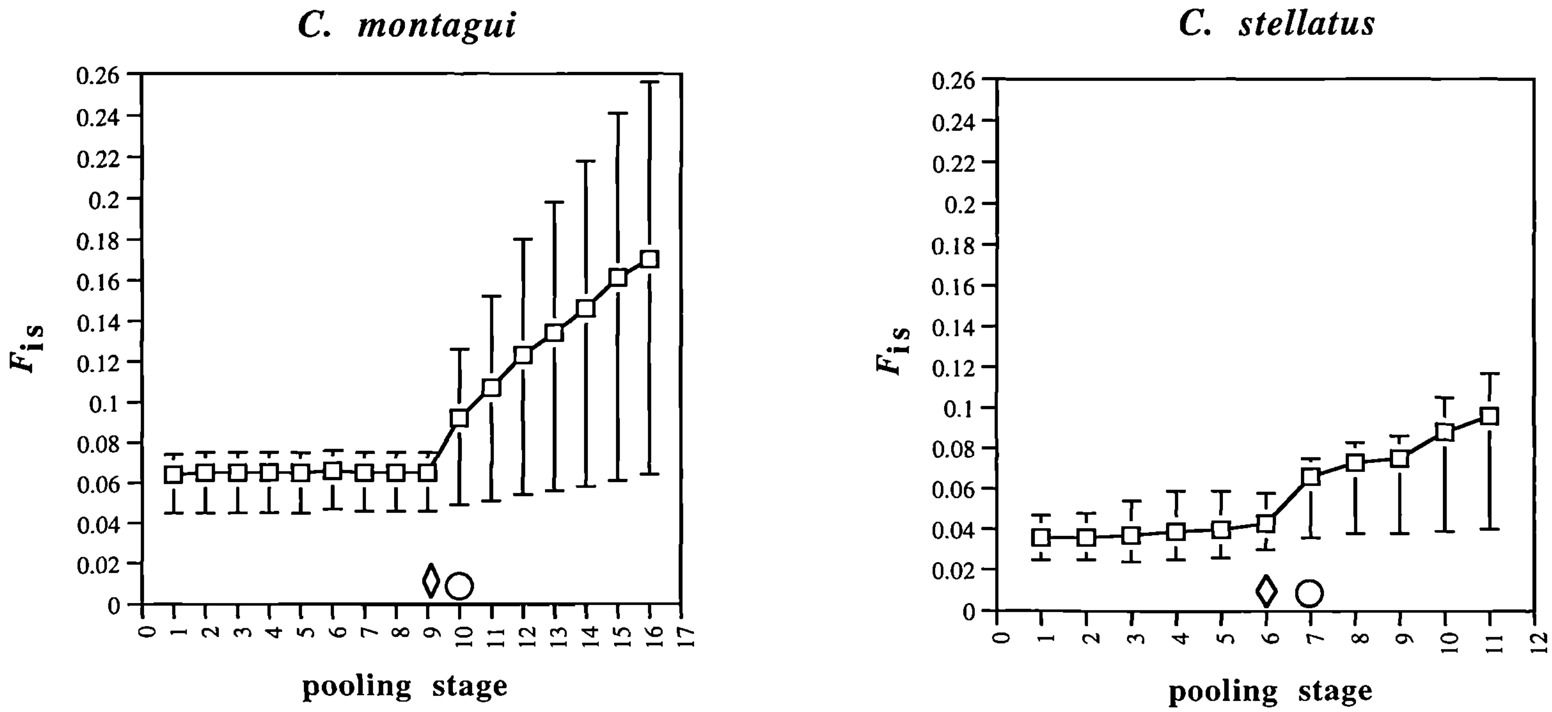


Fig II 3.4: Change of F_{is} with progressive pooling of samples (bootstrapped 95% confidence intervals shown).

Samples were pooled progressively from North to South within the Atlantic and then moving in the Mediterranean from west to east.
The diamond indicates the pooling stage at which the Gibraltar sample was added for each species.
The closed circle corresponds to the stage in which the first Mediterranean samples were added.
For more detailed explanations of the pooling strategy refer to tables II 3.7 and II 3.8.

In *C. montagui* the only discontinuity occurred between pooling stages 9 and 10. At this stage the first two Mediterranean samples were pooled together with the Atlantic ones, causing a sharp increase of the new estimate of F_{IS}. In the subsequent pooling stages the F_{IS} estimates increased monotonically as more Mediterranean samples were added. The bootstrapped 95% confidence intervals of the F_{IS} estimates were very large from pooling stage 10 onwards and overlapped among all pooling stages. These wide intervals resulted from the relatively high F_{IS} values at the *PGM* locus, compared with the other loci, when Mediterranean localities were present in the pooled sample.

C. stellatus samples showed less overall change of F_{IS} values during pooling than *C. montagui*. The first level of structuring could be seen, as in *C. montagui*, when the first Mediterranean samples were pooled with the Atlantic ones (pooling stage 7). Another slight discontinuity could be noticed at pooling stage 10, where the Eastern Mediterranean samples, from Turkey and Israel, were added to the others. Some of the confidence intervals still overlapped among pooling stages but, overall, the error bars were smaller in comparison to those of *C. montagui,* implying that the variation of F_{IS} was distributed more evenly over loci. In both species the confidence intervals were found to be asymmetric with respect to the mean. This is characteristic of the bootstrapping method when the estimators obtained from the resampling technique are not normally distributed.

II 3.6 GENETIC DISTANCE

Two main clusters emerged very clearly in the UPGMA dendrograms (Fig II 3.5), one formed by the Atlantic samples and the other by the Mediterranean ones. *C. montagui* showed a larger separation between its Atlantic and Mediterranean forms (genetic distance = 0.08) than *C. stellatus* (genetic distance = 0.03). The dendrogram for *C. stellatus* largely reflected the variation observed at the *GOT* locus and lost its topology if this locus was removed (not shown). In contrast the two main clusters

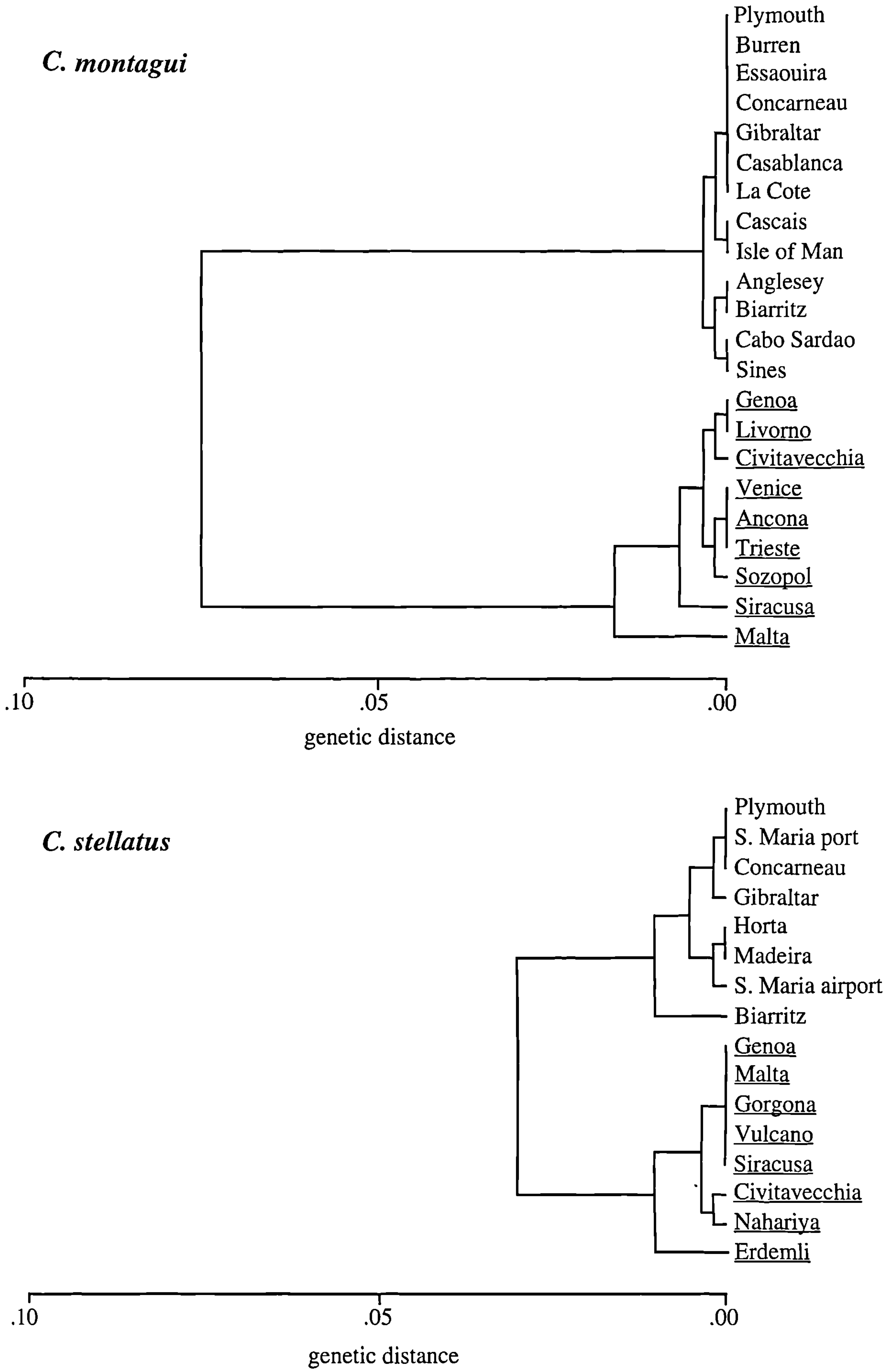


Fig II 3.5: UPGMA dendrogram (based on Nei's unbiased genetic distance) showing relationships among the Atlantic and the Mediterranean populations of the two species were maintained in *C. montagui,* albeit at smaller genetic distances, even after removal of the most variable locus in this species, *PGM* (not shown).

An interesting finding was that the Gibraltar samples in both species were part of the Atlantic cluster, suggesting that the switch from one form to the other was taking place somewhere in the Mediterranean. In the case of *C. stellatus* however, the frequency of the allele GOT^{100} in Gibraltar was found to be of the Mediterranean type (Fig II 3.2) suggesting that other factors, such as heterozygosity and number of alleles, were probably responsible for its belonging to the Atlantic cluster. Given the degree of geographical and hydrographical separation it was also interesting to find a high genetic identity between the Black Sea and the Mediterranean samples of *C. montagui.* In contrast, the Maltese *C. montagui* clustered slightly away from the neighbouring Mediterranean samples.

II 4. DISCUSSION

It must be noted that the relatively high mean per locus heterozygosity values observed in this study are likely to be considerable overestimates because monomorphic loci are underrepresented in my data set. Similarly, the calculated genetic distance values will be influenced by the paucity of monomorphic loci.

This study confirmed Dando and Southward's (1981) results for *Chthamalus montagui,* and demonstrated that *C. stellatus* also shows genetic differentiation between the Atlantic and Mediterranean. This latter finding resulted from the analysis of the *GOT* locus using the staining recipe of Jeremiah *et al.* (1982) which was not yet available at the time that Dando and Southward carried out their work on *Chthamalus.* In *C. stellatus* the differentiation between the two basins is attributable almost entirely to variation at the *GOT* locus. Although this is the most variable locus in *C. stellatus,* its degree of differentiation is smaller than that at the *PGM* locus of *C. montagui,* yielding the apparently greater separation of Atlantic and Mediterranean forms in *C. montagui* seen in the dendrograms (Fig II 3.5). It would be tempting to speculate that, given the suspected greater larval dispersal of *C. stellatus* in comparison to *C. montagui* (Burrows, 1988), the smaller genetic differentiation observed in *C. stellatus* results from greater gene flow. However, it must not be forgotten that the apparent differences are based on the respective levels of variation at a single locus in each species: *GOT* in *C. stellatus* and *PGM* in *C. montagui.* The analysis of F_{IS} values with progressively pooled samples (Fig II 3.4) in fact, indicated some genetic structuring in the Mediterranean *C. stellatus.* In *C. montagui* F_{IS} increases monotonically and shows a single marked point of inflection where the first Mediterranean samples are added. *C. stellatus* shows two points of inflection, where the Genoan and the Turkish samples are added.

Why does genetic differentiation exist between Atlantic and Mediterranean populations in both species? Clines may arise either from the partial mixing of historically differentiated populations, or from ongoing selective pressures. Starting with the first possibility, Dando and Southward (1981) have already hypothesised that the history of the Mediterranean, combined with the present hydrographic pattern, might have promoted and maintained the differentiation of the Mediterranean populations.

During the Messinian Salinity Crisis (5.5 My ago) at the end of the Miocene period, the Mediterranean receded and dried out completely (Hsü *et al.*, 1977) or was reduced to form a series of brackish water lagoons (Karistineos and Ioakim, 1989; McCullach and De-Deckker, 1989). The subsequent re-establishment, in the Pliocene (1.8-5 My ago), of a communication between Atlantic and Mediterranean, at the site of the present Strait of Gibraltar, restored normal marine conditions to the Mediterranean which persist to the present day.

Since the Pleistocene (0-1.8 My ago) and in particular during the Quaternary period, the history of the Mediterranean can be seen as a succession of glacial and interglacial periods with associated regressions and transgressions. The regressions, typical of the glacial periods, were characterised by decrease in air temperature, increase in precipitation and consequent positive water balance. The surface water current flowed out of the Mediterranean while the deep waters flowed in. During transgressions the situation was reversed: the warm climate caused evaporation and negative water balance so that the surface Atlantic waters penetrated the Mediterranean through the Strait of Gibraltar, compensating the water balance (Pérès, 1967; Blanc, 1968). It is possible that during one of the regressions the Mediterranean and the Atlantic populations of *Chthamalus* became physically separated. The isolated populations may have differentiated genetically and when later in geological time they came into contact again, the gradient of differentiation was maintained. It would be interesting to be able to date the time of separation of the two forms and relate this to geological events. Unfortunately, due to the small number of loci analysed in this study and to the fact that the available fossil records for these taxa are scarce, I was not able to calibrate the molecular clock and thus relate the genetic distance between the two forms to evolutionary time.

The present-day divergence between the two forms is probably still maintained by hydrographic barriers, in particular by the Almeria-Oran front. This zone of turbulence in the Alboran Sea (Tintore *et al.*, 1988) may restrict larval dispersal in both directions. The intertidal barnacle *Euraphia depressa* for instance, is practically restricted to the Mediterranean and Black Seas (Crisp *et al.*, 1981); only a few larvae of this barnacle manage to escape and settle just outside Gibraltar (Fischer-Piette, 1935; Kensler *et al.*, 1965). In my study I found that for both species the switch from one form to the other took place somewhere between Gibraltar and Italy. Dando and Southward (1981), who analysed various Spanish samples of *C. montagui,* showed that this changeover was in the area between Salobreña and Calpe on the Mediterranean coast of Spain. The results reported here support the theory of the Almeria-Oran front as the major barrier to gene flow between the Atlantic and Mediterranean forms of the two *Chthamalus* species. If the hydrographic barriers persist, genetic divergence may eventually lead to allopatric speciation.

Focusing on particular cases, barnacles of both species from Gibraltar show the same genetic pattern as those from the Atlantic. It is possible that, due to the barrier created by the Almeria-Oran front, the greatest part of the larval supply to Gibraltar originates from the Atlantic population.

Hydrographic barriers probably also affect Maltese *C. montagui.* Dando (1987), who also analysed samples from Malta, described these barnacles as intermediate between the "Atlantic" and "Mediterranean" forms. He wondered if this was due to an island effect or if the sample was representative of a third differentiated population from the southern shores of the western basin of the Mediterranean. In the present study the Maltese sample also appears to be separated from the Mediterranean cluster (Fig II 3.5); however, the degree of separation obtained is not great enough to consider it intermediate between the Atlantic and the Mediterranean forms. Along the African coast there is an inshore area between the west coast of Cyrenaica and the east coast of Tunisia, where salinities are of the western Mediterranean type (< 39‰), temperatures high and tides are quite pronounced (Sarà, 1985). Malta lies on the edge of this area. It could be that, because of the local current pattern, barnacles from this island cannot exchange larvae with the neighbouring Italian ones, leading to isolation and consequently to the observed differentiation of this sample.

In contrast to the Malta example, the Black Sea sample of *C. montagui* shows great genetic similarity to the Mediterranean population despite their geographical separation and the hydrographic barrier represented by the Bosporus. Various Mediterranean species, possibly including *C. montagui*, are thought to have moved into the Black Sea when communication to the Mediterranean was reopened during the Quaternary period (Ekman, 1953). At present, the predominant movement of water is from the Black Sea into the Mediterranean but, during the autumn season, surface waters can flow in the opposite direction, from the Mediterranean into the Black Sea (Pektas, 1958). Barnacle larvae are probably transported with these currents; the exchange of larvae between the two seas could lead to genetic similarity between Mediterranean and Black Sea samples.

It is also necessary to consider why, in both *Chthamalus* species, the Mediterranean population shows higher genetic variability, in terms of heterozygosity and allelic diversity, than the Atlantic one. The greater richness of the Mediterranean fauna with its many endemic species in comparison to the Atlantic one suggests that the Mediterranean might have acted as a primary centre of evolution and radiation of species (Briggs, 1974). If we accept this theory, the greater genetic variability of the Mediterranean population could be a consequence of its greater age in comparison to the Atlantic one.

The Mediterranean basin underwent a long series of profound changes in recent geological time during the late Tertiary and Quaternary, whilst the Atlantic probably did not experience them to the same degree. This could in itself be a reason why genetic diversity has accumulated in the Mediterranean to a greater extent. It could also be suggested that, given its complex geography and hydrography, the present-day Mediterranean basin is more subdivided than the Atlantic. This subdivision could also help the process of genetic differentiation.

An alternative explanation for the higher number of alleles characteristic of the Mediterranean population is suggested by hydrographic patterns. The unidirectional surface circulation which goes from the Atlantic to the Mediterranean through the Straits of Gibraltar might produce asymmetrical gene flow in this direction and promote differentiation (Endler, 1977). It could be suggested that alleles originating in the Mediterranean population are prevented from spreading to the Atlantic, while those arising in the Atlantic population spread readily into the Mediterranean Sea. This explanation has been put forward by Saavedra *et al.* (1993) for a similar pattern of allelic diversity in the oyster *Ostrea edulis*. For this species they found genetic differentiation among Atlantic and Mediterranean samples and also an increase in the mean number of alleles per locus when going towards the Eastern Mediterranean.

Besides the possible contribution of the history of the two basins and hydrographic barriers, evolutionary processes such as genetic drift and founder effect, and/or selection, must have been involved in producing the observed differentiation. A large and contentious literature discusses the relative importance of stochastic and deterministic processes. Gillespie (1986) suggested that drift and selection might have different relative importances in different times for a given group: neutral processes can predominate in some more stable periods, whilst selection can act more intensely in others.

Selection under different environmental regimes must be considered as a potential process which might have produced the genetic differentiation in *Chthamalus*. Table II 4.1 illustrates how greatly certain physical parameters vary within the range of distribution of the *Chthamalus* species. Data are from McLellan (1965) and Fairbridge (1966).

Despite the much lower salinity and the wider range of variation of the surface temperature of the Black Sea in comparison to the Mediterranean basin, close genetic

Table II 4.1: Variation of some abiotic factors over the distribution range of *Chthamalus* (from McLellan, 1965; Fairbridge 1966)

	NORTH-EAST ATLANTIC		GIBRALTAR	WEST MEDITERRANEAN		EAST MEDITERRANEAN	BLACK SEA
ABIOTIC FACTORS	NORTH	SOUTH		all the rest	ADRIATIC		
Surface temperature (February)	6°C	15°C	15°C	13°C	13°C	15°C	6°C
Surface temperature (August)	15°C	22°C	22°C	25°C	25°C	25°C	28°C
Salinity at the surface	34.0‰	36.0‰	37‰	38.8‰	38.0‰	>39.0‰	18.0‰
Tidal range (max)	≤ 10m	2.8m	0.9m	0.3m	1m	0.3m	0.1m

identity was found between the samples of *C. montagui* in these seas. In contrast, the same abiotic factors do not differ very much between the genetically separated Atlantic and Mediterranean populations. It may therefore be suggested that differences in salinity and water temperature are not primarily responsible for the genetic differentiation of the Mediterranean population.

Although other environmental factors, not listed in Table II 4.1, could be involved, I suggest that differences in the tidal range, which imply different time of exposure to the air, may play a very important role in generating the higher variability in the Mediterranean. On Mediterranean shores the vertical distribution of *Chthamalus* is up to 2-3 metres above mean sea level (Pérès, 1967; Crisp *et al.*, 1981; Pannacciulli, 1991). As the tidal range is normally only 30 cm (maximum 1m in the Adriatic) most of the barnacles rely on wave action and irregular changes in sea level in order to be immersed and carry out their physiological functions. As a consequence of this, Mediterranean barnacles spend more time out of the water, perhaps for a matter of months during the summer, under the influence of the air and its greater variation in temperature. In contrast, the Atlantic barnacles are regularly covered with water at high tide and are therefore less exposed to environmental variability.

Genetic variability could possibly be related to exposure to air and to its great variation in temperature. In this study higher levels of heterozygosity were observed in those barnacles (from the Mediterranean) which experienced a greater exposure to the air (Table II 3.2). Four out of the five loci analysed for *C. montagui*, which is the dominant species at high tide-levels (Southward, 1976), showed significantly higher levels of heterozygosity in the Mediterranean. Only two loci out of four showed significant results in the lower-shore species *C. stellatus*.

The cline in allele frequencies noticed at the *PGM* locus of *C. montagui* and at *GOT* in *C. stellatus* could also be an effect of natural selection enforced by variation in environmental parameters, like tidal range, along the geographical range of the species.

It is also possible to attempt an explanation of the higher values of heterozygosity found in the Mediterranean samples of both species based on environmental differences. Mean per-locus heterozygosity is a function of the proportion of loci that are polymorphic and, at polymorphic loci, the number of alleles present and their relative frequencies. Genetic variability is one of the strategies that may allow species to cope with the variety of habitats they meet both in space and in time (Dobzhansky *et al.*, 1977). The less variable the environment, the lower the polymorphism and consequently the heterozygosity tend to be. Levins' theory (Levins, 1968) of fitness regards heterozygosity as an adaptation to environmental heterogeneity and uncertainty. It seems reasonable that polymorphic organisms are more versatile and better adapted to a changeable environment. It could be argued that Mediterranean intertidal barnacles are exposed to a greater variation and uncertainty than the Atlantic ones.

Another hypothesis to explain the higher variability observed in the Mediterranean could be based on the different length of the breeding season in the two basins. Probably due to the combined effect of water temperature and adaptation to an environment where the time of immersion is relatively unpredictable, chthamalids in the Mediterranean breed almost all year around (Relini, 1983) while those in the Atlantic breed for only 2-3 months a year (Barnes, 1992; Burrows *et al.*, 1992; O'Riordan *et al.*, 1992) and settle over a similarly restricted period. It could be hypothesised that in the Mediterranean, as a consequence of year-round brooding, the time available for promotion of genetic differentiation in terms of allelic diversity is greater than in the Atlantic. It is also possible that Mediterranean barnacles, which breed and settle almost all year round, might be exposed to a more seasonally variable selective regime during the vulnerable larval and post-settlement stages, and show more genetic variation as adults as a consequence of this.

Nevertheless, any enthusiasm for selectionist explanations of the observed patterns must be tempered by the difficulty of making an entirely convincing case for any effect of selection on allozyme genotypes in barnacles (Dando, 1987) despite the previous investigation of populations along strong natural (Flowerdew and Crisp, 1976; Gooch, 1977; Dando *et al.*, 1979) and anthropogenic (Nevo *et al.*, 1977) environmental gradients.

We are thus still some way from understanding why the "Mediterranean" and "Atlantic" forms exist and why genetic differentiation in terms of heterozygosity and allelic diversity is greater in the Mediterranean. The analysis of more samples, in particular from the Eastern Mediterranean and from the North coast of Africa, would certainly help in completing the picture and possibly give a better understanding of the local structuring observed in the Mediterranean basin. Transplantation experiments between basins could also be carried out to test some of the hypotheses mentioned in the discussion. Laboratory experiments on the reproductive affinity of conspecific barnacles from the two basins could help to understand how far the genetic differentiation between the two "forms" has gone.

CONCLUDING REMARKS

The main aim of this study was to investigate in depth some aspects of the population ecology and genetics of European barnacles. These areas had already been the object of attention during the past few decades, but more detailed investigations were required for a better understanding of the biological processes involved. The new information gained by this study has provided answers to some of the questions previously formulated. It has also generated new questions and hypotheses which will, hopefully constitute the base for further work. While working on this project, I was struck by the amount of published work on certain aspects of barnacle ecology (e.g. settlement and recruitment) and by the scarcity of literature on other aspects of their biology (e.g. mating system, larval dispersal, determination of age and longevity). The interpretation of some of the results obtained in this study would have benefited greatly from background information on certain aspects of the barnacle biology which unfortunately were among those neglected previously. To follow are some ideas for future investigations which could broaden our knowledge of barnacles and possibly create the basis for a better understanding of some fundamental marine processes.

A study of the ecology of intertidal species of barnacles across the whole of their range of distribution, would certainly lead to very interesting results. This type of investigation, would provide an insight to the adaptive strategies adopted by different species in different environments.

The ecology of *S. balanoides,* for instance, could be compared along a north-south gradient along the coasts of the Atlantic Ocean (this study will be carried out within the project "Eurorock" financed by the EEC programme MAST III). It could be speculated that this species will encounter greater problems with desiccation and competition in the southern part of its range of distribution, while in the northern part the very low temperatures would represent the major hazard to its survival.

A comparison of the ecology of the two *Chthamalus* species across their range of distribution would also be very rewarding. *C. montagui* and *C. stellatus* are found on both Atlantic and Mediterranean shores. The population dynamics of these species from the Ligurian Sea (Relini, 1983; Relini and Orsi, 1985) and from the British coasts (Southward, 1991) have been monitored for several decades, but different techniques were employed. It would be very interesting to develop a uniform experimental protocol which can be applied to a range of European sites on both Atlantic and Mediterranean coasts, and carry out direct comparisons of the ecology of the two species in the two basins (another aim of the "Eurorock" project). Different environmental factors would certainly influence the ecological strategies adopted by each species. Atlantic barnacles will be less likely to suffer from desiccation as the air temperature and intensity of sunlight are generally lower than in the Mediterranean and above all the pronounced tidal cycle covers them regularly with water. Mediterranean barnacles in contrast will be more subject to desiccation stress because the wetting depends much more on wave action and other less regular changes in water level. The release from desiccation stress encountered on Atlantic shores will probably be counterbalanced by the greater competition and the higher risk of predation experienced by Atlantic barnacles. As already mentioned in the genetic section, differences in the type of environmental pressure applied to the two *Chthamalus* species in the two basins might also account for the existence of the observed intraspecific genetic differentiation. A similar link would be very difficult to determine, but transplantation experiments could be employed to investigate this possibility.

Further investigations on the larval dispersal of the two species of *Chthamalus* are a priority in the study of their biology. Burrows (1988) attempted this study by collecting plankton samples along a transect offshore from Plymouth. His results showed that larvae of *C. stellatus* could be found further offshore than those of *C. montagui*, implying that the former species is capable of a greater dispersal than the latter one. His field experiments were paralleled by laboratory experiments which also showed a longer period of larval development in *C. stellatus*. The problem with Burrows' field study though, was that he described the situation observed along a single transect; his results were therefore an insufficient basis for generalisation. A much broader investigation, involving sampling along several transects and in different years, is required to prove that there are differences between the two species and that *C. stellatus* really disperses further offshore than *C. montagui*. Confirmation of this hypothesis would provide the basis for a better understanding of some of the dissimilarities observed between the two species, e.g. the different geographical distribution, with *C. stellatus* a better coloniser of offshore islands, and the different level of intraspecific genetic differentiation with *C. montagui* apparently more diversified.

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APPENDICES

Appendix II 1: *C. montagui* Allele frequencies and sample sizes

Locus	Population																					
	BUR	IOM	ANG	PLY	CON	BIA	CAS	SIN	SAR	CBL	COT	ESS	GIB	GEN	LIV	CIV	SIR	MAL	ANC	VEN	TRI	SOZ
PGI																						
(N)	(52)	(60)	(48)	(72)	(54)	(53)	(54)	(48)	(59)	(54)	(65)	(58)	(60)	(57)	(60)	(58)	(54)	(53)	(60)	(89)	(59)	(51)
18	-	-	-	-	-	-	-	-	0.881	-	-	-	-	-	-	-	-	-	-	-	0.008	-
25	-	-	-	-	-	-	-	-	-	-	-	-	-	0.018	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.009	-	-	-	-	-
46	-	-	-	-	-	-	-	-	-	-	-	-	-	0.009	-	0.009	-	-	-	0.006	-	-
55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.010
62	-	-	-	-	-	-	0.009	-	-	-	-	-	-	-	-	0.009	-	-	-	-	-	-
68	-	-	-	-	-	-	-	-	-	-	-	-	-	0.009	0.025	0.034	0.065	0.009	0.058	-	0.034	0.039
73	-	0.008	0.021	-	-	-	0.009	0.021	0.008	-	0.008	0.009	-	0.009	-	0.017	-	-	0.025	0.039	0.008	0.020
83	-	-	0.021	-	-	-	-	-	-	-	-	-	-	0.000	-	-	-	-	-	0.017	-	0.010
100	0.875	0.858	0.760	0.840	0.870	0.858	0.880	0.885	0.881	0.852	0.877	0.819	0.917	0.816	0.808	0.707	0.769	0.708	0.783	0.775	0.797	0.814
115	-	-	0.115	-	0.009	0.094	-	-	0.008	0.037	-	0.017	0.008	0.026	-	-	-	-	-	0.028	0.017	0.010
122	0.106	0.133	0.083	0.153	0.093	0.028	0.083	0.073	0.051	0.102	0.085	0.121	0.067	0.061	0.067	0.138	0.037	0.047	0.050	0.112	0.119	0.039
127	0.019	-	-	-	0.028	-	0.019	0.021	0.051	-	0.023	0.026	0.008	0.018	0.067	0.086	0.083	0.189	0.042	0.017	-	0.020
131	-	-	-	0.007	-	-	-	-	-	-	-	-	-	0.035	0.033	-	0.028	0.047	0.033	-	-	0.029
139	-	-	-	-	-	-	-	-	-	-	-	0.009	-	-	-	-	0.009	-	-	0.006	0.017	-
145	-	-	-	-	-	0.019	-	-	-	0.009	0.008	-	-	-	-	-	-	-	0.008	-	-	-
154	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.010
MPI																						
(N)	(37)	(60)	(48)	(54)	(53)	(52)	(46)	(48)	(59)	(52)	(43)	(46)	(48)	(51)	(56)	(45)	(48)	(49)	(58)	(71)	(55)	(54)
66	-	-	-	-	0.009	-	-	-	-	-	-	-	-	-	-	-	0.010	-	-	-	-	-
71	-	-	-	-	0.009	-	-	-	-	-	-	-	0.010	0.010	-	-	-	-	-	0.007	0.018	0.019
76	-	-	0.021	-	0.019	-	0.011	-	-	-	-	-	0.021	-	-	-	-	-	-	0.014	-	-
87	0.054	0.075	0.052	0.056	0.047	0.048	0.054	0.063	0.034	0.029	0.035	0.065	0.052	0.029	0.018	0.078	0.031	0.092	0.026	0.042	0.109	0.065
92	-	-	0.010	0.009	-	-	-	-	-	0.010	-	-	-	0.029	-	-	-	-	-	-	-	-
100	0.919	0.892	0.896	0.907	0.868	0.913	0.848	0.885	0.924	0.904	0.919	0.859	0.833	0.833	0.902	0.867	0.854	0.857	0.879	0.838	0.755	0.759
104	-	-	-	-	-	-	-	-	-	0.038	-	-	-	-	-	-	-	-	-	-	0.009	-
111	0.014	0.008	0.010	0.028	0.019	0.029	0.076	0.031	0.025	0.010	0.047	0.076	0.063	0.098	0.063	0.044	0.094	0.051	0.086	0.092	0.064	0.074
114	-	0.025	0.010	-	0.028	0.010	0.011	0.021	0.017	0.010	-	-	0.021	-	0.009	0.011	0.010	-	-	-	-	0.083
117	0.014	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.009	-	0.036	-
125	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.009	-	-	-	-	0.007	0.009	-
GOT																						
(N)	(50)	(60)	(48)	(71)	(52)	(49)	(52)	(48)	(58)	(53)	(58)	(57)	(60)	(56)	(59)	(58)	(53)	(52)	(59)	(72)	(58)	(54)
-10	-	-	-	-	-	-	-	-	-	-	-	0.009	-	-	-	-	-	-	-	-	-	-
33	-	0.008	-	0.007	-	-	-	0.021	-	-	-	-	-	-	-	0.009	-	-	-	-	0.009	-
50	0.010	-	0.021	0.021	0.010	-	0.019	0.010	-	0.009	0.009	0.018	-	0.018	0.059	0.043	-	0.010	0.008	0.014	0.009	-
90	-	0.008	-	0.007	-	-	0.019	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	0.980	0.958	0.938	0.965	0.971	1.000	0.942	0.969	0.991	0.962	0.974	0.965	0.992	0.911	0.856	0.862	0.849	0.856	0.831	0.889	0.819	0.963
120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.008	-	0.017	-
140	-	0.017	0.042	-	-	-	-	-	-	0.009	-	-	0.008	0.009	0.008	0.026	-	-	0.008	0.035	0.017	-
147	0.010	-	-	-	0.010	-	-	-	-	0.019	0.009	0.009	-	0.036	0.034	0.034	0.009	0.077	0.068	0.049	0.043	-
158	-	0.008	-	-	0.010	-	0.019	-	-	-	0.009	-	-	0.027	0.034	0.017	0.142	0.058	0.068	0.014	0.086	0.037
170	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.008	0.009	-	-	-	-	-	-
196	-	-	-	-	-	-	-	-	0.009	-	-	-	-	-	-	-	-	-	-	-	-	-
220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.008	-	-	-

Appendix II 1 (continued): *C. montagui* Allele frequencies and sample sizes

Locus	Population																					
	BUR	IOM	ANG	PLY	CON	BIA	CAS	SIN	SAR	CBL	COT	ESS	GIB	GEN	LIV	CIV	SIR	MAL	ANC	VEN	TRI	SOZ
PGM																						
(N)	(51)	(40)	(46)	(60)	(43)	(47)	(47)	(46)	(58)	(45)	(42)	(35)	(60)	(48)	(51)	(57)	(43)	(53)	(52)	(55)	(35)	(54)
68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.009	-	-
73	-	-	-	-	-	-	-	-	-	-	-	0.014	-	0.094	0.098	0.044	0.023	-	0.058	0.055	0.029	0.037
80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.019
83	-	0.013	0.022	0.017	0.023	0.011	0.011	-	0.009	-	0.024	0.014	0.017	0.479	0.392	0.535	0.663	0.302	0.548	0.500	0.514	0.509
88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.012	0.094	-	0.018	0.014	0.019
91	0.235	0.275	0.152	0.275	0.267	0.202	0.223	0.207	0.241	0.144	0.167	0.186	0.200	0.250	0.255	0.219	0.244	0.472	0.308	0.327	0.271	0.333
94	0.020	-	-	-	0.035	-	-	-	0.017	0.033	-	0.029	0.017	-	-	-	-	-	-	-	-	-
100	0.706	0.650	0.815	0.683	0.674	0.766	0.755	0.793	0.733	0.800	0.810	0.729	0.725	0.177	0.235	0.193	0.047	0.132	0.077	0.091	0.157	0.074
107	0.039	0.063	0.011	0.025	-	0.021	0.011	-	-	0.011	-	0.029	0.033	-	0.020	0.009	0.012	-	0.010	-	0.014	0.009
116	-	-	-	-	-	-	-	-	-	0.011	-	-	0.008	-	-	-	-	-	-	-	-	-
PFP																						
(N)	(45)	(38)	(14)	(44)	(22)	(12)	(26)	(0)	(5)	(30)	(22)	(22)	(53)	(56)	(6)	(49)	(13)	(40)	(0)	(32)	(20)	(18)
52	-	-	-	-	-	-	-	-	-	-	-	-	-	0.027	-	0.020	-	-	-	-	-	-
73	-	-	-	-	-	-	0.058	-	-	-	-	-	0.009	0.027	-	0.061	-	-	-	-	-	0.028
86	0.122	0.197	0.036	0.136	0.068	0.042	0.192	-	-	0.100	0.114	0.114	0.104	0.018	0.083	0.020	-	-	-	-	-	-
100	0.878	0.803	0.964	0.864	0.886	0.958	0.712	-	1.000	0.850	0.886	0.886	0.868	0.929	0.833	0.898	1.000	1.000	-	1.000	1.000	0.972
114	-	-	-	-	0.045	-	0.038	-	-	0.050	-	-	0.009	-	0.083	-	-	-	-	-	-	-
126	-	-	-	-	-	-	-	-	-	-	-	-	0.009	-	-	-	-	-	-	-	-	-
SOD																						
(N)	(19)	(30)	(33)	(18)	(24)	(28)	(12)	(29)	(23)	(12)	(4)	(10)	(50)	(33)	(26)	(51)	(18)	(54)	(25)	(25)	(30)	(27)
100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Appendix II 2: *C. stellatus* Allele frequencies and sample sizes

Locus	Population															
	PLY	CON	BIA	HOR	SMA	SMP	MAD	GIB	GEN	GOR	CIV	VUL	SIR	MAL	ERD	NAH
PGI																
(N)	(42)	(16)	(30)	(60)	(62)	(54)	(59)	(53)	(54)	(70)	(61)	(54)	(33)	(54)	(51)	(54)
69	-	-	-	-	-	-	-	-	-	0.007	0.008	-	-	-	-	0.028
81	0.012	-	-	0.008	0.008	0.009	-	-	0.009	-	-	0.009	-	0.019	0.020	-
85	-	-	-	0.025	-	-	-	0.009	0.028	0.007	0.008	0.019	-	0.009	-	0.009
89	-	-	-	0.008	-	-	-	-	0.028	0.007	-	-	-	0.009	-	-
100	0.988	1.000	1.000	0.933	0.976	0.991	0.992	0.981	0.926	0.964	0.959	0.926	0.985	0.944	0.931	0.963
106	-	-	-	-	-	-	-	0.009	-	0.007	-	0.019	-	0.009	-	-
112	-	-	-	0.025	-	-	0.008	-	-	-	0.016	0.019	0.015	0.009	0.020	-
117	-	-	-	-	0.016	-	-	-	0.009	0.007	0.008	0.009	-	-	0.029	-
MPI																
(N)	(48)	(16)	(20)	(56)	(55)	(51)	(51)	(53)	(52)	(70)	(58)	(53)	(33)	(46)	(51)	(52)
67	-	-	-	-	0.009	-	-	0.009	-	-	-	-	-	-	-	-
73	-	-	-	0.027	-	-	-	-	-	0.007	-	-	0.015	-	-	-
80	-	0.031	-	-	-	-	-	-	-	-	0.017	-	-	0.011	0.010	0.019
86	0.042	0.031	0.050	0.036	-	0.049	0.029	0.028	0.077	0.071	0.069	0.104	0.045	0.054	0.108	0.058
100	0.948	0.844	0.725	0.875	0.982	0.931	0.931	0.915	0.846	0.879	0.784	0.840	0.924	0.891	0.863	0.894
106	-	-	-	-	-	-	-	-	-	0.007	0.009	-	-	-	-	-
113	0.010	0.094	0.225	0.063	0.009	0.020	0.039	0.047	0.077	0.036	0.121	0.047	0.015	0.033	0.020	0.029
124	-	-	-	-	-	-	-	-	-	-	-	0.009	-	0.011	-	-
PGM																
(N)	(45)	(15)	(9)	(55)	(42)	(49)	(22)	(53)	(53)	(46)	(26)	(54)	(32)	(35)	(51)	(54)
83	0.022	-	-	-	-	-	-	-	0.019	-	-	-	-	-	-	-
91	0.067	-	-	-	-	-	-	0.009	0.019	-	0.019	0.009	0.016	0.014	0.010	0.046
100	0.911	0.967	1.000	0.991	0.917	1.000	0.977	0.981	0.962	1.000	0.962	0.889	0.922	0.986	0.941	0.917
110	-	0.033	-	0.009	0.083	-	0.023	0.009	-	-	0.019	0.102	0.063	-	0.039	0.037
116	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.010	-
GOT																
(N)	(44)	(16)	(29)	(56)	(58)	(54)	(58)	(47)	(54)	(70)	(61)	(54)	(33)	(48)	(51)	(54)
-9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.009
21	-	-	-	-	0.009	0.009	-	-	-	-	0.008	-	-	-	-	0.019
30	-	-	-	-	-	-	-	-	0.009	0.014	-	-	0.030	0.021	-	-
39	-	-	-	-	-	-	-	0.011	0.028	0.007	0.033	0.009	0.030	0.042	0.020	0.046
49	-	-	-	-	-	-	-	-	0.019	0.007	0.008	0.019	0.045	-	0.118	-
60	0.318	0.250	0.293	0.250	0.250	0.296	0.164	0.394	0.481	0.507	0.566	0.546	0.470	0.500	0.627	0.593
82	-	-	-	-	-	-	0.009	-	-	-	0.025	-	-	0.010	-	0.009
90	-	-	-	-	-	-	-	-	-	0.007	-	0.009	-	-	0.020	-
100	0.648	0.656	0.655	0.750	0.741	0.676	0.793	0.543	0.454	0.457	0.352	0.417	0.409	0.417	0.206	0.306
127	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.009
135	0.034	0.094	0.052	-	-	0.019	0.034	0.053	0.009	-	0.008	-	0.015	0.010	0.010	0.009