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The ecology of the mussel Mytilus edulis chilensis from three sites in the Falkland Islands.

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The ecology of the mussel *Mytilus edulis chilensis* from three sites in the Falkland Islands

by

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Summary

An ecological study of populations of *Mytilus edulis chilensis* from three study sites in the Falkland Islands.

Mytilus edulis chilensis is dioecious with no recorded incidence of hermaphroditism and with approximately equal numbers of males and females. Reproductive development is initiated during spring (August - September) whilst a single major spawning period occurs during the southern summer (December - March). The timing of peak reproductive condition broadly coincides with mean maximum summer temperatures (12 - 14°C). Whilst settlement of *M.e.chilensis* spat (190 - 2000 µm) onto artificial filamentous substrate units occurs at low levels throughout the year with a single peak during the austral summer, relatively low numbers (> 2 mm) settle into the established populations in a sporadic fashion, often bearing little relation to the timing of the reproductive cycle. Reproductive output of the three study populations ranged between 0.439 x 10^9 eggs.m^{-2}, at Camilla Creek, and 1.771 x 10^9 eggs.m^{-2}, at Darwin.

The deposition of microgrowth bands appears to be under the control of the spring - neap lunar cycle. The age and growth rate of individual mussels as well as the age composition of the study populations were determined from winter growth checks identified within the prismatic shell layer. Size and growth rate increase with decreasing tidal elevation, whilst longevity exhibits no such relationship with tidal level. L_∞ and k values range between 36.3 and 91.4 mm and 0.198 and 0.702, respectively. The populations from Darwin and Goose Green consisted mainly of older mussels (3 - 8 years old), whilst those from Camilla Creek were generally younger (1 - 2 years old). Allometric growth of *M.e.chilensis* appears to be controlled principally by age and food supply.

A hitherto undescribed association between the valviferan isopod, *Edotia doellojuradoi*, and *M.e.chilensis* is reported. The relationship between a green alga, *Coccomyxa parasitica* (Chlorococcales, Coccomyxaceae) and its host *M.e.chilensis* is also documented.

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Contents

List of Plates

layer of a *Mytilus edulis chilensis*, which was file-marked and emersed for 24 hours (large arrow) and grown at 0.82 m above chart datum at Camilla Creek for 56 days. P, periostracum, PL, prismatic layer, S, spring period; N, neap period; the large arrow indicates the 24 hour emersion band, whereas the smaller arrows indicate the position of this band within the shell section; the medium-sized arrow indicates a prominent band deposited at low tide during a period of anomalously high air temperature, whilst small arrows highlight this band within the shell section. Scale bar = 100 µm. B. Schematic diagram of the photomicrograph in A above to highlight the 24 hour emersion band, the proximity of the growth banding during spring and neap tides and the clearly defined band deposited during emersion when an anomalously high air temperature occurred. C. Predicted tidal cycle during the experimental period; solid line marks position the of the mid shore experimental cage, c.d. = + 0.82 m. D. Continuous seawater and air temperature records logged by TinyTalk temperature logger (Orion Ltd). Asterix highlights the anomalously high temperature (20 th January 1996) which occurred when the mussel was emersed for several hours during a spring low water. On other occasions the mussels were not emersed when there were unusually elevated air temperatures.

4.3 Photographs of the shell surface of *Mytilus edulis chilensis;* A. with clear surface checks, from the low shore population at Goose Green; B. with severe abrasion, from the mid shore population at Goose Green; C. with blisters on the shell surface due to the infestation by shell boring algae. Scale bar = 10 mm.

4.4 Photographs of the shell surface of *Mytilus edulis chilensis;* A. with an intact periostracum, but no clear surface checks, from the low shore population at Darwin; B. with a poor record of surface checks in a relatively old, slow growing individual from the high shore population at Goose Green. Scale bar = 10 mm.

4.5 A. Photograph of the shell surface of a high shore *Mytilus edulis chilensis* from Darwin, which was grown for one year in a subtidal cage suspended from the bridge at Darwin. The arrow denotes the time of marking and transplantation. Scale bar = 10 mm. B. Growth of the natural population of mussels from the high shore at Darwin (closed circles), together with the mean (± 1 standard error) length of mussels subsequently transplanted to subtidal cages at Darwin (open circles), FIPASS in Stanley (open triangles) and the 'Vicar of Brae' at Goose Green (open squares).

5.1 *Edotia doellojuradoi*, dorsal view (A, B, C) and a ventral view (D, E, F) of male, female and juvenile isopods, respectively; P position of penis, O indicates one of three pairs of marsupial oostigites on the second - fourth thoracic segments. Scale bar = 1 mm

5.2 *Edotia tuberculata*, dorsal view (A, B, C) and a ventral view (D, E, F) of male, female and juvenile isopods, respectively; P position of penis, O indicates one of three pairs of marsupial oostigites on the second - fourth thoracic segments. Scale bar = 2 mm

6.1 A. A photomicrograph of a section of *Mytilus edulis chilensis* mantle tissue showing the presence of a 'colony' of *Coccomyxa parasitica* within the connective tissue. Scale bar = 100 µm. B. and C. Transmission electron microscope images of *C.parasitica* cells within *M.e.chilensis* connective tissue. c, chloroplast; m, mitochondrial profiles; s, starch grain; v, electron dense vesicle; rer, rough endoplasmic reticulum; n, nucleus. Scale bar = 500 nm.

6.2 A. Transmission electron microscope images of a dividing *Coccomyxa parasitica* cell. The large double arrows highlight the animal cell membrane; the single large arrow indicates the algal cell parental membrane; small double arrows show the algal daughter cell membrane. B. and C. low power transmission electron microscope images of mussel tissue with leukocytes containing algal cells. mv, multi-vesiculate body; ml, multi-lamellar body; l, leukocyte. Scale bar = 500 nm.

List of Tables

List of Figures

Chapter 1

General Introduction

The genus *Mytilus* Linné 1758 belongs to the family Mytilidae which dates back to the Devonian era some 400 million years ago (Soot-Ryen, 1969). The family Mytilidae includes many important byssally attached genera such as *Choromytilus, Perna, Modiolus* and *Aulacomya*, as well as *Mytilus* itself. *Mytilus* is of relatively recent origin, just 2 million years old, with no records older than the Pliocene (Soot-Ryen, 1955). Numerous reviews of the mytilids following the Systema Naturae (Linneaus, 1758 in Gosling, 1992a) have been compiled including those of Dodge (1952) and Soot-Ryen (1955, 1969). *Mytilus* is differentiated from other genera in the family, for example *Perna* and *Choromytilus*, by the presence of a pitted resilial ridge, several hinge teeth, the presence of an anterior adductor muscle and a more or less continuous posterior byssus and foot retractor muscle scar.

Several comprehensive reviews have recognised the following as either distinct species or subspecies of *Mytilus. Mytilus edulis* Linnaeus, 1758 from northern temperate latitudes, *Mytilus galloprovincialis* Lamarck, 1819 from the Mediterranean Sea, *Mytilus trossulus* Gould, 1850 from the Pacific coast of North America, *Mytilus chilensis* Hupe, 1854 from Chile, *Mytilus platensis* Orbigny, 1846 from Argentina, *Mytilus planulatus* Lamarck, 1819 from Australia, *Mytilus desolationis* Lamy, 1936 from the Kerguelen Islands, *Mytilus coruscus* Gould, 1861 from Japan and China, *Mytilus californianus* Conrad, 1837 from the Pacific coast of North America, *Mytilus aoteanus* from New Zealand and *Mytilus edulis kussakini* and *Mytilus edulis zhirmunskii*, from the Pacific coast of Asia (Lamy, 1936; Soot-Ryen, 1955; Fleming, 1959; Scarlato & Starobogatov, 1979). However, until recently, *Mytilus* systematics have been based solely on morphological shell characteristics, which are known to be highly variable, being influenced by several factors including age, density of mussels, tidal level and habitat (Seed, 1968; Kautsky *et al.*, 1990; Stirling & Okumus, 1994). Biochemical techniques, such as protein electrophoresis, DNA-DNA hybridisation, mitochondrial DNA analysis, immunology and amino acid sequencing have recently proved invaluable in quantifying genetic differences between species (Ferguson, 1980). McDonald *et al.* (1991) have used such genetic differences in combination with

statistical techniques applied to both enzyme and morphological phenotypes to clarify the systematic status of some of the species within the genus *Mytilus*. Electrophoretic evidence from eight loci indicated that the Northern Hemisphere samples consisted of three electrophoretically distinguishable species, *Mytilus edulis* from eastern North America and western Europe, *Mytilus galloprovincialis* Lamarck, 1819 from the Mediterranean Sea, western Europe, California and eastern Asia; and *Mytilus trossulus* Gould, 1850 from the Baltic Sea, eastern Canada, western North America and the Pacific coast of Siberia. Mussels from Chile, Argentina, the Falkland Islands and the Kerguelen Islands contained alleles characteristic of all three Northern Hemisphere species, but because they were most similar to *M.edulis* from the Northern Hemisphere, they were included in *M.edulis*. Mussels from Australia and New Zealand were found to have similar allele frequencies and morphometric characters to *M.galloprovincialis* from the Northern Hemisphere. Other *Mytilus* taxa, *M.californianus* and *M.coruscus* are distinguished from the other taxa by the presence of radiating ribs on the shell. Although previously thought to be separate species, Vermeij (1989) has recently suggested that *M.californianus* and *M.coruscus* may in fact be a single species. Electrophoretic analyses of *M.coruscus* will resolve this taxonomic anomaly. Although *M.desolationis* was included with *M.edulis* by McDonald *et al.* (1991), the fact that the genetic differences between the two are in the same order as those between *M.edulis* and *M.galloprovincialis* (Gosling, 1992b) suggests that *M.edulis* and *M.desolationis* are perhaps different species. Electrophoretic analysis has therefore served to reduce the original number of taxa in the genus from 12 to about five or six, *M.edulis, M.galloprovincialis, M.trossulus, M.desolationis, M.californianus* and possibly *M.coruscus*.

Mussels belonging to the genus *Mytilus* are widely distributed throughout the cooler waters of both the northern and southern hemispheres (Soot-Ryen, 1955). The range of *M.edulis* has previously been cited as extending all over the Atlantic littoral of Europe, as far south as the North African coasts, but not into the Mediterranean, and from the Arctic southwards to California and Japan on the Pacific coasts and North Carolina on the Atlantic coast (Seed, 1976; 1978; Suchanek, 1985 and references therein). However, it is now known that in the northern hemisphere this mussel occurs in European waters extending from the White Sea, U.S.S.R. (McDonald *et al.*, 1990) as far south as the Atlantic coast of southern France (Seed, 1972; 1978; McDonald *et al,* 1991); in North America, from the Canadian Maritimes southwards to Cape Haltteras in north Carolina (McDonald & Koehn, 1988; McDonald *et al.*, 1990) and in

Iceland (Varvio *et al.*, 1988). Sanjuan *et al.* (1990) have recently shown that mussels in North-west Spain and probably the entire Iberian peninsula are *M.galloprovincialis* and not *M.edulis,* whilst McDonald and Koehn (1988) and McDonald *et al.* (1990) have shown that *M.edulis* is absent from both Pacific coasts and has a more restricted distribution on the east coast of North America. In the southern hemisphere mussels from South America, the Falkland Islands and the Kerguelen Islands have all been classed as *M.edulis* (McDonald *et al.*, 1991). *Mytilus trossulus* (oldest lineage) is thought to have originated in the northern Pacific and subsequently spread to the northern Atlantic after the Bering Strait opened during the upper Pliocene period. Following this trans-Arctic migration, *M.trossulus* may then have given rise to *M.edulis* which subsequently dispersed along coasts of Europe and North America (Seed, 1994). The presence of *M.trossulus* in parts of eastern Canada and in the Baltic Sea is consistent with this biogeographical interpretation. *Mytilus galloprovincialis*, however, appears to be a more recently derived mussel, having evolved from *M.edulis* in the warmer, semi-enclosed waters of the Mediterranean, as well as the coasts of California and eastern Asia (Seed, 1994).

Species of *Mytilus* have solid shells which are generally wedge-shaped, elongate and equivalve, with inequilateral, terminal, anterior beaks. *Mytilus* is a typical heteromyarian bivalve with extreme expansion of the posterior margin, which possibly serves to elevate the posterior current flow (Yonge & Campbell, 1968), and reduction in the anterior region of the shell (Morton, 1992). As a result, the posterior adductor muscle is large relative to the anterior adductor and the byssal retractor muscles are similarly different, with the posterior byssal retractor being divided into subunits in *M.edulis*. The evolution of this heteromyarian form, coupled with the neotenic retention of the primitive larval attachment organ the byssus, have enabled mytilid mussels to successfully exploit hard or semi-consolidated substrata and to dominate many rocky shore habitats on all continents of the world (Yonge, 1976; Morton, 1992).

Mytilus edulis is mytiliform being triangular in form and possessing a posterior inhalant stream rather than an anterior inhalant stream as is typified by its less acutely triangular modiolised relatives, such as *Modiolus*. Ventral flattening in *Mytilus* results in a stable shell that is widest basally with a low centre of gravity and a broad base for fixation. However, shell form in *M.edulis* is known to be highly variable according to habitat and age (Seed, 1968; Kautsky *et al.*, 1990; Stirling & Okumus, 1994). A posterior pair of pedal retractor muscles is present, the anterior pair having been lost

at the expense of the heteromyarian form. There is a large posteriorly elongate ligament which is comprised of two layers, the outer ligament and the posterior inner ligament layer, both of which are covered by periostracum (Yonge & Campbell, 1968). The anterior hinge plate has several denticulate hinge teeth and the pallial line is entire with no pallial sinus.

The shell of *M.edulis* consists of three layers: 1) a thin outer periostracum, consisting of the quinone tanned protein, conchiolin; 2) a middle prismatic layer, made up of simple prisms of calcite, each unit of calcium carbonate being separated by a wall of conchiolin; and 3) the inner nacreous layer, the microstructure of which consists of layers of aragonitic tablets separated by an interlamellar organic matrix. Individual tablets consist of even smaller blocks each surrounded by intracrystalline organic material (Taylor, 1969). The periostracum, which is usually worn away at the umbones, is often blue in colour, giving rise to the common name 'blue mussel', although colour can range from brown to blue-black (Seed, 1976). The periostracum and the prismatic layers are secreted by the mantle epithelium around the growing margin of the shell whilst the nacreous layer is deposited by the general outer surface of the mantle and thus efficiently thickens and strengthens the shell (Seed & Richardson, 1990).

Mytilus is the most diverse and widely distributed genus within the Mytilidae and its representatives are often dominant space occupiers of many intertidal habitats on most major continents. The ability of *M.edulis* to withstand wide fluctuations in environmental variables such as salinity, desiccation, temperature and oxygen tension, serves to widen its distribution to the extent that it is the most widely distributed species of the genus. *Mytilus edulis* therefore occupies a broad variety of microhabitats ranging from estuaries to fully oceanic sites, and mild subtropical locations to ice-scoured and frequently frozen habitats (Seed & Suchanek, 1992). The zonational range of *M.edulis* is from the high intertidal to the subtidal, where physical factors such as temperature and desiccation control the upper distributional limit (see Suchanek, 1985 for review) and biological factors such as predation and competition, and to a lesser extent, physical factors such as burial by fine sediment, control the lower limit (Paine, 1974; Daly & Mathieson, 1977; Suchanek, 1978;1981).

Mytilus edulis is dioecious with approximately equal numbers of males and females (Seed, 1976; Kautsky, 1982a; Sprung, 1983). The colour of the reproductive tissue

varies considerably, typically females are orange/apricot whilst males are creamy white (Chipperfield, 1953). Gametes are generated primarily within the extensive mantle folds, although small amounts of reproductive tissue also extend into the visceral mass and mesosoma posterior to the foot. Paired gonoducts lead into five major canals with convoluted walls forming longitudinal ciliated ridges. These in turn lead into a series of smaller canals where part of the walls consist of a ciliated columnar epithelium. Each of these fine ducts eventually terminates in a genital follicle. Early oocytes and spermatogonia are budded off from the germinal epithelium of these follicles. Early oocytes are connected to the epithelium by a broad stalk which gradually becomes more slender and finally ruptures to leave the mature ova free within the follicular cavity. Spermatogonia give rise, in turn, to concentric bands of spermatocytes, spermatids and spermatozoa, the latter converging towards the centre of the follicles in the form of dense lamellae. Mature gametes are discharged into the mantle cavity on papillae situated between the mesosoma and the inner gill lamellae (Seed, 1969a; Wilson & Seed, 1974; Seed & Suchanek, 1992).

Once released into the open water, eggs and sperm meet and fertilisation takes place. Fertilised eggs usually range from 60 - 90 µm in diameter and the initial cleavage division usually takes place within one hour of fertilisation (Lutz *et al.*, 1991). Subsequent cleavage yields an embryo that begins to swim when cilia first appear at 4 - 5 hours and a ciliated trochophore stage is reached approximately 24 - 48 hours after fertilisation. A shell gland secretes the first larval shell, the prodissoconch I (Bayne, 1976) which is D-shaped with a length of 100 to 120 µm (Jablonski & Lutz, 1980). A second larval shell, the prodissoconch II, is subsequently secreted by the mantle, and has concentric growth lines (Millar, 1968). This planktotrophic stage, commonly known as the 'veliger' due to the presence of the ciliated swimming organ the 'velum', lasts for several weeks and is characterised by rapid larval growth from approximately 120 µm to 250 µm in shell length (Bayne, 1976), as well as the formation of an umbo when 140 - 150 µm. The pedal organ or 'foot' is developed at ≈ 200 µm and is well defined in mature larvae at 210 - 300 µm. These 'pediveligers' are now capable of metamorphosis (Carriker, 1961). However, if a suitable settlement substratum is not located the pediveliger can delay metamorphosis for several weeks (40 days at 10°C for *M.edulis*, Bayne, 1965). On locating a suitable settlement substrate, crawling behaviour followed by the secretion of byssal threads, occurs (Lutz & Kennish, 1992 and references therein). Secretion of the byssus marks the end of the pelagic larval life and the initiation of metamorphosis. Shell deposited after

metamorphosis (dissoconch) is usually substantially different from the prodissoconch and on the outer shell surface an abrupt demarcation line is present at the prodissoconch-dissoconch boundary (Jablonski & Lutz, 1980). Postlarval mussels which have successfully completed settlement and metamorphosis are frequently referred to as 'plantigrades' (Bayne, 1976).

Mussels have been recognised as an important food resource for some time. Exploitation of wild mussel stocks where they are abundant on rocky shorelines, is almost universal, and in many cases has a history extending back thousands of years (Chanley & Chanley, 1991). The culture of these animals also has a significant history, with considerable cultivation taking place on the west coast of France since the thirteenth century (Audouin, 1954). Today mussel culture is a well-developed industry, taking place in 45 different countries around the world. The world production in 1993 stood at a formidable 1,192,000 metric tonnes, slightly lower than the maximum to date, 1,332,220 mt in 1992 (FAO, 1993). Production is currently dominated by China, who provide 43 % of the total production; the next highest producers, Spain and Italy contribute 9 and 8% respectively.

Mussels are particularly well suited for aquaculture for a number of reasons: 1) the natural availability of seed resources; 2) rapid growth rates; 3) a tolerance to high densities; 4) they are cheap to feed, relying on natural phytoplankton and detritus; 5) they have a relatively high resistance to disease; and 6) they have the ability to attach and reattach to any firm surfaces using the byssal apparatus (Hickman, 1992). With the knowledge of these valuable attributes, several methods for the cultivation of mussels have been developed. Typically two basic types of cultivation are employed; on-bottom and off-bottom. Off-bottom cultivation includes pole cultivation (commonly known as 'bouchot' in France), fixed suspended cultivation (poles joined by a series of 'racks') and floating suspended cultivation (rafts and longlines). Although the most widely used method is currently floating suspended cultivation (Hickman, 1989; 1992; Nie, 1991), the oldest method, is that using poles or 'bouchot', which is still used successfully in France today. Extensive reviews by Mason (1976) and Hickman (1992) and references therein provide a detailed insight into the above mentioned methods of cultivation along with the typical farming process, from seed collection to the supply of mussels to the consumer.

As a result of the diverse systems employed and equipment used during cultivation, direct comparisons of the different culture methods is often difficult. Moreover, it is not

realistic to relate directly the quantities produced without reference to the area or volume of water that has provided the supply of food for the mussels. Nevertheless, several studies have calculated the productivity of mussels in culture situations and *Perna canaliculus* grown on ropes suspended from rafts in New Zealand were found to have the highest productivity with 20 kg m^{-1} of substrate (Hickman, 1992 and references therein).

The problems of pollution are peripheral to the main focus of this study which is the reproduction and growth of *Mytilus edulis chilensis*. However, increased anthropogenic activities, along with the possibility of a future hydrocarbon industry in the Falkland Islands make it necessary to mention the important role that mussels play in monitoring environmental pollution. The two main reasons for assessing the chemical contamination of coastal waters are to protect human health and to protect valuable living resources. In the mid 1970's the establishment of a 'mussel watch' programme was proposed following the realization that the geographical extent and severity of marine environmental contaminants and the associated biological impact was largely unknown (Goldberg, 1975). The 'mussel watch' programme is now successfully employed in many countries around the world, monitoring spatial and temporal trends in chemical contamination in estuarine and coastal areas. Bivalves generally, and mussels in particular, are important indicator organisms for a number of reasons: 1) they are dominant members of coastal and estuarine communities, with a wide geographical distribution; 2) they are sedentary; 3) they are relatively tolerant of a wide range of environmental conditions, including moderately high levels of many types of contaminants; 4) they are suspension feeders, pumping large volumes of water and concentrating chemicals in their tissues; 5) the ability to measure chemicals in bivalve tissue provides an assessment of biological availability; 6) in comparison to fish and crustaceans, bivalves have very low enzyme activity with regard to those enzymes responsible for metabolising organic contaminants; 7) mussel populations are relatively stable and can be sufficiently large for repeated sampling; 8) they can be readily transplanted to sites of interest and maintained in cages; and 9) they are commercially important seafood species and measurement of chemical contamination is of interest for public health (see Widdows and Donkin, 1992 for review).

Unfortunately, although mussels are known to accumulate a wide range of organic and inorganic contaminants, there are biotic and abiotic factors, and complex interactions between these, that influence the relationship between the contaminant

levels in the environment (water, suspended particulates and sediments) and contaminant levels in the mussel tissue. Until these relationships are fully understood it is difficult to quantify the true levels of contaminants in estuarine and coastal waters (see Widdows & Donkin, 1992 for review). Programmes that are concerned with assessing levels of contaminants in mussels and associated biological effects are, however, solely interested in the bioaccumulated and potentially toxic contaminants.

The degree of accumulation of both organic and inorganic contaminants within mussel tissue depends upon both biotic factors (eg pumping activity, growth, reproductive condition, biochemical composition and metabolism/elimination) and abiotic factors (eg physiochemical properties of contaminants and their speciation), and these in turn are affected by environmental variables such as temperature and salinity (see Widdows & Donkin, 1992 for review).

Contaminants such as hydrocarbons (polyaromatic hydrocarbons, PAH), organochlorines and pesticides, organometals (tri- and dibutyltin), radionuclides and metals (Ag, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn) have all been observed in significant quantities within the tissues of *M.edulis* (Widdows & Donkin, 1992 and references therein). Other potentially harmful contaminants include toxins associated with algal blooms (red tides) and faecal contamination from domestic discharges both of which may be accumulated and therefore harmful to the organism and/or the consumer (Shumway, 1990; 1992). Although most bacteriological and viral diseases can be avoided through depuration and/or adequate cooking of shellfish (van den Broek *et al.*, 1979), toxins remain active following cooking and there are no known antidotes; mussels, should therefore only be eaten from areas known to be monitored regularly by an authorized and recognised public health agency.

Thus the process of monitoring the marine environment is far from simple, with a large number of important factors and interactions to be considered before any quantifiable results can be produced. Widdows and Donkin (1992) and Livingstone and Pipe (1992) and references therein provide comprehensive reviews of the 'mussel watch' concept, the role that mussels play in monitoring environmental pollution, factors affecting bioaccumulation and the deleterious effects on physiological, molecular and cellular responses.

Mytilus edulis chilensis is potentially important, both economically as a food resource,

and as a biomonitor of coastal water quality. It is also an ecologically dominant member of the intertidal zone around the coast of the Falkland Islands. Although widely distributed and locally abundant, there is surprisingly little information on *Mytilus* in this part of its geographic range. There is clearly a need to study the ecological aspects of this animal in the Falkland Islands. The present study therefore concentrates principally on reproduction and growth of *M.e.chilensis* from three intertidal sites in the Falkland Islands.

The first chapter deals with a general introduction of the mytilids, in particular *M.edulis*. Chapter two describes the study sites including some environmental characteristics, general sampling techniques used during fieldwork, population density, pattern of dispersion and criteria for statistical methods employed. Chapter three details the reproduction of *M.e.chilensis* using quantitative and qualitative histological preparations of the reproductive tissues, assessment of changes in the dry tissue weight as well as condition index, monitoring settlement densities on natural and artificial substrata and population reproductive output. Chapter four is concerned with absolute and allometric growth, the latter assessed from seasonal changes in population length frequency samples, as well as growth patterns within the shells of *M.e.chilensis*. Chapter five provides an insight into the relationship between the host mussel, *M.e.chilensis,* and the infesting isopod, *Edotia doellojuradoi*, detailing its occurrence, abundance, size and distribution, as well as its effect upon the host mussel. Chapter six provides a description of the green alga *Coccomyxa parasitica* as well as its occurrence, abundance and effect upon its host.

Each aspect of the subject matter of the study is introduced and discussed separately in the individual chapters whilst a general discussion at the end attempts to synthesise the information concerning the reproduction and growth of *M.e.chilensis*, comparing and contrasting patterns in relation to the habitat studied. An attempt is also made to apply the ecological knowledge of *M.e.chilensis* gained throughout this study to the possible cultivation and the potential use of this mussel as a biomonitor of water quality and environmental change in the Falkland Islands.

Chapter 2

Site description, environmental characteristics and general methods

2.1. Site descriptions

The Falkland Islands lie on the edge of an extension of the Patagonian Shelf in the south-west Atlantic, approximately 450 km north-east of Tierra del Fuego and 600 km due east of Patagonia (Figure 2.1). A small branch of the circumpolar current (West Wind Drift) loops off and moves northwards after passing through Drake Passage, to form the Falkland Current. Considerable upwelling occurs around the archipelago particularly to the north west giving rise to a nutrient-rich body of water which supports a large and thriving ecosystem around the islands (Strange, 1992).

The study sites selected for this investigation lie on the relatively sheltered isthmus which joins north and south East Falkland (Figure 2.2). Darwin and Goose Green are coastal beaches on the eastern side of the isthmus and Camilla Creek is a tidal estuary on the west.

2.1.1. Darwin

The foreshore lies adjacent to an uninhabited farm 2.5 km north of the main settlement at Goose Green. The shore faces north-east and has strong tidal currents (tidal correction +00:04 hours after Stanley) running perpendicular to the shore, a result of a narrow inlet which opens into a small lagoon adjacent to the shore (Plate 2.1A and B). The substratum consists of shingle and shell debris overlying anoxic mud. Despite being partially protected by a headland on the opposite side of the bay, the shore is exposed to the full force of northerly winds which roll across the relatively flat stretch of land from the Wickham Heights in the north. This combined with the strong tidal currents give rise to a somewhat less sheltered site. The mussel bed is fairly narrow, reaching a maximum width of 6 m between the bridge and jetty and a minimum of 4 m at the jetty and around the headland (Plate 2.1A and B). Increasing algal cover is

Figure 2.1 A map to illustrate the position of the Falkland Islands in relation to South America and Antarctica.

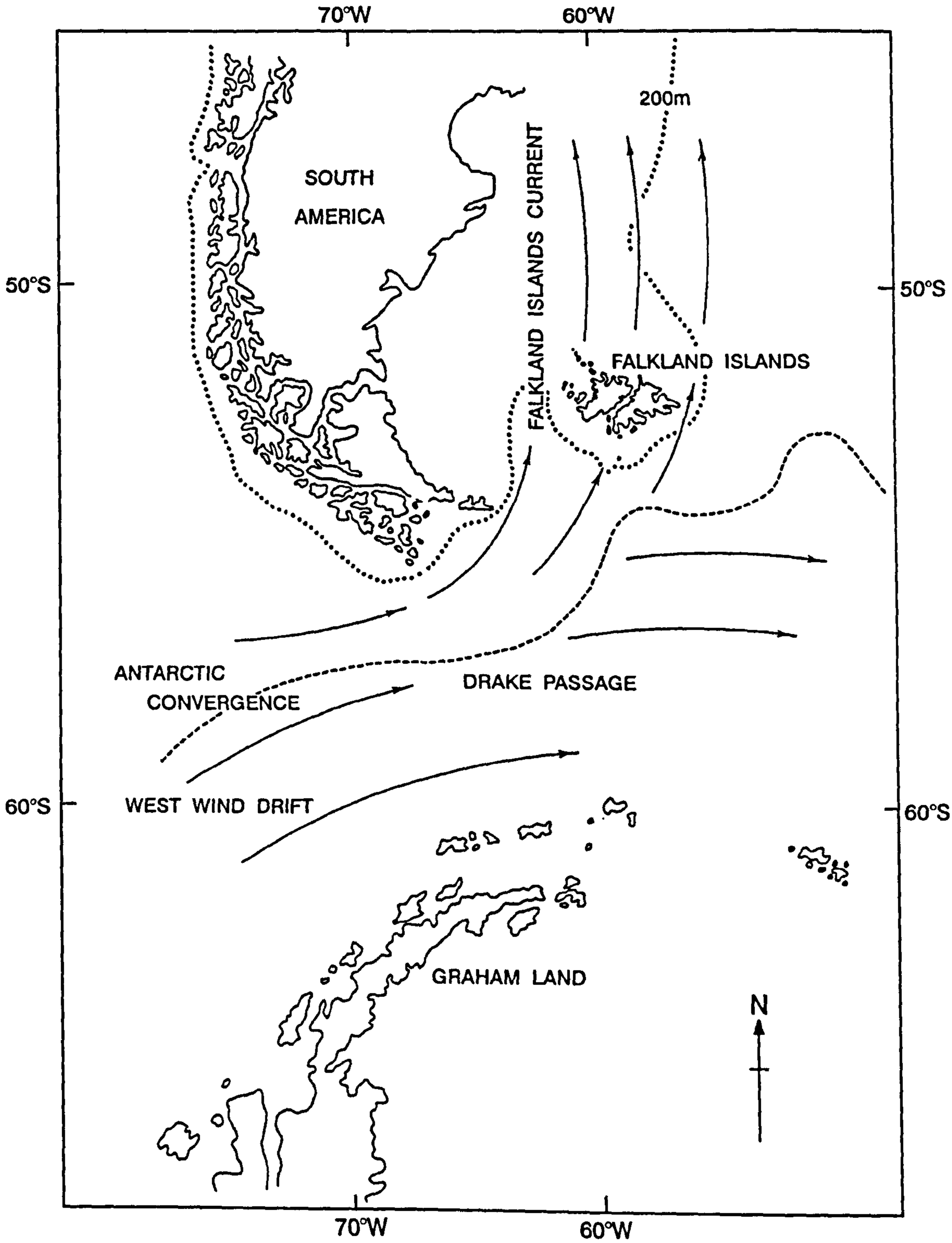

70°W
60°W
200m
SOUTH
AMERICA
FALKLAND ISLANDS CURRENT
FALKLAND ISLANDS
50°S
50°S
ANTARCTIC
CONVERGENCE
DRAKE PASSAGE
WEST WIND DRIFT
60°S
60°S
GRAHAM LAND
N
70°W
60°W

Figure 2.2 Locations of the study sites in the Falkland Islands (51 - 53°S, 57 - 62°W)

61°
60°
59°
58°
51°S
51°
N
West
Falkland
East
Falkland
Stanley
52°
52°
Camilla Ck.
Darwin
Goose G.
0 km 25
61°W
60°
59°
58°

observed with decreasing shore level.

2.1.2. Camilla Creek

Camilla Creek is a tidal estuary 6 km north of Goose Green. The main body of the estuary is approximately 3 km long and 100 m wide at the study site (Low Pass), running north-east to south-west and opening into Brenton Loch water on the west side of the isthmus (Figure 2.2). The tides in this area are difficult to predict from published tide tables due to the nature of the creek, and strong south-westerly winds often force the tide up the creek resulting in a considerably reduced low water or none at all. Observations over the study period revealed that low water on relatively calm days is approximately +05:00 hours after Stanley, although it may take up to 6 hours to uncover the entire mussel bed and only 2 hours to cover the same area. The substratum consists of shingle and fine anoxic mud, and towards the lower reaches of the shore increasing levels of silt deposition are observed. The site is relatively sheltered and the sides of the main estuary fairly steep, in some cases up to 16.5 m high. The main body of the mussel bed is situated on a shingle spit which protrudes into the estuary (Plate 2.2A and B). At the landward base mussels are distributed around the edge of the spit and further seawards they make an almost continuous cover. There is an increasing cover of algae over the mussel bed with decreasing shore level.

2.1.3. Goose Green

A coastal beach which is adjacent to the sheep farm, Goose Green, approximately 96 km west of Stanley. Low tide is +00:04 hours after Stanley. The shore faces north-east and is relatively sheltered, gently sloping seawards (Plate 2.3A and B). On spring low tides a small island approximately 250 m offshore is accessible by foot, with a water depth of about 1.5 m. Mussels occur fairly patchily and cover a relatively wide area (approximately 50 m from upper to lower shore); only in the lower reaches does the bed form a continuous covering, stretching subtidally to the offshore island. The substratum consists of exposed bed-rock, coarse shingle and mud, with a dense algal covering in the lowest reaches of the shore.

2.2. Mussel population density and dispersion indices

Routine monthly samples of between 1 and 3 random quadrats (0.17 m^2) were collected

Plate 2.1 Darwin

A. Photograph of the beach taken from the bridge at mid-low water;

B. Schematic diagram of the study site. Scale bar = 30 m

A

B

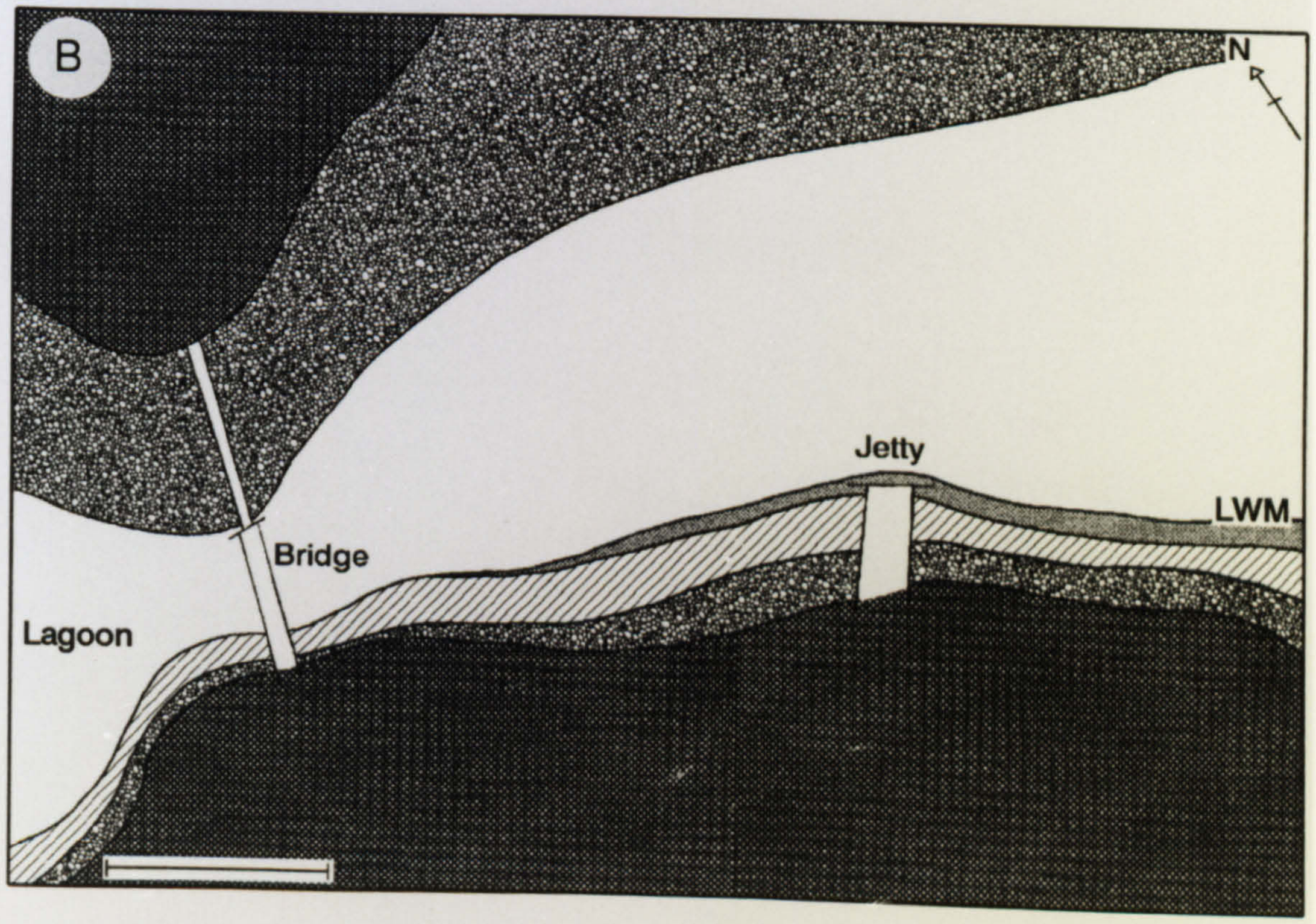


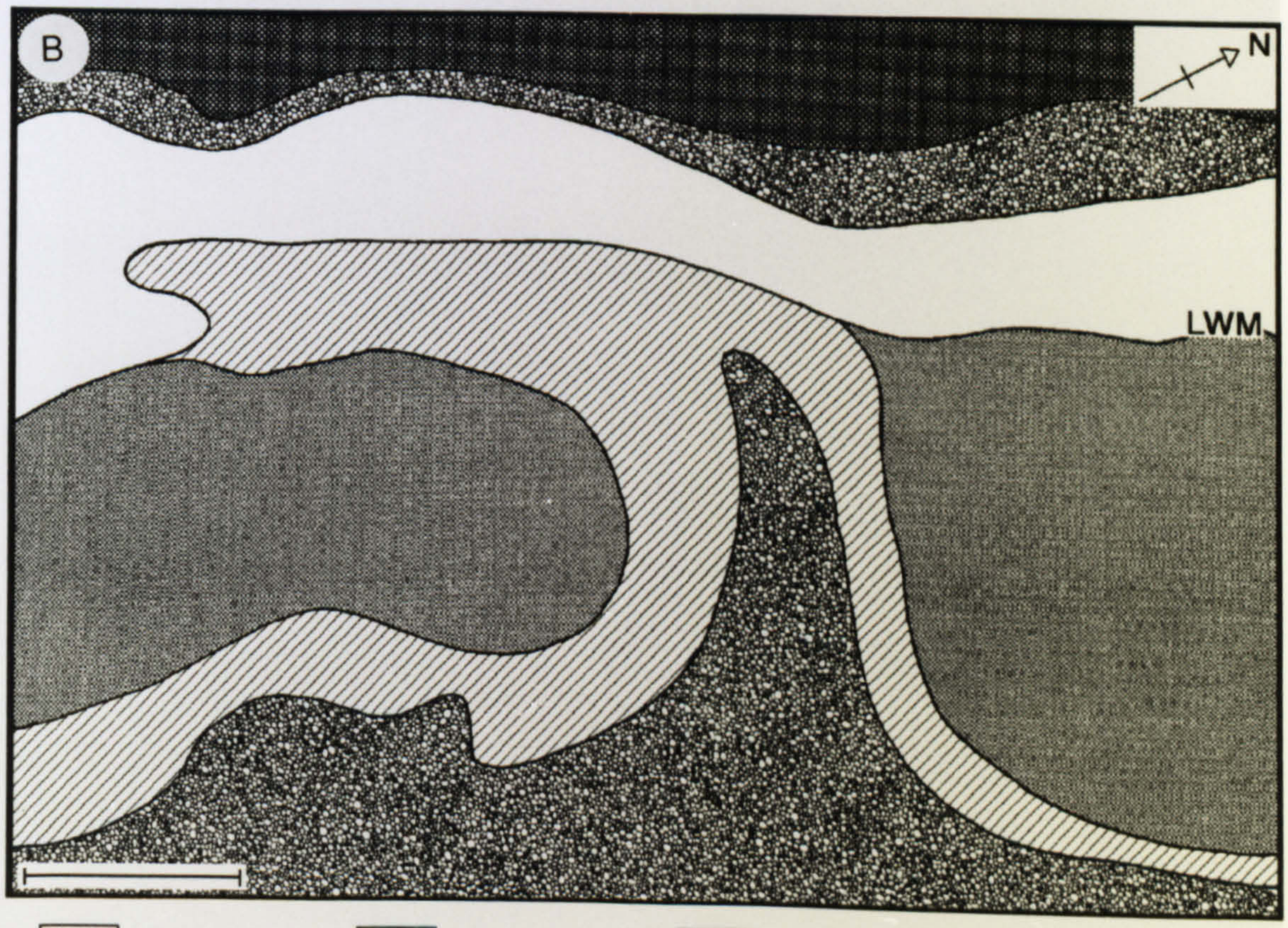
Plate 2.2 Camilla Creek

A. Photograph of the gravel spit at mid water;

B. Schematic diagram of the study site. Scale bar = 25 m

A

B



Water
Mud
Gravel
Land
Mussel Bed

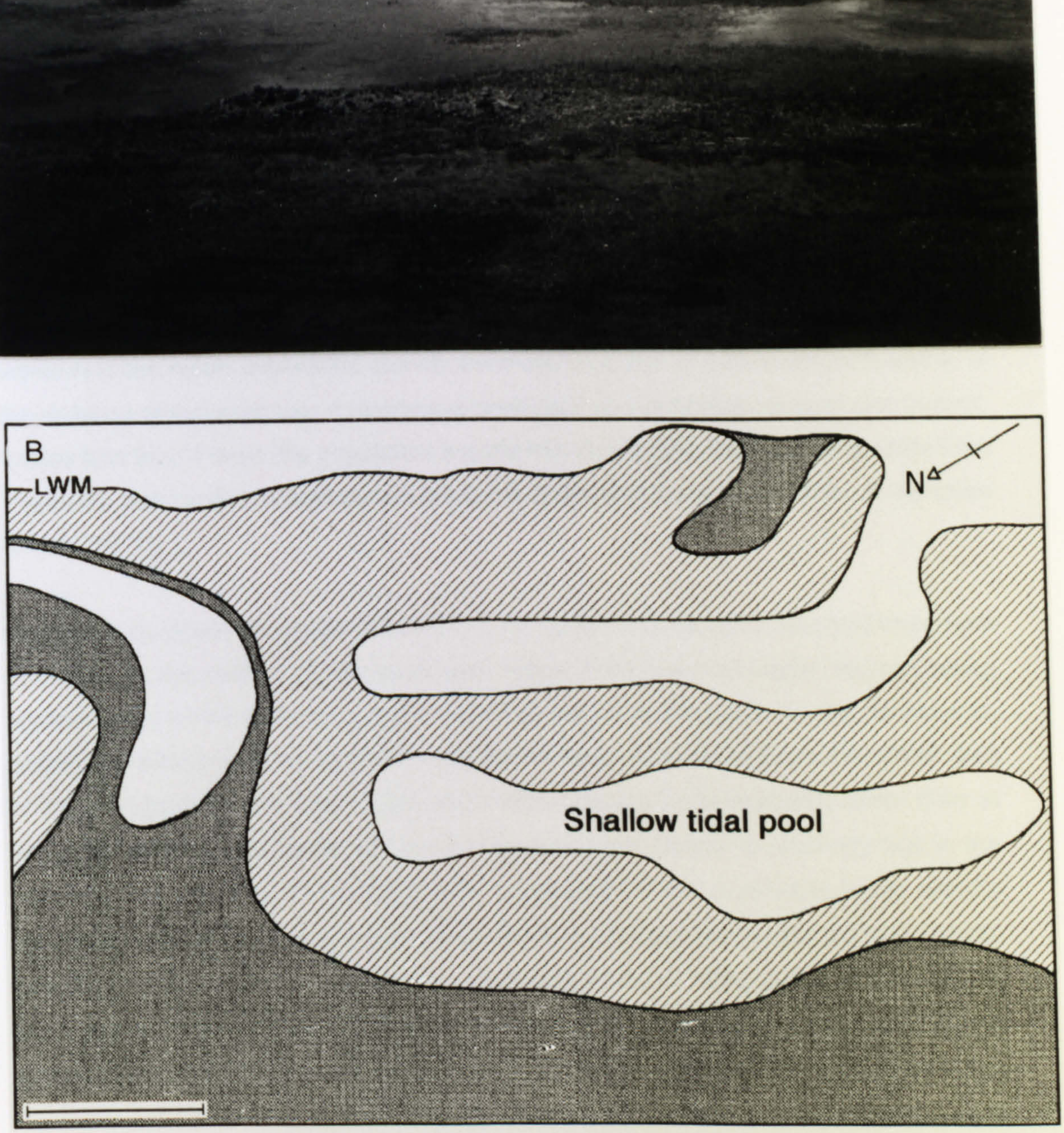
Plate 2.3 Goose Green

A. Photograph of the beach at low water;

B. Schematic diagram of the study site. Scale bar = 30 m

A

B



Water
Mud
Gravel
Land
Mussel Bed

from the mid part of the mussel bed at each site during daylight hours, at low tide, over a 30 month period (September 1993 to February 1996). These samples were collected to investigate the growth, reproduction and general ecology of *Mytilus edulis chilensis* populations in the Falkland Islands. On two occasions, September 1994 and November 1995, sufficient samples (between 3 and 10 quadrats) were collected from the low, mid and upper reaches of the mussel bed at each site in order to determine the density, biomass and pattern of dispersion of the mussel populations. Mussels from each quadrat were counted and the whole sample subsequently weighed (to the nearest 25 g) using a spring balance.

The degree of aggregation/spatial dispersion was assessed using a simple index of dispersion :

$$Id = \frac{s^2}{N} \qquad \text{Equation 2.1}$$

where N is the mean population density estimate (number of individuals.m^{-2}) and s^2 is the variance of that estimate. A dispersion coefficient of 1 indicates random distribution. Values less than 1 imply the population is uniformly distributed, and values greater than 1 indicate patchiness, maximum contagion is represented by the number of samples (Elliott, 1971).

Population densities are given in Table 2.1. At Darwin the mussels reached maximum densities in the middle of the zone with those from low and upper regions being approximately a third of those from the middle of the zone. Biomass, on the other hand, despite remaining relatively high in the mid shore region, is even higher in the lower part of the mussel bed. Biomass in the upper shore region is considerably lower than at either mid or low shore levels. At Camilla Creek mussel density is relatively high in the low and mid areas of the mussel bed, compared to the somewhat lower density observed in the upper region (approximately 15 % of those in the mid and low zones). Biomass follows a similar pattern, with the highest values being found in the mid and low regions of the mussel bed and the lowest values in the upper zone. Densities at Goose Green exhibit a similar pattern to those at Darwin with maximum densities in the mid region of the mussel bed and considerably lower densities in the lower and upper zones. Biomass does not, however, follow this pattern, with the highest values being recorded from the low zone where mussel density is relatively low, and only marginally

lower in the mid zone where mussel density is considerably higher. In the upper part of the bed biomass is somewhat lower than at either mid and high shore levels.

Overall densities and biomass appear to have declined with time, the reason for this is not known, although it could be speculated that if the populations are relatively slow growing and recruitment poor, routine monthly samples could be in part responsible for this decline. Coefficients of dispersion for all sites and levels are not only higher than one but also considerably higher than the value of maximum contagion, suggesting that the populations are extremely patchy and contagious in their distribution.

Table 2.1. Density, biomass and indices of dispersion for *Mytilus edulis chilensis* at three study sites in the Falkland Islands

Site		September 1994				November 1995			
		n	density ($no.m^{-2}$)	biomass ($kg.m^{-2}$)	*Id*	n	density ($no.m^{-2}$)	biomass ($kg.m^{-2}$)	*Id*
Darwin	(high)	7	682	1.42	41.6	-	-	-	-
	(mid)	7	1828	6.07	20.4	5	1438	7.09	16.2
	(low)	7	569	3.93	37.7	5	282	-	24.6
Camilla Creek	(high)	6	280	1.32	17.1	10	220	1.13	9.6
	(mid)	6	1834	3.59	26.1	5	1330	3.97	6.3
	(low)	6	1824	3.02	46.1	3	1177	2.83	82.1
Goose Green	(high)	7	279	1.78	22.2	3	66	-	16.1
	(mid)	7	997	7.49	19.7	5	638	4.82	11.9
	(low)	8	383	9.35	19.8	3	233	-	18.3

Id = dispersion index of mussel population

n = number of quadrats examined

2.3. Meteorological data

The Falkland Islands experience a rather cool oceanic climate dominated by westerly winds a high proportion of which are north-westerly in direction. Lying on the northern edge of the depression belt which passes through Drake Passage, the islands experience fairly continuous variations in weather, with some warming and drying influence from the South American continent, despite lying nearly 400 km offshore.

During the course of the study seawater temperatures were monitored initially (October 1993 - January 1995) at Goose Green on a weekly basis by mercury thermometer (to the nearest 0.1°C) and for the latter part of the study (January 1995 - February 1996) by TinyTalk (Orion) temperature loggers (to 0.01°C, range -5 to 37°C) deployed at the three study sites. The loggers were attached to fixed frameworks on the shore in the mid zone of the mussel bed at each of the sites, and launched for periods of between one and six months. The temperature was recorded at intervals of between 24 and 150 minutes according to the deployment period (duration of deployment), after which they were retrieved and the data down loaded into Quattro Pro and appropriate temperature plots created (raw temperature data for the three sites are given in Appendix 1). Both air and seawater temperatures were extracted from the TinyTalk temperature records by comparing temperature data with the tidal records. Extracted data were subsequently averaged on a monthly basis for each study site (Figure 2.3). A complete record of the seawater temperature over the study period was assembled by combining TinyTalk temperature data with that collected by mercury thermometer (Figure 2.4B).

The seawater temperatures displayed a cycle typical of temperate waters (Figure 2.4B), with maximum temperatures (11.5 - 13.5°C) being reached in the summer months (December - February) and minimum temperatures (1.5 - 2.0°C) during the winter (July - August). The temperature loggers recorded minimum air temperatures between June and September as low as -5°C (even though lower temperatures may have occurred, but -5°C was the lower limit of the loggers) at Darwin and Camilla Creek (Figures 2.3A and B), whilst air temperatures as high as 30°C were reached at Darwin and Camilla Creek during January and February. Overall, the average monthly seawater temperatures at the three sites do not appear to differ from each other in any obvious way, although those at Goose Green (Figure 2.3C) do not show the extremes encountered at the other two sites, the reason for this may be due to differences in shore aspect.

The Meteorological Office at Mount Pleasant Airport in the Falkland Islands provided mean monthly minimum and mean monthly maximum air temperatures (Figure 2.4A), total monthly sunshine hours (Figure 2.5A), monthly precipitation (Figure 2.5B and C) and monthly wind speed (Figure 2.6A) records over the 30 month study period as well as ten year averages (1987-1996). Additional information on wind speed and direction (Figure 2.6B) was provided by the National Meteorological Library (Bracknell, UK).

Average monthly minimum and maximum air temperatures exhibit a seasonal cycle over

Figure 2.3 Mean monthly seawater temperatures (closed circles) and mean, minimum and maximum monthly air temperatures (open circles and bars) derived from TinyTalk data loggers deployed at

A. Darwin,

B. Camilla Creek,

C. Goose Green.

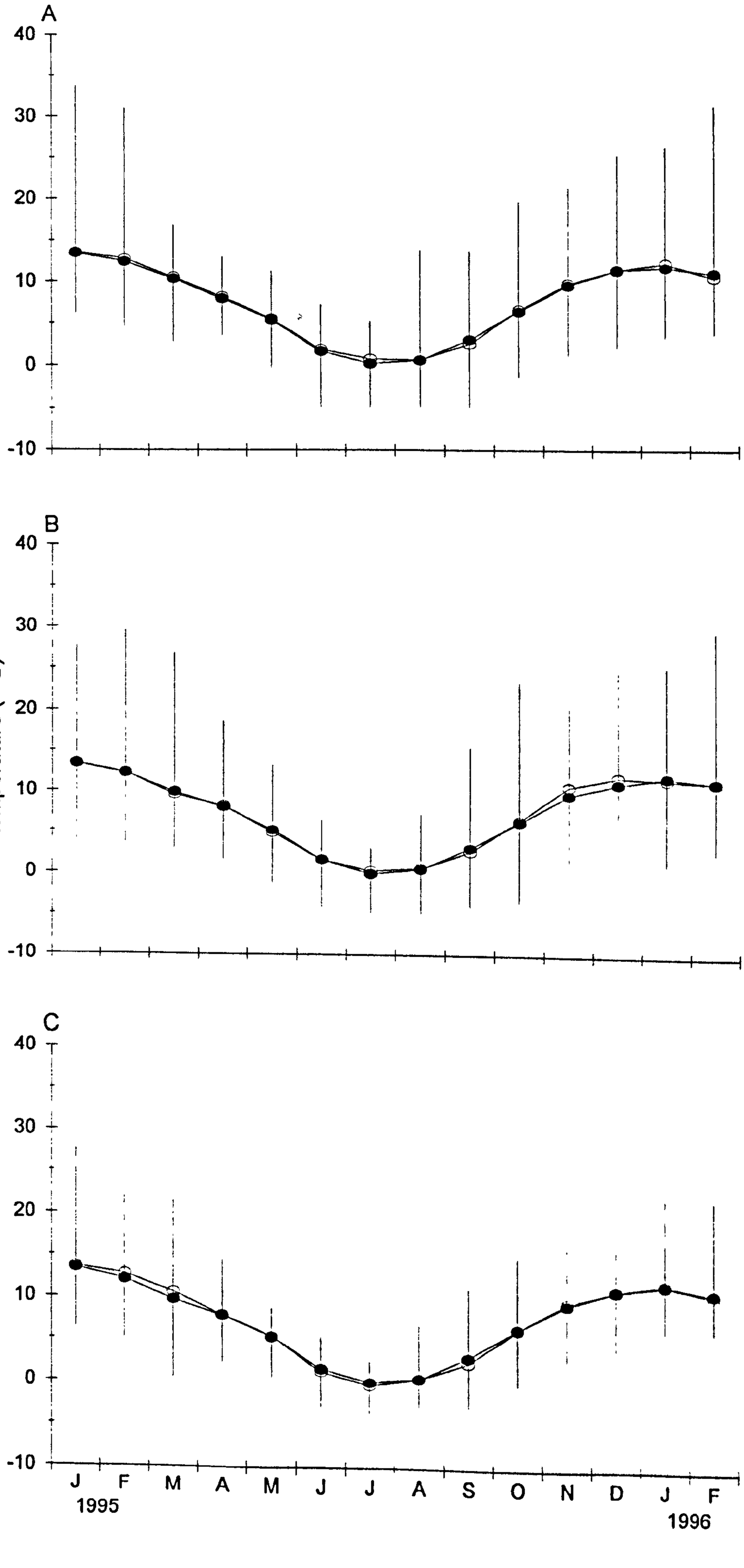

A
40
30
20
10
0
-10
B
40
30
20
10
0
-10
temperature (°C)
C
40
30
20
10
0
-10
J
F
M
A
M
J
J
A
S
O
N
D
J
F
1995
1996

Figure 2.4

A. Mean minimum (open circles) and mean maximum (closed circles) monthly air temperatures, together with the 10 year average (broken lines) recorded at Mount Pleasant Airport, Falkland Islands.

B. Mean monthly seawater temperatures recorded at the study sites throughout the study period.

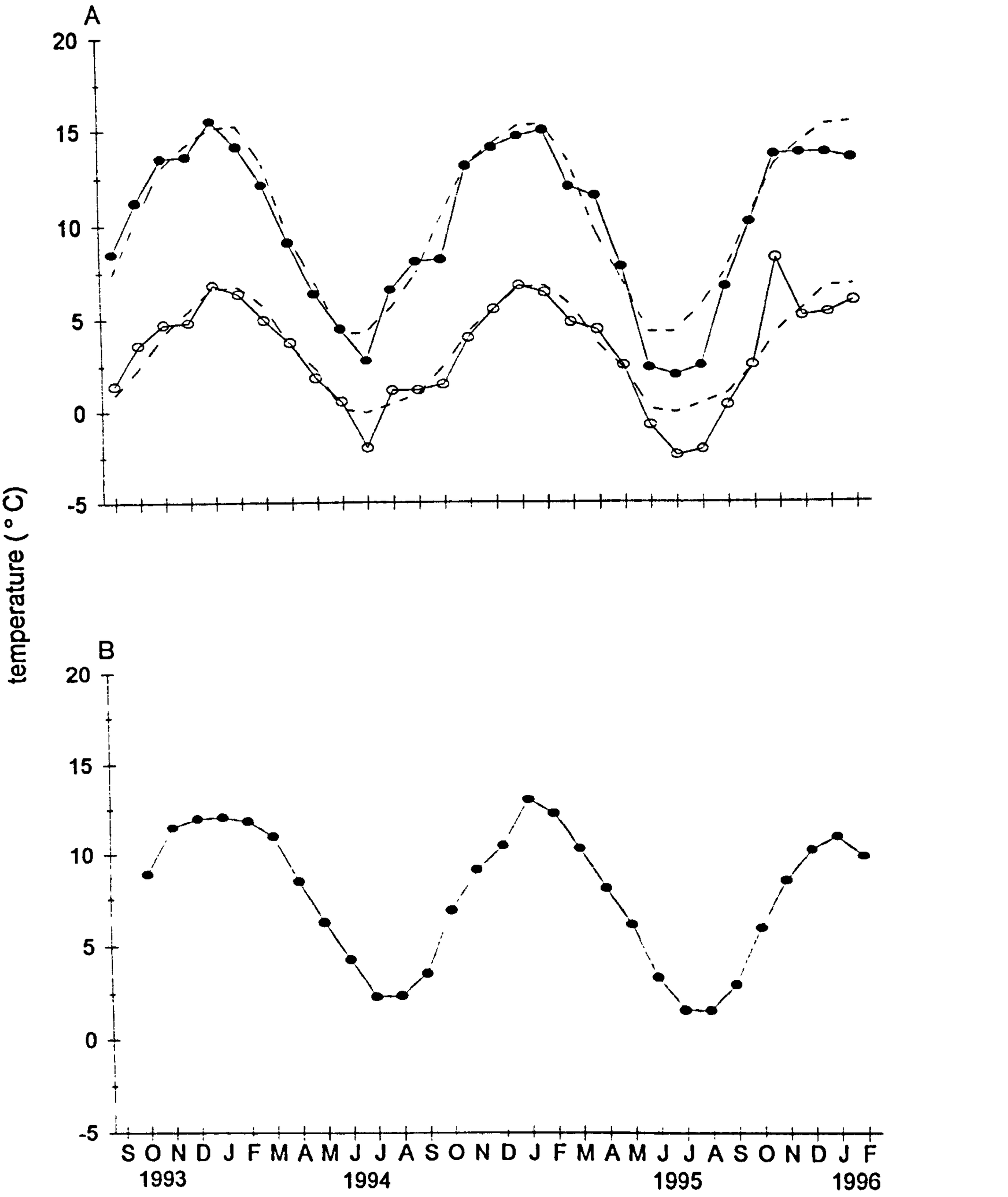

A
20
15
10
5
0
-5
B
20
15
10
5
0
-5
temperature (° C)
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996

Figure 2.5

A. Total monthly sunshine hours during the study period (closed circles) and 10 year average (broken line);

B. Total monthly rainfall during the study period (open bars) and 10 year average (broken line);

C. Number of days with snow recorded during the study period (closed circles) and 10 year average (broken line).

Data collected at Mount Pleasant Airport, Falkland Islands.

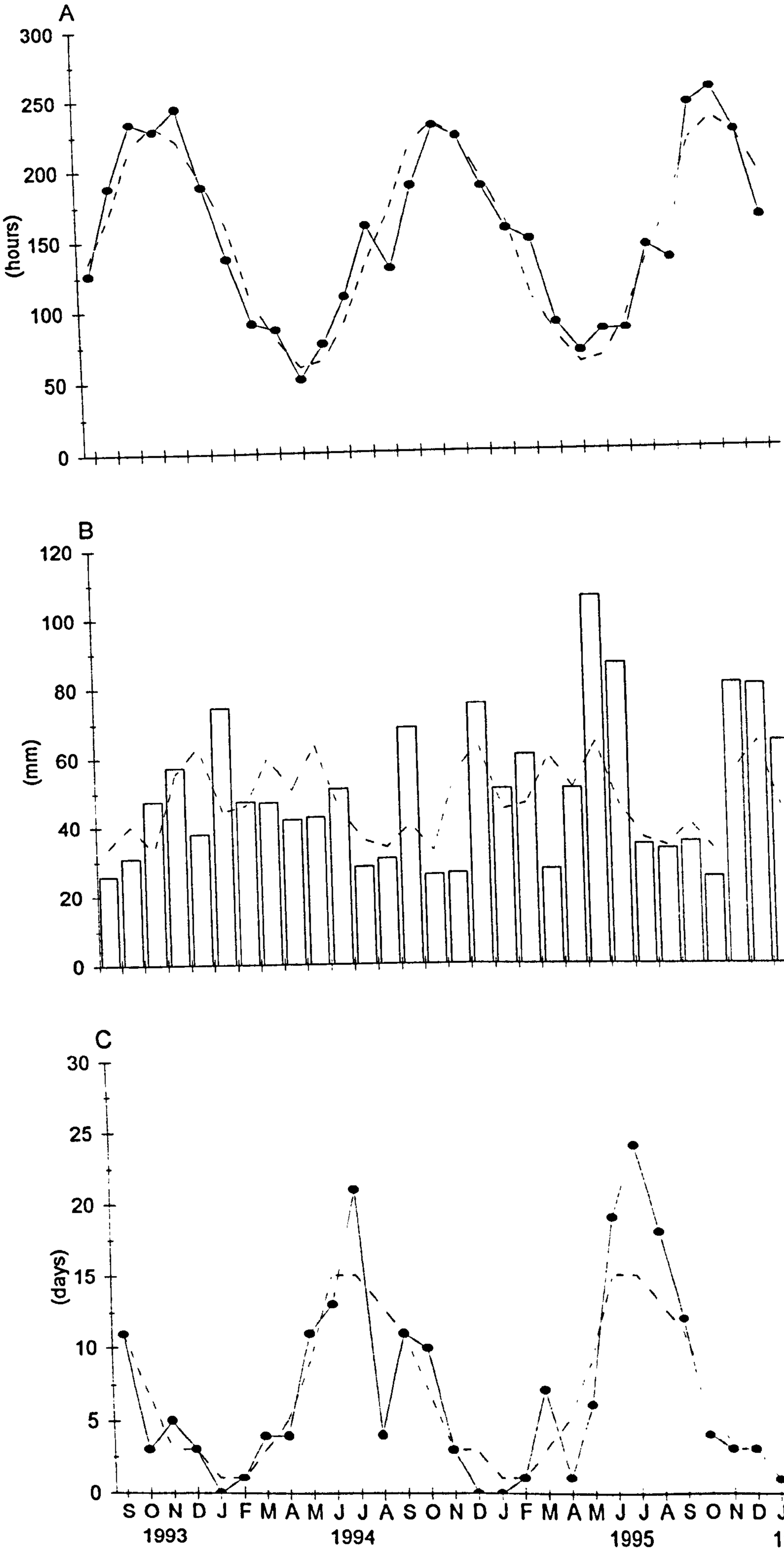
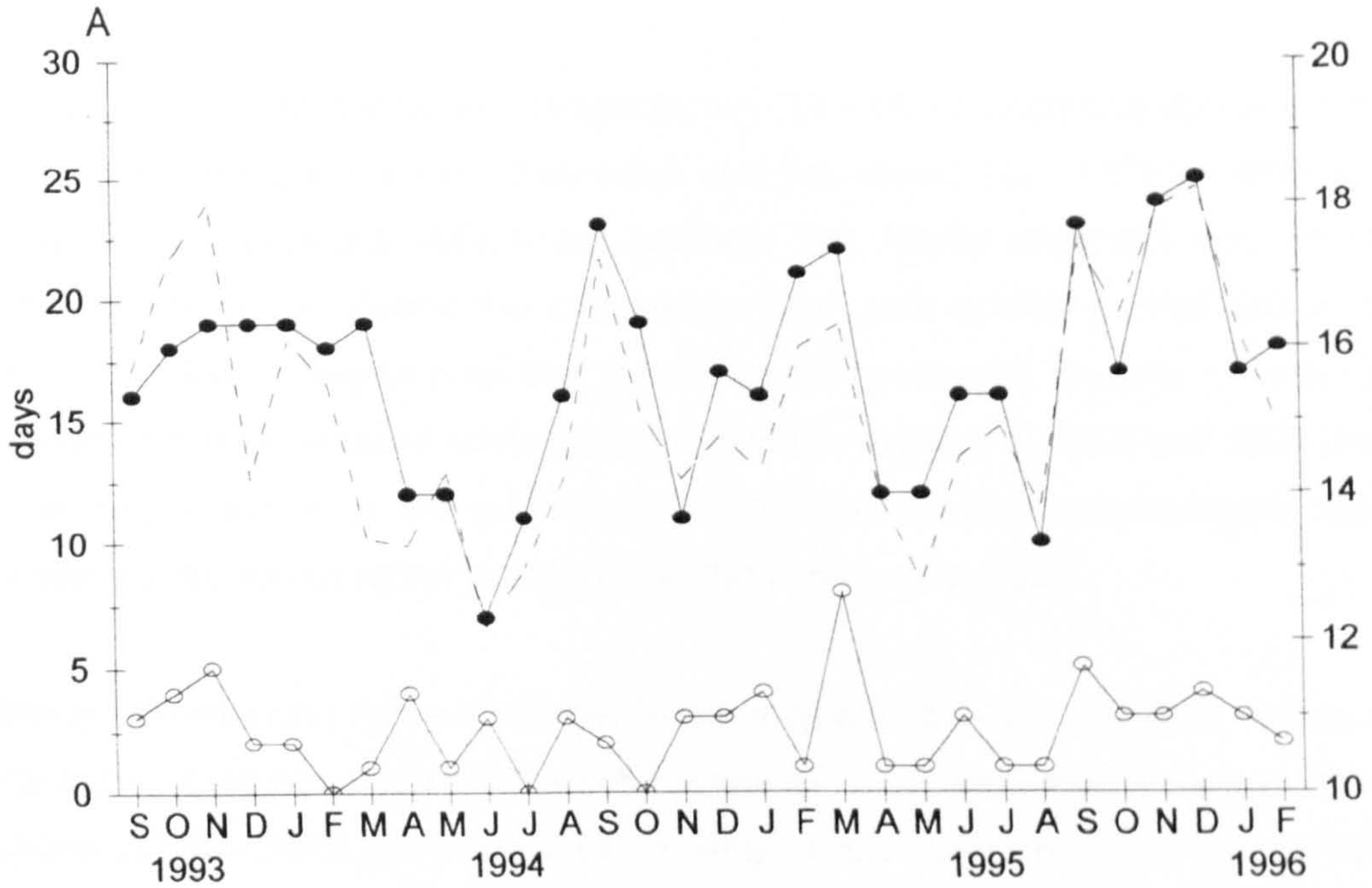

A
300
250
200
150
100
50
0
(hours)
B
120
100
80
60
40
20
0
(mm)
C
30
25
20
15
10
5
0
(days)
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996

Figure 2.6

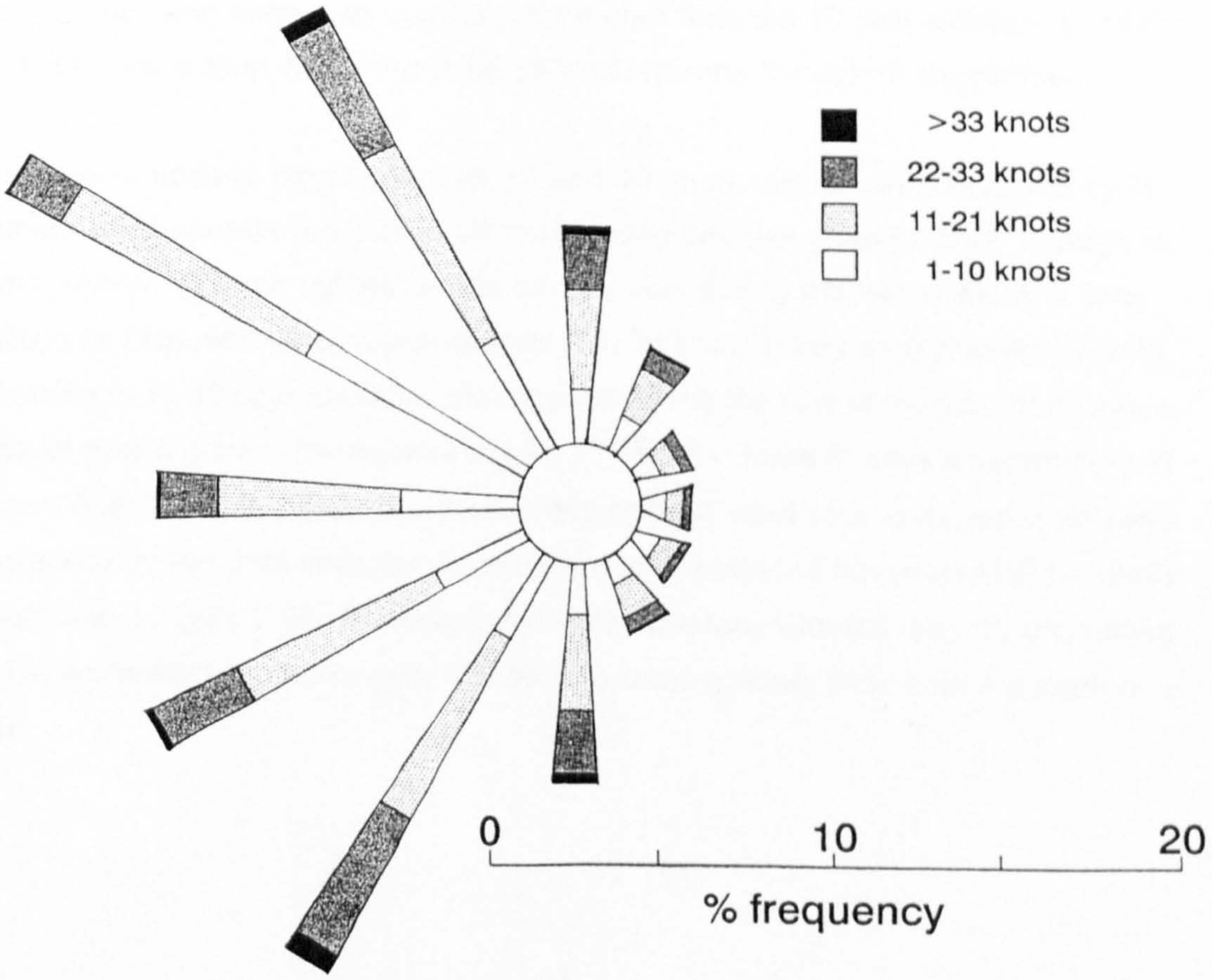
A. Number of days with wind speeds > 33 knots (closed circles), number of days with Force 8 winds (open circles) and average monthly wind speeds (broken line). Data collected at Mount Pleasant Airport, Falkland Islands.

B. Wind frequency diagram showing the direction and strength of winds over a ten year period (1971 - 1980). Data collected at Stanley, Falkland Islands.

A


30
25
20
15
10
5
0
days
20
18
16
14
12
10
speed (knots)
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996

B


>33 knots
22-33 knots
11-21 knots
1-10 knots
0
10
20
% frequency

the study period, with the highest temperatures (13 - 16°C) occurring during the austral summer months (November - February) and the lowest (-3 - 1°C) in winter (June - August). The greatest difference between the mean minimum and maximum temperatures occurs during the summer months, with relatively small differences in winter. It is interesting to note that the mean minimum and maximum temperatures reached considerably lower levels during the winter months of 1994 and 1995 than the overall 10 year averages and coincided with severe weather conditions which rendered the study sites inaccessible in July 1994 and July/August 1995.

Sunshine hours in the Falkland Islands follow a typical temperate latitude annual cycle, with a minimum of between 50 and 80 hours of sunshine a month during winter and maxima of 220 - 260 hours in summer. Monthly rainfall ranged between 20 and 105 mm during the 30 month study period with annual totals of 520 and 659 mm in 1994 and 1995, respectively. In 1995 there appears to be a slight seasonal cycle with the highest rainfall during the summer and winter months, although during 1994 this cycle was not apparent. Interestingly rainfall during the winter months of 1995 and the following summer was considerably higher than the 10 year averages.

The number of days with snow follows a clear seasonal pattern with between 11 and 25 days of snow each month during the austral winter. During 1994 and 1995 the number of days with snow was considerably higher than the 10 year average. In 1995 snow fell for more than 12 days a month from May/June through to September.

Average wind speeds range between 12 and 19 knots with a slight seasonal cycle. Maximum wind speeds are observed from spring (August /September), through to autumn, (April), with the lightest winds usually only during the winter months (May - July/August). Days with wind speeds greater than 33 knots follow a very similar pattern, with minima of 7 - 10 days in winter and maxima during the rest of the year of between 19 and 26 days a month. The number of gale (≥ Beaufort force 8) days a month ranged between 0 and 6, with no evidence of seasonality. A wind rose compiled from wind speed and direction data collected in Stanley over a period of ten years (1971 - 1980) is presented in Figure 2.6B. Prevailing winds are westerly with the majority originating from the south-west and north-west. Winds ≥ 33 knots typically blow from the north and south.

2.4. Tidal pattern and aerial exposure

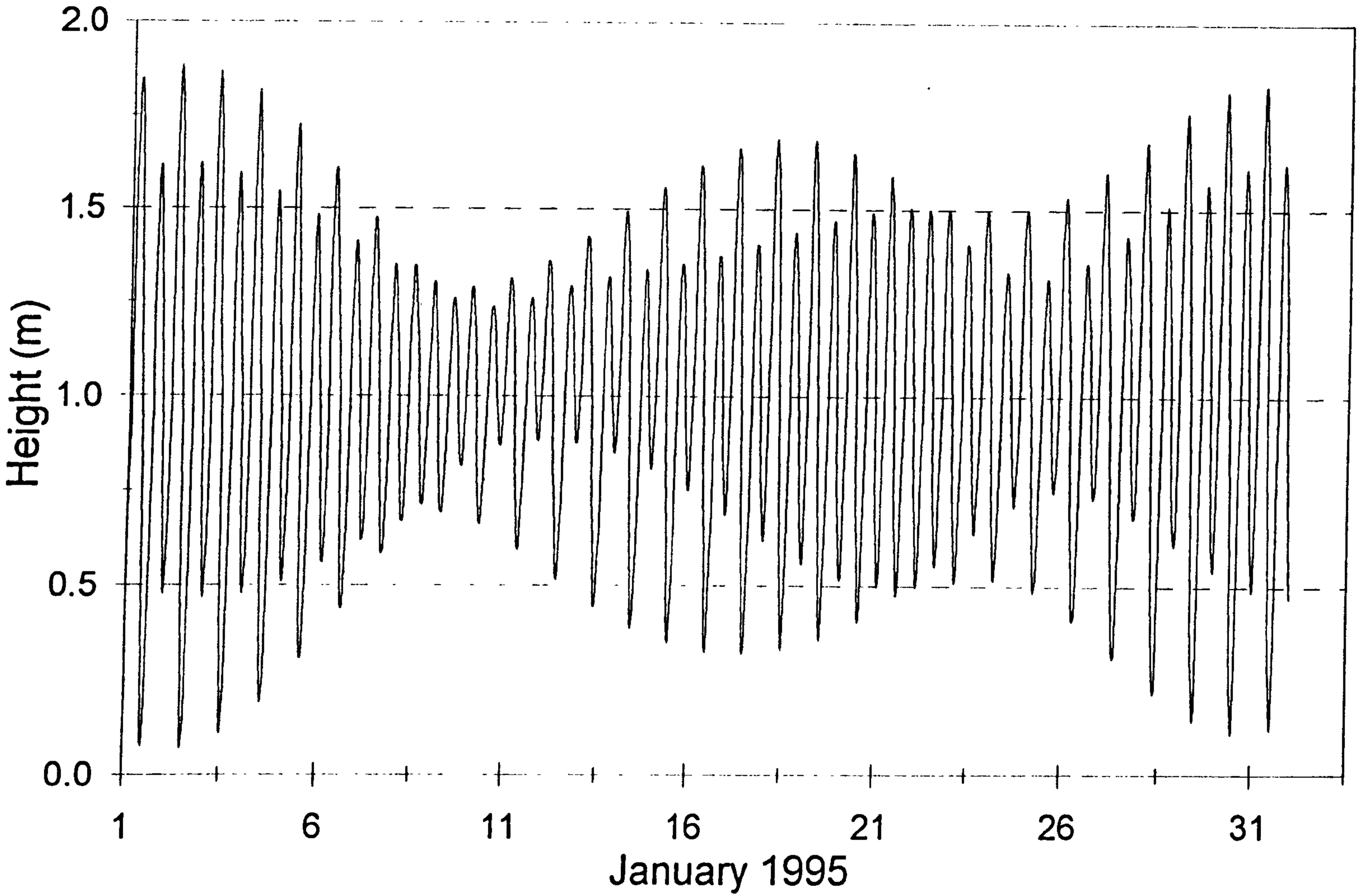
Tide tables and the tidal prediction programme 'Poltips' provided by the Proudman Oceanographic Laboratory were used to identify routine sample collection times at the three study sites and to observe the tidal pattern for the waters around the Falkland Islands. Figure 2.7 illustrates a typical example of the tidal pattern over a lunar cycle. The tidal regime has a mixed semi-diurnal component with two spring tides occurring each month and a tidal range of approximately 1.7 metres. However, on some neap tides the amplitude may fluctuate by as little as 0.5 m. The aerial exposure and tidal heights of the mussel beds were estimated by timing the covering and uncovering of marked areas of the mussel bed over several days during spring tide periods. By plotting the daily tidal pattern for each site an approximate estimate of height above chart datum for the three zones of the mussel bed could be determined. The percentage aerial exposure and estimated heights of the mussels above chart datum are provided in Table 2.2.

Table 2.2. Aerial exposure (%) and estimated height above chart datum (c.d.) for high, mid and low regions of the mussel beds at the three study sites in the Falkland Islands.

Site		Aerial exposure (%)	Height above c.d. (m)
Darwin	(high)	59	1.07
	(mid)	43	0.87
	(low)	18	0.38
Camilla Creek	(high)	55	0.95
	(mid)	47	0.82
	(low)	40	0.64
Goose Green	(high)	63	0.99
	(mid)	52	0.66
	(low)	28	0.23

Tidal height and aerial exposure are closely related, with the upper shore levels experiencing longer periods of aerial exposure. Although the high, mid and low zones of the mussel bed at Camilla Creek occur at different tidal heights, they are in fact only separated by 0.3 m in vertical height, whereas similar zones selected at Darwin and

Figure 2.7 Mixed semi-diurnal tidal pattern predicted for the Falkland Islands


2.0
1.5
1.0
0.5
0.0
Height (m)
1
6
11
16
21
26
31
January 1995

Goose Green are separated by 0.6 m and 0.8 m respectively. Although the difference in the upper and lower tidal height of the zones appears small, the fact that the maximum tidal range is only 1.7 m suggests that small differences in height above cd may be sufficient to have a significant effect on the mussel populations. The upper limits of the mussel beds appears to coincide with a level of aerial exposure of between 55 and 63 %, which is comparable to the limits suggested by other authors. Baird (1966), for example, predicted zero growth in *Mytilus edulis* at 56% aerial exposure, whilst Seed (1969b) observed zero growth of *M.edulis* at 75% aerial exposure. Although an aerial exposure of 80% was predicted as the point of zero growth for *M.edulis* observed by Gillmore (1982).

2.5. Salinity, currents and food supply

Salinity and water flow were monitored over 12 hour periods at sites where two sub-tidal cages were deployed for use in the growth experiments, in order to investigate whether there were any fluctuations in these environmental variables during a tidal cycle. The cages were situated in a narrow channel beneath a bridge at Darwin (Plate 2.1B) and inside the submerged hull of the 'Vicar of Brae' which is moored along-side a jetty at Goose Green on the opposite side of the peninsula to the main mussel beds. Measurements of water flow and salinity were made at thirty minute and one hour intervals at Darwin and Goose Green respectively. Water velocity was determined by taking three ten second revolution counts with a Valeport 'Braystoke' BFM002 miniature current flow meter suspended approximately one metre below the water surface. Revolution counts were converted into velocity using the calibration chart for the BFM002 current metre and mean velocities were subsequently plotted for both sites (Figure 2.8A and C). Salinity was measured to the nearest ppt using a refractometer, and plotted with time (Figure 2.8B and D).

Water velocity varied considerably over the tidal cycle at Darwin, but at Goose Green, the water inside the hull of the 'Vicar of Brae' is sheltered, with little or no measurable water movement at any stage of the tidal cycle. Maximum velocities of 0.35 $m.sec^{-1}$ were recorded two hours prior to and following both high and low tide at Darwin. At the times of high and low tide as well as mid way through the flood and ebb of the tide, velocity dropped to zero. Fluctuations in salinity according to the state of the tide were observed. At Darwin lower salinities were observed during low tide, whilst water from the 'Vicar of Brae' was found to reach minimum salinities around the time of high tide.

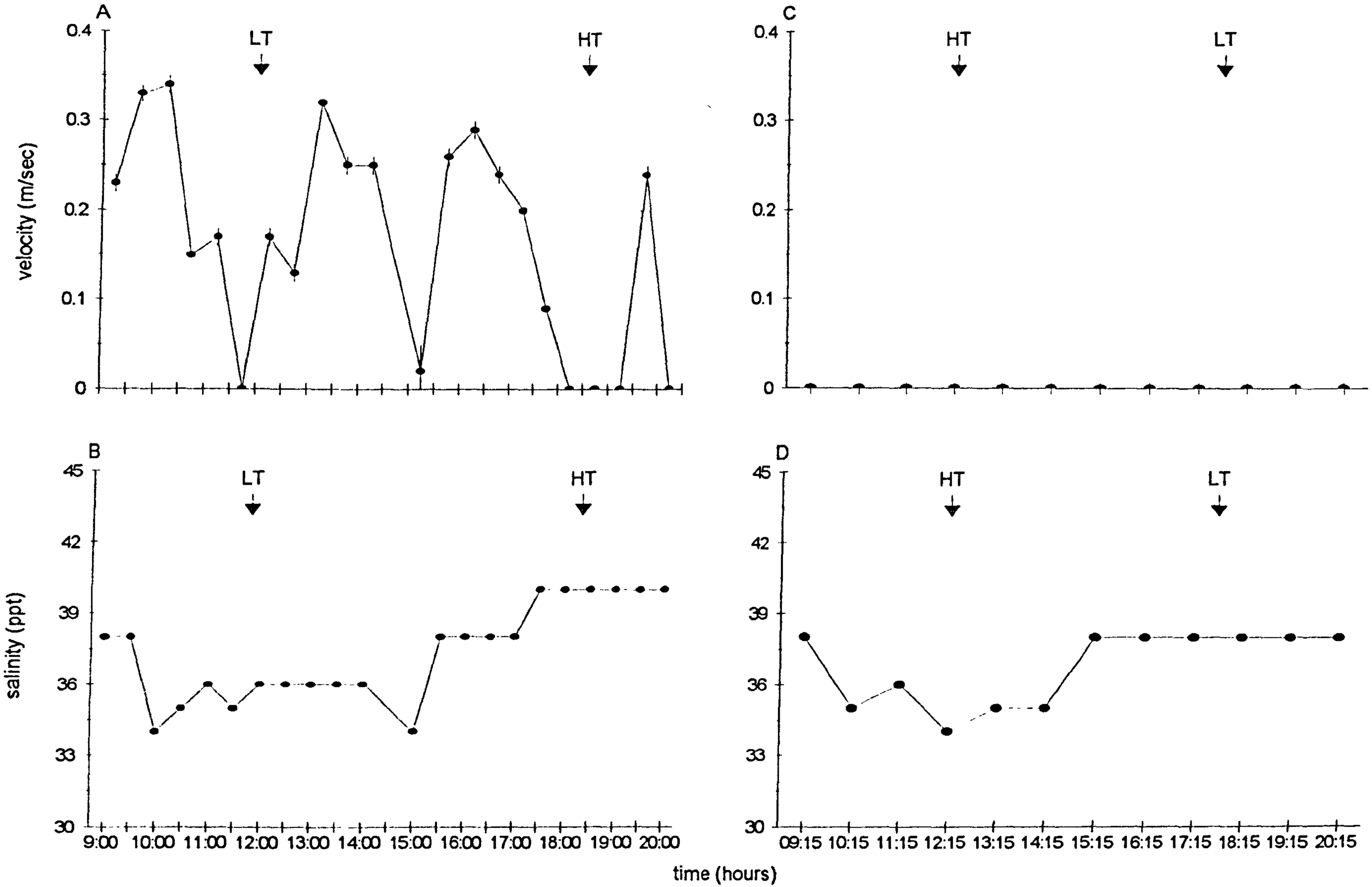
Figure 2.8

A. Water velocity (mean ± 1 standard error) measured over one tidal cycle at Darwin bridge;

B. Variations in salinity at Darwin bridge over one tidal cycle;

C. Water velocity (mean ± 1 standard error) measured over one tidal cycle at the 'Vicar of Brae';

D. Variations in salinity at the 'Vicar of Brae' over one tidal cycle data.


A
B
C
D
LT
HT
velocity (m/sec)
salinity (ppt)
time (hours)
0.4
0.3
0.2
0.1
0
45
42
39
36
33
30
9:00
10:00
11:00
12:00
13:00
14:00
15:00
16:00
17:00
18:00
19:00
20:00
09:15
10:15
11:15
12:15
13:15
14:15
15:15
16:15
17:15
18:15
19:15
20:15

Further measurements of salinity are required in order to ascertain whether these fluctuations are directly linked to the tidal pattern or not. Average salinities over the 12 hour period were found to be slightly higher at Darwin (37.4 ppt) than at Goose Green (36.8 ppt).

Any differences in food supply during the spring-neap lunar cycle between the two sites were investigated daily by determining the amount of total suspended solids (TSS) and chlorophyll a concentrations for a period of eight days.

Six 2 litre water samples were collected from 0.5 m below the water surface 3 hours after low tide during daylight hours. Three of the replicate 2 litre samples used to determine TSS were filtered onto pre-weighed (to 0.1 mg) glass fibre filters (Whatman GF/C) using Millipore filtration equipment. Filters were stored frozen for up to 5 weeks before being dried in an oven at 65°C for 24 hours. Filters were then re-weighed and TSS ($mg.l^{-1}$) calculated. Table 2.3 shows the mean, minimum and maximum TSS at both sites. Despite the average TSS at Darwin being higher (13.21 $mg.l^{-1}$) than that found at Goose Green (11.43 $mg.l^{-1}$), a comparison of TSS at the two sites using the non-parametric Kruskal-Wallis test revealed no significant differences ($H = 0.09$, $p > 0.05$).

Table 2.3. An eight day average of the total suspended solids and chlorophyll a concentrations in water samples collected from Darwin bridge and the 'Vicar of Brae' in January 1995.

Site	Total Suspended Solids ($mg.l^{-1}$)			Chlorophyll a ($mg.m^{-3}$)		
	mean	minimum	maximum	mean	minimum	maximum
Darwin bridge	13.21	2.15	29.40	0.118	0.018	0.321
'Vicar of Brae'	11.43	1.10	30.20	0.154	0.018	0.707

The three remaining replicate 2 litre samples of water destined for chlorophyll analysis were kept cool and in the dark (to prevent any deterioration of the photosensitive chlorophyll pigments) and similarly filtered using Millipore filtration equipment with GF/C glass fibre filters, before being stored frozen for approximately 5 weeks until further analysis could be undertaken. Chlorophyll extraction was carried out following the method outlined by Parsons *et al.* (1983). Each filter was placed in a 15 ml graduated centrifuge tube with 10 ml of 90% acetone, thoroughly shaken and left for 20 hours in

the dark at 5°C to allow pigment extraction to occur. The contents of each tube were subsequently centrifuged at room temperature for 10 minutes before the supernatant was decanted and the extinction coefficients determined spectrophotometrically. Measurements were made at 750, 664, 647 and 630 nm. All extinction coefficients were corrected for by cell to cell blanks (90% acetone) and turbidity (extinction at 750 nm). Only concentrations of chlorophyll a were determined as this is the most prominent pigment in algae. At those wavelengths for other pigments the extinction coefficients were negligible. Chlorophyll a concentrations were calculated using equations provided by Parsons *et al.* (1983) :

$$\text{Chl a} = 11.85\ E_{664} - 1.54\ E_{647} - 0.08\ E_{630} \qquad \text{Equation 2.2}$$

where Chl a is $mg.m^{-3}$ if a 1 cm light path cuvette is used and E is the absorbency at different wavelengths in the spectrophotometer;

$$mg\ chl.m^{-3} = \frac{C \times v}{V \times 10} \qquad \text{Equation 2.3}$$

where v is the volume of acetone in ml and V is the volume of seawater in litres. The eight day average and minimum and maximum chlorophyll concentrations are presented in Table 2.3. Concentrations in water from the 'Vicar of Brae' at Goose Green were generally higher than those from Darwin Bridge, however in both cases minimum values dropped below the lower limit of detection (0.02 $mg.m^{-3}$) for this method. The average values were also relatively low, 0.118 and 0.153 $mg.m^{-3}$, at Darwin and the 'Vicar of Brae' respectively. The fact that the precision of this method is relatively poor (± 10 - 30%), together with the extended periods between sample collection and analysis (which would result in lower than normal results and extraction being more difficult) suggest that the results obtained during this study should perhaps be viewed with caution.

The effects of temperature, tidal level, aerial exposure, salinity, water flow and food supply on reproduction and growth of *M.e.chilensis* at the three study sites are discussed in Chapters 3 and 4.

2.6. Statistical methods

Parametric statistical methods rather than non-parametric methods were employed where possible. Certain assumptions must be met before parametric testing can be used (Fry, 1993). The most important of these assumptions is that the data are normally distributed and that they have homogeneous variance. Data were tested for normality and homogeneity of variance using the statistical software package Minitab. Data were transformed and re-tested where necessary (Fry, 1993), usually by $\log_{10}$ methods. When data deviated from normality and heterogeneity was high, less sensitive non-parametric tests were used. Multiple comparison tests were performed where significant differences were observed. The non-parametric Dunn test (Whitaker, 1990) was chosen to identify significant differences between levels.

Least squares linear regression analysis was used to provide prediction equations and to understand the relationship between paired variables, x and y, in data sets where x and y are linearly related for n number of observations. The more sensitive least squares linear regression was chosen in preference to other more robust methods (Robust Linear Regression), mainly due to the fact that it is more powerful and provides considerably more information (Fry, 1993). Regression lines were compared by analysis of covariance using the General Linear Model command in the statistical software package Minitab.

Chapter 3

Reproduction, Condition and Settlement

3.1. Introduction

Reproduction in *Mytilus edulis* has been extensively studied, largely due to its relatively ubiquitous distribution and its commercial value (for reviews see Bayne, 1976; Seed, 1976; Sastry, 1979; Griffiths & Griffiths, 1987; Seed & Suchanek, 1992). *M.edulis* is typically dioecious with most populations containing approximately equal numbers of males and females, with only occasional incidences of hermaphroditism (Seed, 1976; Sunila, 1981; Kautsky, 1982a; Brousseau, 1983; Sprung, 1983).

The reproductive cycle of *M.edulis* varies both spatially and temporally, and generally exhibits a marked seasonal pattern (Seed & Suchanek, 1992). The timing and duration of this cycle probably results from complex interactions between several endogenous and exogenous factors, for example nutrient reserves, hormonal cycles, genotype, temperature, food and salinity (Newell *et al.*, 1982; de Zwaan & Mathieu, 1992). Typically gametogenesis is initiated in late autumn or early winter and proceeds throughout winter so that the gonads are morphologically ripe by early spring. The first major spawning takes place during the spring and summer months, and may be followed by a period of redevelopment prior to a second spawning in late summer or early autumn. Mussels then enter a reproductively quiescent phase during which time nutrient reserves are accumulated in order to fuel gametogenesis throughout winter when food supplies may be limited (Wilson & Hodgkin, 1967; Seed, 1975; Suchanek, 1981; Kautsky, 1982a; Lowe *et al.*, 1982; Brousseau, 1983; Sprung, 1983; Dix & Ferguson, 1984; Rodhouse *et al.*; 1984a; Emmett *et al.*, 1987; Kennedy, 1987; King *et al.*, 1989). Although temperature and food supply are known to be principal regulators of gametogenesis (Bayne, 1975; Newell *et al.*, 1982) mussels will develop gametes even when temperatures are close to zero (Kautsky, 1982a), suggesting that the quantity and quality of the food supply are the principal limiting factors (Bayne & Worrall, 1980; Newell *et al.*, 1982). Several methods, both direct and indirect, are available to monitor the reproductive cycle of *Mytilus*. Direct observations of spawning in natural or laboratory populations may be made, although the former is often difficult or impossible (Seed & Suchanek, 1992). The macro- and microscopic appearance of

the gonad may be monitored by taking routine samples from the natural population throughout the year. Resulting histological preparations generally provide reliable and detailed information regarding the reproductive cycle (Wilson & Seed, 1974; Kautsky, 1982a; King *et al*, 1989; Kimball & McElroy, 1993). Indirect methods include monitoring the appearance of larvae in the plankton (Wilson, 1988), however, these methods are generally less reliable since larvae and spat may have been transported by currents over considerable distances from parental stocks which have quite different environmental conditions (Seed & Suchanek, 1992).

It is well known that energy which is surplus to metabolic requirements can be utilised for somatic growth and/or gamete production (Hawkins & Bayne, 1992). Reproductive output or fecundity is a useful index that estimates the proportion of the energy budget which is allocated to reproductive processes. Reproductive output of several *M.edulis* populations has been estimated and mussel populations from contrasting sites compared (Thompson, 1979; Bayne & Worrall, 1980; Kautsky, 1982a; Sprung, 1983; Rodhouse *et al.*, 1984a). Environmental factors affecting reproductive output include variables such as temperature, food supply and tidal exposure (Thompson, 1979; Bayne & Worrall, 1980; Bayne *et al.*, 1983). Reproductive output can account for a substantial proportion of both total production and the standing crop of mussel populations (Griffiths & Griffiths, 1987). It can also represent a significant energy subsidy to the pelagic system. Kautsky (1982a), estimated that the reproductive output of Baltic *M.edulis* was equivalent to half the zooplankton production of the area, and was thus an important food source for herring larvae and carnivorous zooplankton.

Condition indices, in which the amount of flesh is related to the quantity of shell, of either individual or groups of mussels, have been used extensively in scientific research as well as being applied to the commercial shellfish industry (Dare & Edwards, 1976; Lutz, 1980; Aldrich & Crowley, 1986). Condition indices are commonly used to predict the time of maximum yields in cultured mussels (Lutz, 1980; Hickman, 1992). Once the general trend in yield is known, harvesting and marketing strategies can be developed accordingly. Condition indices and dry flesh weights of standard sized mussels are often used to determine the timing of spawning together with the reproductive and nutrient storage cycles of mussel populations (Dare & Edwards, 1975; 1976; Bayne & Worrall, 1980; Rodhouse *et al.*, 1984a, 1986; Thompson, 1984; Mallet & Carver, 1993). However, it is important to note that uncoupling of tissue and shell growth, which has been observed in some *M.edulis* populations (Hilbish, 1986;

Mallet & Carver, 1993), may seriously affect the results obtained from tissue weights adjusted to a standard length. A wide range of methods are available to determine the condition index of mussels (Lutz, 1980; Aldrich & Crowley, 1986). Davenport and Chen (1987) provide a number of examples and conclude that methods using dry tissue weight as a proportion of either shell weight or internal cavity volume of the shell are preferable. Methods utilising wet flesh weight or volume are less sensitive largely due to the difficulty in standardising the degree of wetness (Seed & Suchanek, 1992). Condition indices vary according to body size (Baird, 1958), season (Mason, 1976; Dix & Ferguson, 1984; Rodhouse *et al.*, 1984a; Emmett *et al.*, 1987; Kimball & McElroy, 1993; Schluter & Josefsen, 1994), level of parasitic infection (Kent, 1979; Thiesen, 1987; Ambariyanto & Seed, 1991; Tablado & Gappa, 1995), and local environmental conditions, in particular food availability and aerial exposure (Baird, 1966; Seed, 1980).

Following spawning and external fertilization, developing mussel larvae passively drift by water currents usually for a period of 2 - 4 weeks. In temperate waters this pelagic phase typically takes place throughout the spring and summer months. Some studies (Seed, 1969a; Rodhouse *et al.*, 1985), however, have recorded *M.edulis* in the plankton throughout much of the year. The settlement of plantigrades (postlarval mussels which have settled and metamorphosed, usually ≥ 260 µm in shell length) is generally via two phases, the primary settlement of early plantigrades <500 µm and the secondary settlement of late plantigrades > 500 µm. Settling plantigrades typically attach to a wide variety of filamentous substrata including the byssal filaments of conspecific adults (Petraitis, 1978; Eyster & Pechenik, 1987; McGrath *et al.*, 1988), filamentous algae (Paine, 1974; Suchanek, 1978; King *et al.*, 1989), fibrous ropes (Mason, 1976; Lutz, 1980; Rodhouse *et al.*, 1984a), or any of a number of artificial substrata, for example, rubberised hair (Seed, 1969a; Davies, 1974) and nylon domestic pan scourers (King *et al.*, 1990). Following settlement onto the adult mussel bed, whether directly (Bøhle, 1971; Kautsky, 1982a; McGrath *et al.*, 1988; King *et al.*, 1990) or indirectly (de Blok & Geelen, 1958; Bayne, 1964), recruiting juvenile mussels, having undergone some post-settlement mortality, may be observed as a new cohort joining the established mussel community when samples from the mussel population are routinely collected. The onset and duration of settlement and recruitment exhibits considerable spatial and temporal variation (see reviews Seed, 1976; Suchanek, 1985), and the main controlling factors include temperature, food supply and the availability of a suitable settlement surface.

Although widely distributed and locally abundant throughout much of the Falkland Islands there is surprisingly little information on *Mytilus* in this part of its geographic range. This chapter details some aspects of the reproductive biology of *Mytilus edulis chilensis* at a number of sites on the Falkland Islands. Results were determined from histological preparations of gonads taken at monthly intervals. The reproductive output of the populations was determined by counting and weighing the eggs collected from artificially spawned mussels. The seasonal variation in the condition index and dry tissue weight of a standard sized mussel are presented together with data on the settlement of juvenile mussels onto artificial substrata and into the established adult populations.

3.2. Materials and Methods

3.2.1. Gonad condition, index and gamete volume fraction

3.2.1.1. Sample collection and treatment

The three study sites selected for investigation (Chapter 2) were sampled on a monthly basis. To avoid any variation in reproductive condition resulting from mussel size, samples of between 25 and 30 animals of medium size (35 - 45 mm in shell length) were selected for histological examination. Any effect on reproduction caused by mussel size was investigated by collecting 50 small (25 - 35 mm), 50 medium (35 - 45 mm) and 50 large (45 - 55 mm) mussels in early January 1995, at the estimated time of their peak reproductive condition. Each mussel was opened and the entire mantle removed from the right valve of the shell. In the Falklands, mantle tissues were initially fixed in 5% saline buffered formalin (for up to 6 months), and on return to the UK, 1 cm^2 tissue samples were taken from the centre of each mantle and subsequently fixed in Bouins fluid for 12 hours. Samples were washed and stored in 70% alcohol prior to dehydration through increasing concentrations of ethanol (70% - 100%) terminating in toluene prior to embedding in paraffin wax at 60°C. Sections were cut from the paraffin wax blocks at a thickness of 7 µm on a rotary microtome, placed onto glass microscope slides, which were covered in a thin film of glycerol albumin solution, and allowed to dry over 24 hours on a copper hot-plate (30 - 40°C). Wax was removed from the sections, using Histoclear, and the tissues subsequently rehydrated through decreasing concentrations of ethanol before staining in aqueous

Ehrlich's haematoxylin. Samples were again dehydrated to enable mounting in non-aqueous DPX. After drying (7 days) slides were examined microscopically and the different reproductive stages photographed.

3.2.1.2. Assessment of gonad condition

Although several methods are available for monitoring changes in the reproductive cycle of mussels (Seed & Suchanek, 1992), during the course of this study the direct method of observing histological changes in gonad tissue was used. The timing of gamete development and spawning were monitored by calculating the gonad index and gamete volume fraction. Changes in the appearance of male and female gonads during gametogenesis were quantified by applying a simple scheme to microscopic sections. The arbitrary scheme initially used by Orton *et al.* (1956) for assessing the reproductive condition in *Patella vulgata*, and which was subsequently adapted by Seed (1969a) and Wilson and Seed (1974), to observe reproduction in *M.edulis*, is outlined below and used in the present study.

Four main phases in the reproductive cycle were identified; developing, ripe, spawning and spent. The developing and spawning phases were further divided into four stages. A gonad description for males and females follows:

Resting or spent

Stage 0: No traces of sexuality can be observed. During this period nutrient stores are accumulated in the connective tissue (Plate 3.1G).

Developing

Stage 1: The onset of gametogenesis; small areas of germinal tissue appear within the connective tissue. At this stage it is often difficult to separate individuals into males and females. No ova or spermatozoa are present (Plate 3.1A and 3.2A).

Stage 2: Some ripe gametes may appear in the centre of the follicles although these are mainly occupied by small oogonia attached to the germinal epithelium in females and spermatogonia in males (Plate 3.1B).

Plate 3.1 Photomicrographs of sectioned male gonads of *Mytilus edulis chilensis* at various stages of development, x 250.

A. Developing male stage 1, islands of germinal tissue within the connective tissue filled with spermatogonia.

B. Developing stage 2, slightly larger follicles containing spermatocytes and spermatids.

C. Developing stage 3, a few darkly stained nuclei of spermatozoa are scattered between the larger cells.

D. Ripe stage 5, follicles packed with spermatozoa arranged in lamellae, a few residual spermatocytes and spermatids are present around the periphery.

E. Spawning stage 4, partial release of gametes has taken place, although remaining gametes retain lamella arrangement.

F. Spawning stage 1, follicles are virtually empty, and considerably reduced in size.

G. Resting or spent stage 0, no follicles are present within the connective tissue.

H. Redeveloping stage 2, a layer of undifferentiated early stage spermatogonia line the empty follicle.

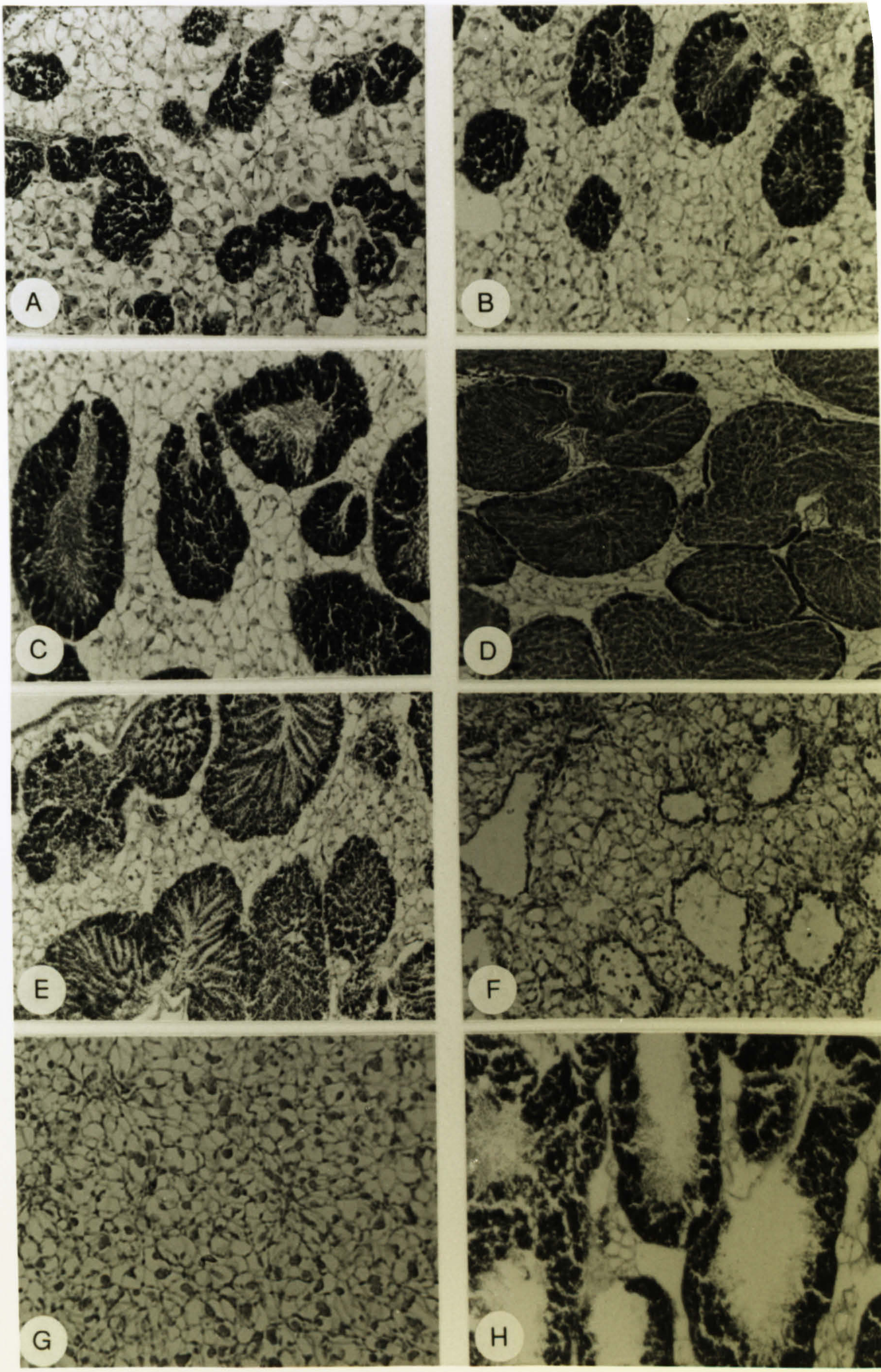

A
B
C
D
E
F
G
H

Plate 3.2 Photomicrographs of sectioned female gonads of *Mytilus edulis chilensis* at various stages of development, x 250.

A. Developing stage 1, islands of germinal tissue with a few small oogonia basally attached to the follicle wall.

B. Developing stage 3, larger oocytes still basally attached to the germinal epithelium.

C. Ripe stage 5, little or no connective tissue visible, oocytes at maximum size and compressed into polyhedral shape due to increased pressure within follicles.

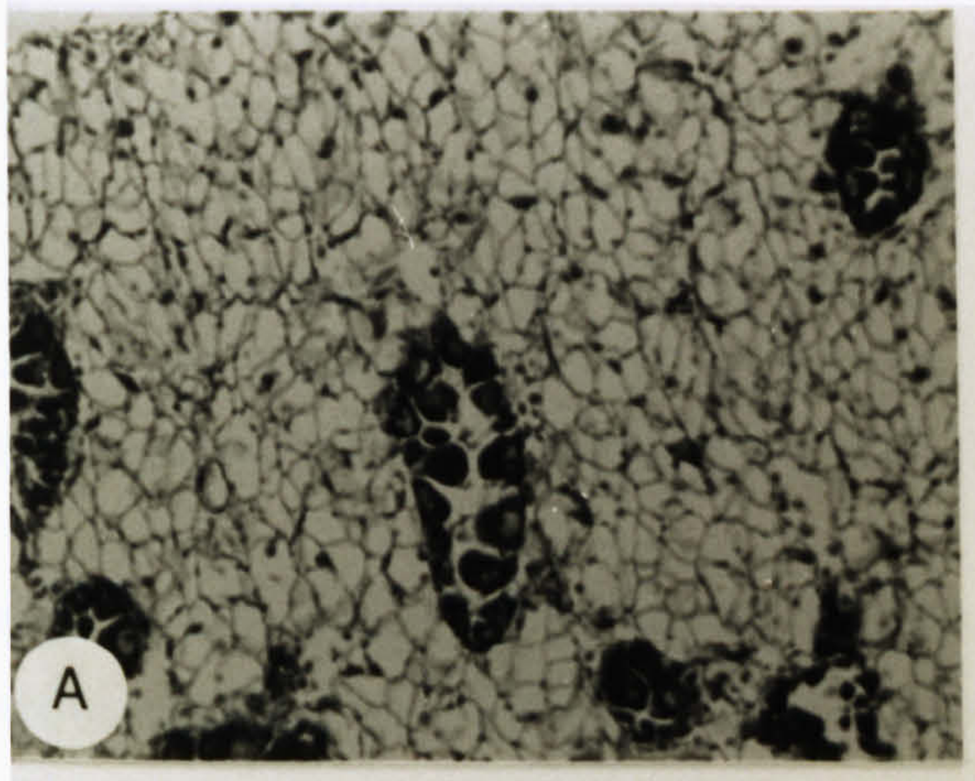
D. Spawning stage 4, the release of some gametes has reduced the pressure within the follicles and remaining oocytes have become spherical in shape.

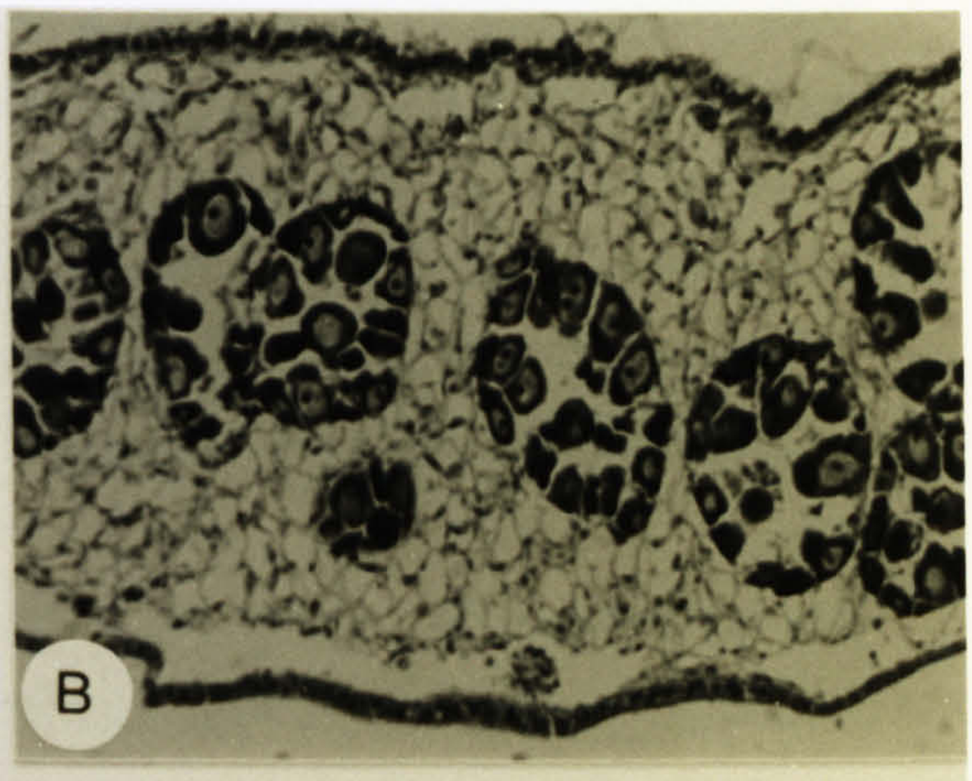
E. Spawning stage 2, a few large oocytes remain and some cytolysis is taking place.

F. Spawning stage 1, follicles have collapsed and only residual ova remain, considerable cytolysis can be observed.

G. Redeveloping stage 1, a layer of undifferentiated early stage oogonia line the empty follicles.

H. A ripe gonad which has partially spawned as a result of chemical stimulation, x 100.


A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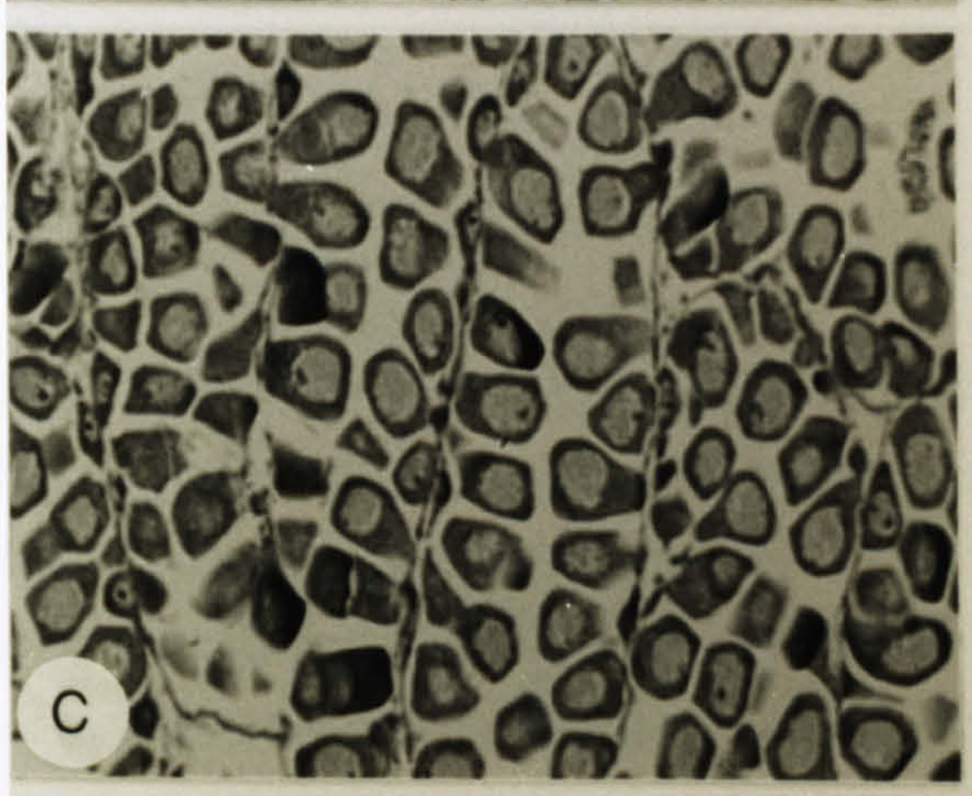
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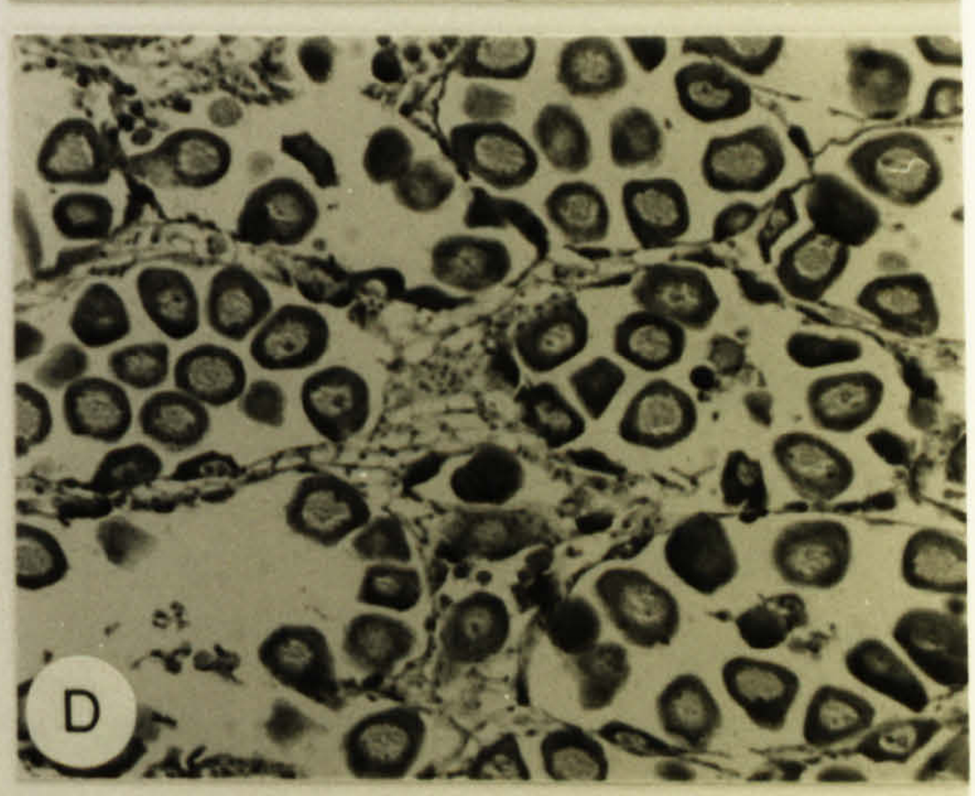
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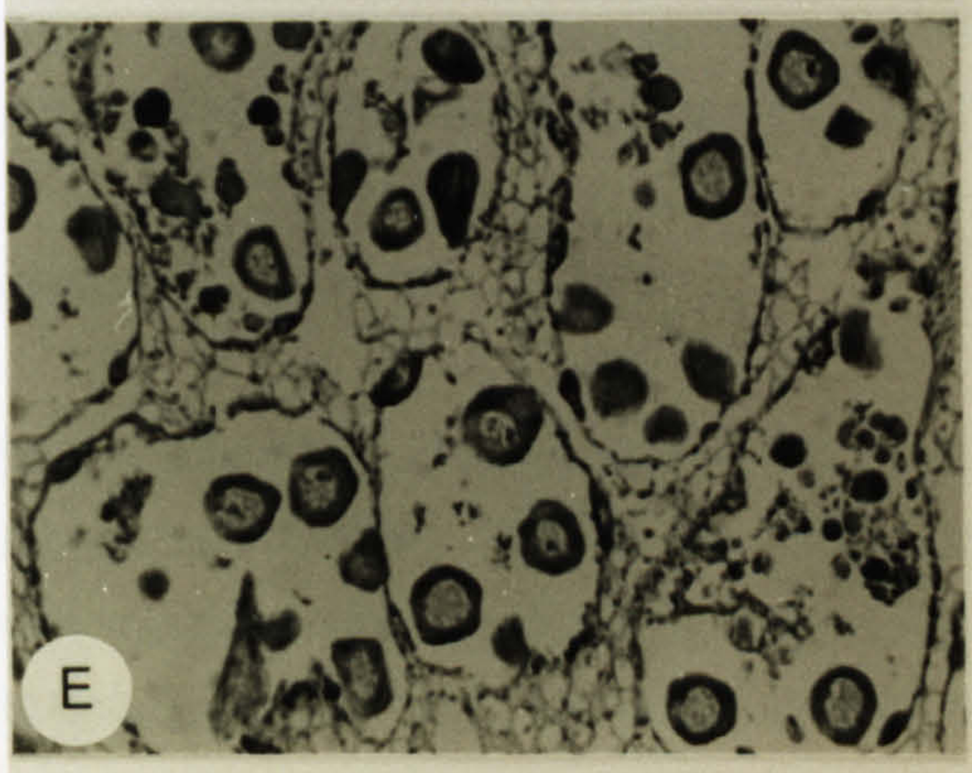
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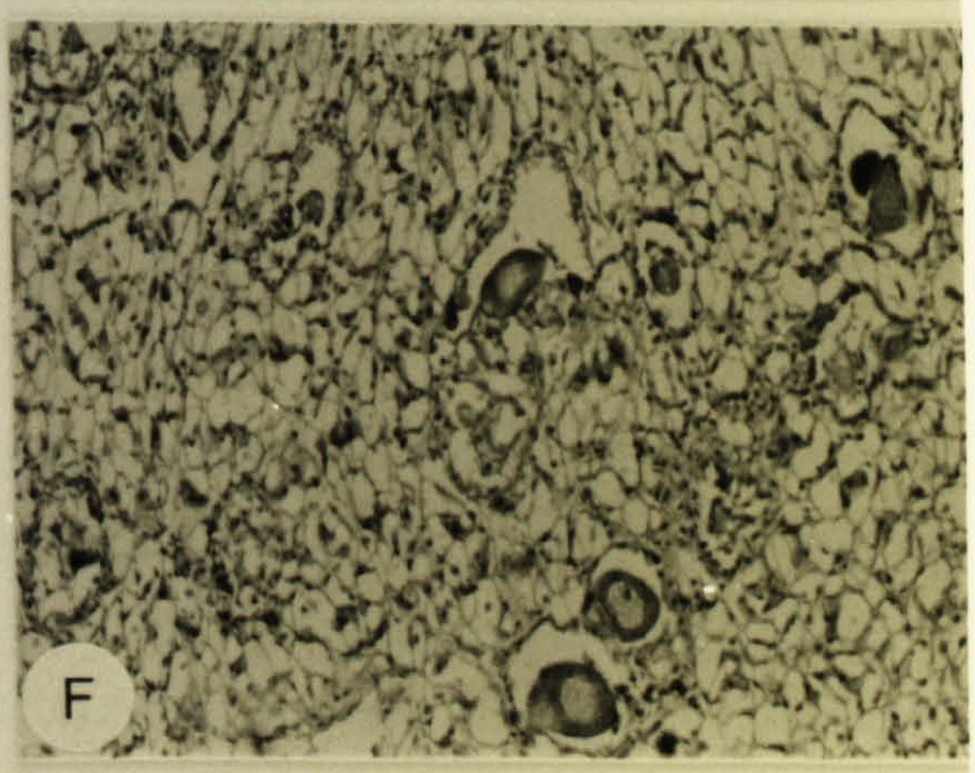
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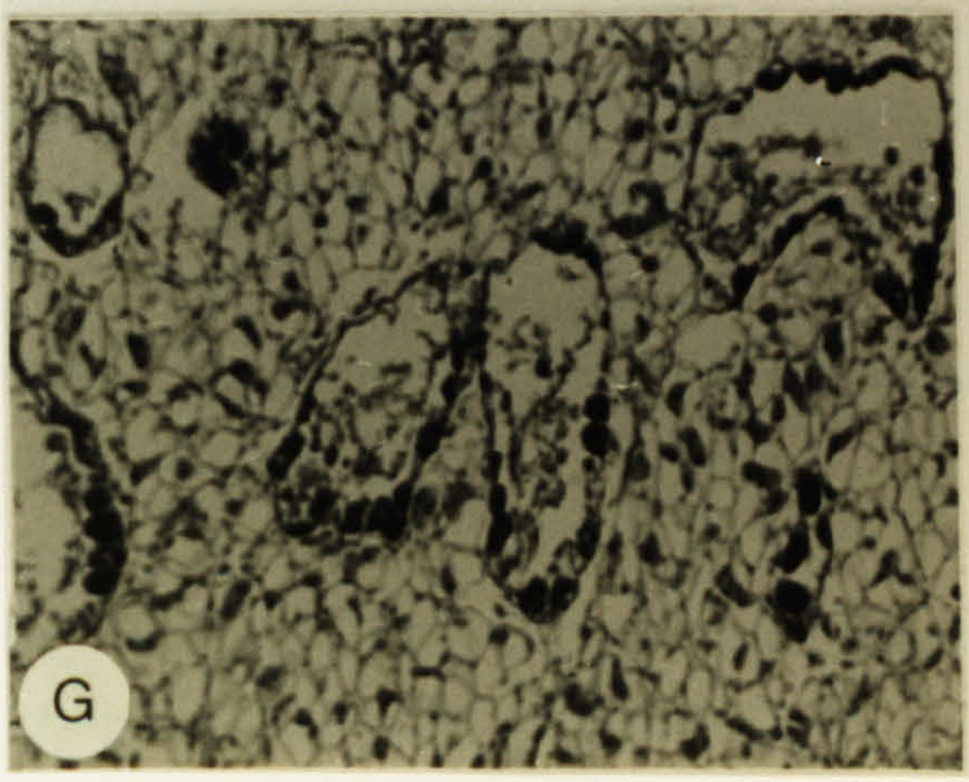
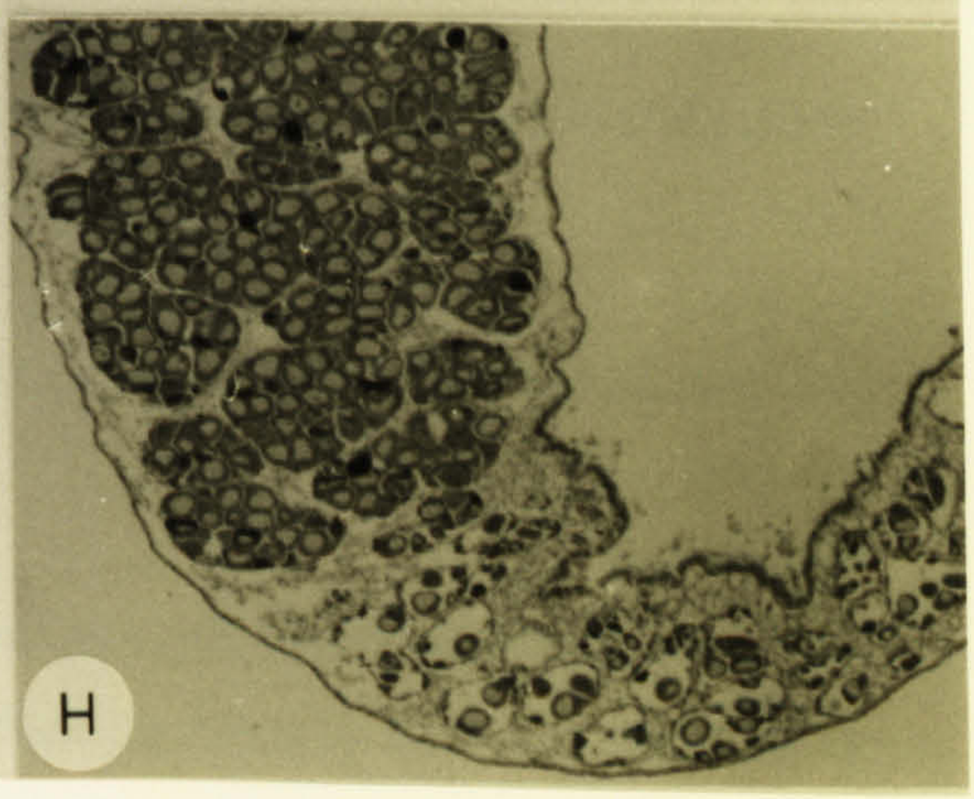

G


H

Stage 3: A substantial increase in gonad size is evident. In males primary and secondary spermatocytes and spermatids fill the follicles with a few darkly stained nuclei of spermatozoa scattered between the larger cells (Plate 3.1C). In females oocytes have begun to accumulate yolk and have grown considerably. Some larger oocytes are still attached to the follicular epithelium by a slender stalk of cytoplasm which eventually ruptures to leave the oocytes free within the follicle (Plate 3.2B).

Stage 4: The gonad tissue almost reaches maximum volume. Ripe gametes are predominant within the follicles, although gametogenesis is still in progress (Plate 3.1D).

Ripe

Stage 5: The gametes are now morphologically ripe. In males the follicles are packed with spermatozoa arranged in lamellae which converge towards the centre of the lumen. A few residual spermatocytes and spermatids may be present. In females the majority of oocytes have reached their maximum size and increased pressure within the follicles compresses the oocytes into polyhedral forms (Plate 3.2C). Connective tissue, which has now given up most of its nutrient reserves for gametogenesis, is almost completely obscured by the swollen follicles.

Spawning

Stage 4: The release of gametes has started. Large numbers of ripe oocytes are still present in the follicles, although those remaining are spherical in shape, a result of the decrease in pressure (Plate 3.2D). Large numbers of spermatozoa line the follicles but the arrangement of the lamellae is considerably reduced (Plate 3.1E).

Stage 3: The follicles are now approximately half empty and there are no visible signs of gametogenesis, only mature gametes remain (Plate 3.2E).

Stage 2: There is a general reduction in the area occupied by genital tissue, and few gametes remain.

Stage 1: Follicles are beginning to collapse and degenerate, although residual ova and spermatozoa may still be present and can often be seen undergoing cytolysis by

amoeboid phagocytes (Plate 3.1F and 3.2F). In some extreme cases follicles do not collapse and are still present at the onset of gametogenesis the following year (Plate 3.1H).

3.2.1.3. Sex ratios

The ratios of male to female mussels were calculated after microscopic examination of the tissue sections.

3.2.1.4. Gonad index

The gonad index, an arbitrary scheme which provides an estimate of the reproductive condition of the population, was calculated for both sexes of *M.e.chilensis* at the three study sites using the method outlined by Seed (1969a). Mean gonad indices were determined by multiplying the number of individuals in each stage by the arbitrary numerical factor assigned to each stage (0, 1, 2, 3, 4 or 5), and dividing the sum of these products by the total number of individuals in the sample. The resulting value ranges from 0 when all individuals in the sample are spent or resting, to a maximum of 5 when all individuals are sexually mature or ripe. Seasonal variations in the reproductive cycle were examined by plotting the mean gonad index over the study period. Gametogenesis could be identified by an increase in the index and spawning by a decrease.

3.2.1.5. Gamete volume fraction

Arbitrary classifications such as gonad indices are rather subjective and do not necessarily recognise the intermediate stages of reproductive development. More importantly they provide no information on nutrient storage within the mantle tissue cells. Hence, in the present study the more quantitative stereological method (Bayne *et al.*, 1978; Lowe *et al.*, 1982) of volume fraction determination has been used to supplement information provided by the nominal gonad indices. Ten histological sections were randomly selected from the routine monthly samples in order to determine the relative quantities of tissue components, such as developing gametes, ripe gametes, spent follicles and connective tissue. A statistically derived grid, comprising 45 random points, was applied to the tissue sections when viewed by a TV camera mounted on a light microscope, and the tissue component present at each

random point recorded. Four replicate point counts were made for each individual and volume fractions, ie the percentages of mantle tissue occupied by developing gametes, ripe gametes, empty follicles or connective tissue, determined. Volume fractions varied in proportion according to the stage of the reproductive cycle. Average volume fractions of particular tissue components were calculated for male and female *M.e.chilensis* at each of the three study sites. The effect of mussel size on the gamete volume fraction was assessed by comparing small, medium and large individuals using the non-parametric Kruskal-Wallis test and Dunn's test for multiple comparisons where significant variation was observed (Whitaker, 1990).

3.2.2. Assessment of condition index and tissue weight

Seasonal changes in gametogenesis were also assessed at the population level by monitoring changes in condition indices and dry tissue weights of standard-sized individuals.

3.2.2.1. Sample collection and treatment

Monthly samples of 50 mussels over the size range of the population were collected from each site (see Chapter 2) and frozen at -25°C for up to 6 months awaiting transportation to the UK or following field visits. The length of each mussel was subsequently measured to 0.1 mm using vernier calipers, opened and the soft tissues removed and placed into pre-weighed aluminium boats. Shell and tissue weights were determined (to the nearest 0.1 mg) after oven drying at 65°C for 24 hours and 3 days respectively.

3.2.2.2. Analysis of data

Condition index (C.I.) was determined using the statistically robust and convenient formula (equation 3.1) used by Davenport and Chen (1987):

$$Condition\ Index\ (\%) = \frac{dry\ tissue\ weight}{shell\ weight} \times 100 \qquad \text{Equation 3.1}$$

Seasonal variations in condition index were plotted against shell length and regression

lines calculated. Non-linear dry tissue weights were linearised by log transformation and similarly plotted against shell length. Regression equations were subsequently calculated. Any seasonal changes in population condition indices as well as dry tissue weights were tested by applying the general linear model (Fry, 1993) with a single covariate (GLM in Minitab). Wherever possible, the monthly condition index and dry flesh weight of a standard-sized, 40 mm, mussel (= average size mussel) were calculated from the regression equations relating condition index and dry tissue weight to shell length. In cases where the data did not significantly fit the least squares linear regression model, or when the fitted regression slopes were heterogeneous (ie rotating), condition indices and tissue weights were calculated from monthly sub-samples of 10 animals (around 40 mm in shell length). Differences in condition between the three study sites were determined by comparing condition indices and tissue weights of 10 standard-size animals from monthly samples using the non-parametric Kruskal-Wallis test (Whitaker, 1990).

The degree of correlation between total body condition (condition index, dry tissue weight), reproductive condition (gonad index, gamete volume fraction) and temperature were assessed over the study period using the Spearman Rank Order correlation (Minitab).

3.2.3. Settlement

In order to assess the level of settlement of mussel spat at the three study sites two methods were employed. Firstly, the deployment of artificial substrate units (ASU) adjacent to the adult population, and secondly, the examination of length frequency distributions of samples of the mussel populations.

3.2.3.1. Artificial substrate units (ASU)

The Vileda Industrial Super Scourer was routinely used as an ASU during this study. Fresh (without naturalising) ASU (15.0 x 11.5 x 0.9 cm) were deployed in the mid region of the mussel bed at each study site for the period October 1994 through to February 1996 to monitor settlement of *M.e.chilensis* spat. ASU attachment was initially onto wooden boards (0.7 x 0.4 m) which were secured horizontally on the shore by vertical steel rods. However, when weather conditions resulted in the loss of the wooden boards pads were fixed directly to the vertical steel rods using cable

ties. Davies (1974) has previously shown that no significant difference between settlement intensities of mussels onto horizontally and vertically positioned 'Hairlok' pads occurred in the Menai Strait, UK. Pads were deployed and replaced after periods of one month, and occasionally longer if sites were rendered inaccessible during severe winter conditions. On collection, pads were frozen until further analysis could be carried out.

Each pad was thawed, thoroughly washed using a high pressure water jet and the residual sample of detritus and organisms retained by a 190 µm filter, to prevent loss of spat. The sample, made up to approximately 200 ml, was subsequently "split" using a meiofauna sample splitter (250 ml total capacity) following a design by Jensen (1982). The chambers of the sample-splitter, labelled 1 to 8, were tested for bias by splitting ten 200 ml samples containing 50 *Mytilus* spat provided by Miguel Angel del Rio at the School of Ocean Sciences and subsequently applying a 2-way ANOVA (Fry, 1993) to the resulting data. Three chambers were selected randomly using random numbers tables, and the number of mussels present in each chamber determined using a Bogorov tray and binocular microscope. Numbers (mean ± standard error) of spat (0.2 - 2.0 mm in shell length) per square metre were counted and plotted over time for each site. Any overall differences in settlement intensity between the three sites were determined by comparing pooled data using the non-parametric Kruskal-Wallis test (Whitaker, 1990). Significant differences were identified using the Dunn statistic for non-parametric multiple comparisons (Whitaker, 1990).

3.2.3.2. Length frequency distribution

Settlement into the established mussel population was monitored by observing seasonal changes in the length frequency distributions of the mussels. Random monthly, bi-monthly or quarterly samples were collected by quadrat (0.17 m^2) from each study site. All mussels removed from the substratum were measured to 0.1 mm by vernier calipers and the frequency distributions of each population determined.

3.2.4. Fecundity and Reproductive output

There is a wide range of both direct and indirect methods available for assessing the reproductive output of *Mytilus* populations (Seed & Suchanek, 1992). The direct method of artificial spawning was employed during this investigation.

Twenty-five mussels over the size range present within the population were collected several weeks prior to the expected time of natural spawning and artificially induced to spawn by subjecting them to both physical and chemical shocks (Bayne *et al.*, 1983). Mussels were cooled to 5°C for approximately 3 hours, injected, between the shell valves into the mantle cavity, with 2 ml of 0.5M KCl, and thoroughly shaken. These 'shocked' mussels were then left out of water for one hour before being placed into individual containers containing filtered seawater at 17°C. If after 12 hours no spawning had occurred, the water inside the individual containers was changed and mussels left for a further 6 hours before being discarded. Males, which were identified by a milky colouration of the water, were not required and therefore discarded. Female mussels produced negatively buoyant orange eggs which were visible on the bottom of the container. Mussels were induced to spawn until the eggs from 25 females were successfully collected. Detritus, such as faecal material, was separated from the eggs by filtering the suspension of eggs through an 80 µm sieve. The eggs were retained by a 20 µm sieve, washed and a stock solution, normally of one litre, made up using filtered seawater. Wherever possible, three replicate 100 ml sub-samples were decanted from the homogenised stock solution. The number of eggs present in each 100 ml sub-sample was estimated from counts made from five replicate 20 µl samples. The remainder of each of the three sub-samples were subsequently filtered using a Millipore filter apparatus fitted with a pre-weighed GF/C filter. The residual salt retained on the filter paper was removed by washing with 5 ml of 0.1M ammonium formate. Filters were frozen at -25°C and stored until they could be dried for 3 days at 65°C and re-weighed to 0.1 mg.

In order to determine whether or not the mussels had completely spawned both visual and quantitative (volume fraction) estimates were made. Following visual examination of the opened mussel, a section of mantle tissue from each spawned individual was removed, fixed and resulting wax sections examined histologically (see section 3.2.1.). Simultaneously, samples of mantle tissue from 10 females from each site, which were not artificially induced to spawn, were fixed and examined histologically. Mean volume fractions of ripe gametes were calculated from 4 random point counts for each mussel. The proportion of gametes spawned artificially was determined by comparing the gamete volume fractions of spawned individuals with unspawned mussels from the natural population. The proportion of the mussel tissue weight which is comprised of reproductive material (gametes) was calculated after the dry weights of the remaining body and mantle tissues, together with the dry weight of eggs that were

artificially induced to spawn, were determined.

Maximum fecundity, in terms of the number of eggs for each spawned mussel, was determined and regressed on shell length by least squares linear regression (Fry, 1993). Differences between the maximum fecundities of a standard-sized (40 mm) mussel from each site were identified, where possible, using the general linear model with a covariate (Minitab). Alternatively the average of six artificially spawned mussels of approximately 40 mm in shell length was calculated. The reproductive output of the population was determined as the product of the predicted fecundity over the population size range (calculated either from regression constants or from the actual spawned animals) and the density of mussels in 5 mm size classes predicted from population size frequency distributions.

3.3. Results

3.3.1. Gonad maturation, sex ratios and mussel size

Histological preparations of mantle tissue provided an insight into the pattern of gonad maturation in *Mytilus edulis chilensis* from the Falkland Islands. The onset of gametogenesis was characterised by islands of germinal tissue developing within the connective tissue matrix of the mantle. Sex determination was not possible until individual spermatogonia and oogonia could be identified within the follicles (Plate 3.1A and 3.2A).

In males, spermatogonia gave rise to large primary and secondary spermatocytes which subsequently matured into spermatids and reached maximum maturity as considerably smaller spermatozoa. The darkly stained spermatozoa were observed with their acrosomes positioned towards the follicle periphery and their tails occupying the central position of the lumen. Thus the classical formation was established with the earliest stages adjacent to the follicle wall and increasingly mature stages towards the follicle centre (Plate 3.1). Even when males reached maximum maturity a thin layer of early spermatogenic stages could still be observed lining the follicle wall.

Early oogonia lay flattened against the follicle wall, but as they grow and build up yolk reserves they became progressively more elongate and basally constricted (Plate

3.2C). Eventually ripe oocytes detached from the follicle wall and rounded off within the follicle lumen as the gonad matured. Early stages of oogonia were present around the follicle periphery even after spawning had started (Plate 3.2D). Immature gametes lining old follicles appeared to mark the onset of a new gametogenic cycle; however these redeveloping stages were often observed, apparently frozen in time, for several months over the austral winter. The occurrence of old follicles which had not fully spawned, but which also had not regressed, was relatively common in male *M.e.chilensis* (Plate 3.1H). These follicles often remained at their maximum size until the onset of the following gametogenic cycle.

Mytilus edulis chilensis is dioecious and no cases of hermaphroditism were observed during this study. Overall sex ratios of males : females at the three sites were 1.07:1, 1.48:1 and 1:1 at Darwin, Camilla Creek and Goose Green respectively. Departure from a 1:1 ratio was tested for by applying a Chi-squared approximation, which identified the population at Camilla Creek as the only site where departure was significant, and in this case in favour of males ($\chi^2 = 16.98$, d.f. = 1, $p < 0.05$).

Mussel size had a significant effect upon the reproductive condition of populations of *M.e.chilensis* from Camilla Creek ($H = 15.32$, d.f. = 2, $p < 0.01$) and Goose Green ($H = 9.22$, d.f. = 2, $p < 0.05$), but not on those from Darwin ($H = 4.87$, d.f. = 2, $p > 0.05$). At Camilla Creek, however, small mussels had significantly higher gamete volume fractions than either medium ($D = 3.32$, $p < 0.05$) or large ($D = 3.47$, $p < 0.05$) mussels, whereas at Goose Green the reproductive condition of small individuals was significantly lower than that of large mussels ($D = 2.89$, $p < 0.05$).

3.3.2. Seasonal changes in reproductive condition

Seasonal changes in the reproductive condition of mussels from the three study sites were observed using mean gonad indices, numbers of ripe and spent follicles and relative volume fractions of different tissue components of the mantle (Tables 3.1, 3.2, 3.3 and Figures, 3.1, 3.2 and Appendix 2). Reproductive condition appeared to be highly seasonal with no obvious differences in timing between the three study populations.

An increase in mean gonad index and developing gamete volume fraction marked the onset of gametogenesis in early spring (August - September) as seawater

Table 3.1 Distribution of gonad stages in monthly samples of *Mytilus edulis chilensis* from Darwin

Date	No. of mussels examined	Males										Females									Gonad Index
		Developing				Ripe		Spawning			Spent	Developing				Ripe		Spawning			
		1	2	3	4	5	4	3	2	1	0	1	2	3	4	5	4	3	2	1	
21/09/93	23	0	2	1	0	0	0	0	3	1	4	1	4	4	0	0	0	0	2	1	1.74
16/10/93	25	3	6	0	0	0	0	0	2	1	5	0	2	2	1	2	0	0	0	1	1.80
14/11/93	28	0	0	4	3	5	2	0	0	1	1	0	2	3	1	4	0	0	1	1	3.50
11/12/93	27	0	0	0	0	3	3	2	1	0	3	0	0	0	0	11	2	0	1	1	3.74
15/01/94	28	0	0	0	0	9	2	0	1	2	6	0	0	0	0	1	0	1	2	4	2.61
12/02/94	27	0	0	0	0	0	0	1	3	0	16	0	0	0	0	0	0	2	0	5	0.74
13/03/94	24	0	0	0	0	2	2	1	0	1	18	0	0	0	0	0	0	0	0	0	0.92
09/04/94	23	2	0	0	0	0	0	0	1	1	12	7	0	0	0	0	0	0	0	0	0.52
15/05/94	27	7	0	0	0	0	0	1	0	3	4	12	0	0	0	0	0	0	0	0	0.93
14/06/94	22	1	1	0	0	0	0	0	2	1	1	3	9	2	0	0	0	0	0	2	1.68
06/08/94	18	0	0	0	0	0	0	0	1	5	4	2	6	0	0	0	0	0	0	0	1.17
05/09/94	23	2	1	0	0	0	0	0	1	3	2	10	4	0	0	0	0	0	0	0	1.17
06/10/94	33	3	11	3	0	0	0	0	0	0	0	5	6	5	0	0	0	0	0	0	2.00
03/11/94	27	1	3	3	3	1	1	0	0	0	0	2	2	5	3	3	0	0	0	0	3.15
08/12/94	22	0	0	0	0	1	2	2	0	0	0	0	0	1	7	8	1	0	0	0	4.27
02/01/95	51	0	0	0	1	0	6	11	3	1	1	0	0	0	0	1	6	5	8	8	2.67
01/02/95	28	0	0	0	0	0	0	2	1	3	19	0	0	0	0	0	0	0	0	3	0.50
02/03/95	29	0	0	0	0	0	0	1	0	3	22	3	0	0	0	0	0	0	0	0	0.31
14/04/95	49	8	0	0	0	0	0	2	0	3	35	1	0	0	0	0	0	0	0	0	0.37
13/05/95	26	10	2	0	0	0	0	1	0	0	4	9	0	0	0	0	0	0	0	0	1.00
18/06/95	32	10	2	0	0	0	1	0	1	1	8	9	0	0	0	0	0	0	0	0	0.94
14/08/95	24	8	4	0	0	0	0	0	0	0	0	5	6	1	0	0	0	0	0	0	1.50
23/09/95	30	5	11	5	0	0	0	0	0	0	1	3	2	3	0	0	0	0	0	0	1.93
26/10/95	29	0	0	7	8	0	0	0	0	0	0	0	0	2	9	3	0	0	0	0	3.79
24/11/95	28	0	0	0	0	11	0	0	0	0	0	0	0	0	4	13	0	0	0	0	4.86
21/12/95	30	0	0	0	1	4	9	1	0	0	1	0	0	0	0	0	5	5	3	1	3.50
20/01/96	30	0	0	0	0	0	0	1	2	7	14	0	0	0	0	0	0	0	2	4	0.73
18/02/96	29	0	0	0	0	0	0	0	6	1	21	0	0	0	0	0	0	0	0	1	0.48

Table 3.2 Distribution of gonad stages in monthly samples of *Mytilus edulis chilensis* from Camilla Creek

Date	No. of mussels examined	Male										Female									Gonad Index
		Developing				Ripe	Spawning				Spent	Developing				Ripe	Spawning				
		1	2	3	4	5	4	3	2	1	0	1	2	3	4	5	4	3	2	1	
15/09/93	24	4	5	2	0	0	1	0	0	1	3	3	3	2	0	0	0	0	0	0	1.67
16/10/93	18	4	2	4	0	0	1	0	0	0	0	3	2	1	1	0	0	0	0	0	2.11
14/11/93	25	5	1	1	2	1	1	0	0	0	3	8	1	0	1	1	0	0	0	0	1.84
11/12/93	18	1	1	0	0	3	2	2	1	0	1	0	0	0	0	3	2	0	1	1	3.33
15/01/94	27	0	0	0	0	0	6	3	2	1	9	0	0	0	0	0	1	1	2	2	1.89
12/02/94	23	0	0	0	0	0	4	2	2	0	14	0	0	0	0	0	0	0	0	1	1.17
13/03/94	23	0	0	0	0	0	0	2	1	1	18	0	0	0	0	0	0	0	0	1	0.43
09/04/94	24	3	0	0	0	0	0	2	0	2	13	4	0	0	0	0	0	0	0	0	0.63
15/05/94	28	4	0	1	0	0	3	4	0	2	10	4	0	0	0	0	0	0	0	0	1.32
14/06/94	23	6	1	0	0	0	0	0	0	4	9	3	0	0	0	0	0	0	0	0	0.65
06/08/94	23	6	0	0	0	0	0	1	1	3	8	4	0	0	0	0	0	0	0	0	0.78
05/09/94	30	5	1	0	0	0	0	0	0	0	16	6	2	0	0	0	0	0	0	0	0.57
06/10/94	25	6	3	1	0	0	0	0	0	2	4	4	2	3	0	0	0	0	0	0	1.36
03/11/94	27	7	5	4	3	0	0	0	0	0	2	1	2	2	1	0	0	0	0	0	2.07
08/12/94	24	0	5	1	0	2	1	1	1	0	5	1	1	3	1	0	0	2	0	0	2.25
03/01/95	57	0	2	1	0	0	1	6	6	5	9	1	0	0	0	0	2	7	7	10	1.75
01/02/95	26	0	0	1	0	0	0	0	1	3	18	0	0	0	0	0	0	0	0	3	0.42
02/03/95	29	0	0	0	0	0	0	3	1	3	20	2	0	0	0	0	0	0	0	0	0.55
14/04/95	49	5	0	0	0	0	0	1	0	2	40	1	0	0	0	0	0	0	0	0	0.22
13/05/95	24	1	0	0	0	0	0	1	1	2	18	1	0	0	0	0	0	0	0	0	0.38
18/06/95	28	4	0	0	0	0	0	1	3	0	15	4	1	0	0	0	0	0	0	0	0.68
23/09/95	22	8	1	0	0	0	0	0	0	0	5	4	4	0	0	0	0	0	0	0	1.00
26/10/95	26	2	5	6	1	0	0	0	0	0	0	4	1	5	2	0	0	0	0	0	2.42
24/11/95	30	0	2	8	3	1	0	0	0	0	0	0	1	7	6	0	1	1	0	0	3.30
22/12/95	34	0	0	0	1	10	2	1	1	0	3	0	0	3	4	2	2	3	1	1	3.59
20/01/96	28	0	0	0	0	0	1	1	4	3	11	0	0	0	1	0	1	3	2	1	1.43
18/02/96	31	2	0	0	0	0	1	3	2	6	13	0	0	0	0	0	0	0	0	4	0.94

Table 3.3 Distribution of gonad stages in monthly samples *Mytilus edulis chilensis* from Goose Green

Date	No. of mussels examined	Male									Spent	Female									Gonad Index
		Developing				Ripe		Spawning				Developing				Ripe		Spawning			
		1	2	3	4	5	4	3	2	1	0	1	2	3	4	5	4	3	2	1	
27/09/93	23	4	8	2	0	0	0	0	0	0	1	2	3	3	0	0	0	0	0	0	1.87
16/10/93	24	0	0	1	7	2	1	0	0	0	0	0	0	0	4	8	1	0	0	0	4.38
14/11/93	26	0	0	0	0	7	2	0	0	0	0	0	0	0	0	12	5	0	0	0	4.73
11/12/93	27	1	0	0	0	3	4	0	0	0	2	0	0	0	0	3	9	3	1	1	3.52
15/01/94	26	0	0	0	0	0	8	1	2	2	1	0	0	0	0	0	3	4	0	5	2.69
12/02/94	23	2	0	0	0	0	3	1	0	2	13	0	0	0	0	0	0	1	0	1	1.00
13/03/94	27	3	0	0	0	0	1	1	2	5	14	0	0	0	0	0	0	0	1	0	0.78
09/04/94	29	5	0	0	0	0	3	0	4	1	3	13	0	0	0	0	0	0	0	0	1.34
15/05/94	18	3	0	0	0	0	0	0	0	0	14	1	0	0	0	0	0	0	0	0	0.22
14/06/94	26	4	5	0	0	0	0	0	0	0	0	14	3	0	0	0	0	0	0	0	1.31
06/08/94	21	6	1	0	0	0	0	0	0	0	5	8	1	0	0	0	0	0	0	0	0.86
05/09/94	27	5	7	2	0	0	2	0	0	0	0	7	4	0	0	0	0	0	0	0	1.78
06/10/94	28	3	7	2	0	0	0	0	0	0	0	2	3	10	1	0	0	0	0	0	2.32
03/11/94	30	0	0	0	0	0	5	5	2	0	0	0	0	5	12	0	1	0	0	0	3.53
08/12/94	24	0	0	0	1	8	3	0	0	0	0	0	0	0	6	6	0	0	0	0	4.58
01/01/95	53	0	0	0	0	0	12	10	2	1	0	0	0	0	0	2	9	11	5	1	3.26
01/02/95	31	0	0	0	0	0	0	0	1	2	25	0	0	0	0	0	0	0	0	3	0.23
02/03/95	30	2	0	0	0	0	0	0	1	2	24	0	0	0	0	0	0	0	0	1	0.23
14/04/95	49	6	0	0	0	0	0	0	0	2	41	0	0	0	0	0	0	0	0	0	0.16
13/05/95	19	3	3	0	0	0	0	0	0	2	1	7	3	0	0	0	0	0	0	0	1.26
18/06/95	28	11	1	1	0	0	0	0	0	1	6	7	1	0	0	0	0	0	0	0	0.93
23/09/95	23	1	4	3	0	0	0	0	0	0	0	2	8	5	0	0	0	0	0	0	2.22
26/10/95	30	0	0	3	13	1	0	0	0	0	0	0	0	3	9	1	0	0	0	0	3.87
24/11/95	30	0	0	0	0	12	2	0	0	0	0	0	0	0	4	12	0	0	0	0	4.80
21/12/95	30	0	0	2	1	9	4	0	0	0	0	1	0	0	3	5	3	1	1	0	4.20
20/01/96	29	0	0	1	1	2	8	0	0	0	2	0	0	0	1	3	4	2	2	3	3.34
18/02/96	30	0	0	0	1	6	3	0	0	0	9	1	0	0	0	0	0	3	3	4	2.20

Figure 3.1 Gonad index of male (closed circles) and female (open circles) *Mytilus edulis chilensis* and the percent of the population in ripe (closed bar) and spent (open bar) condition at

A. Darwin

B. Camilla Creek

C. Goose Green.

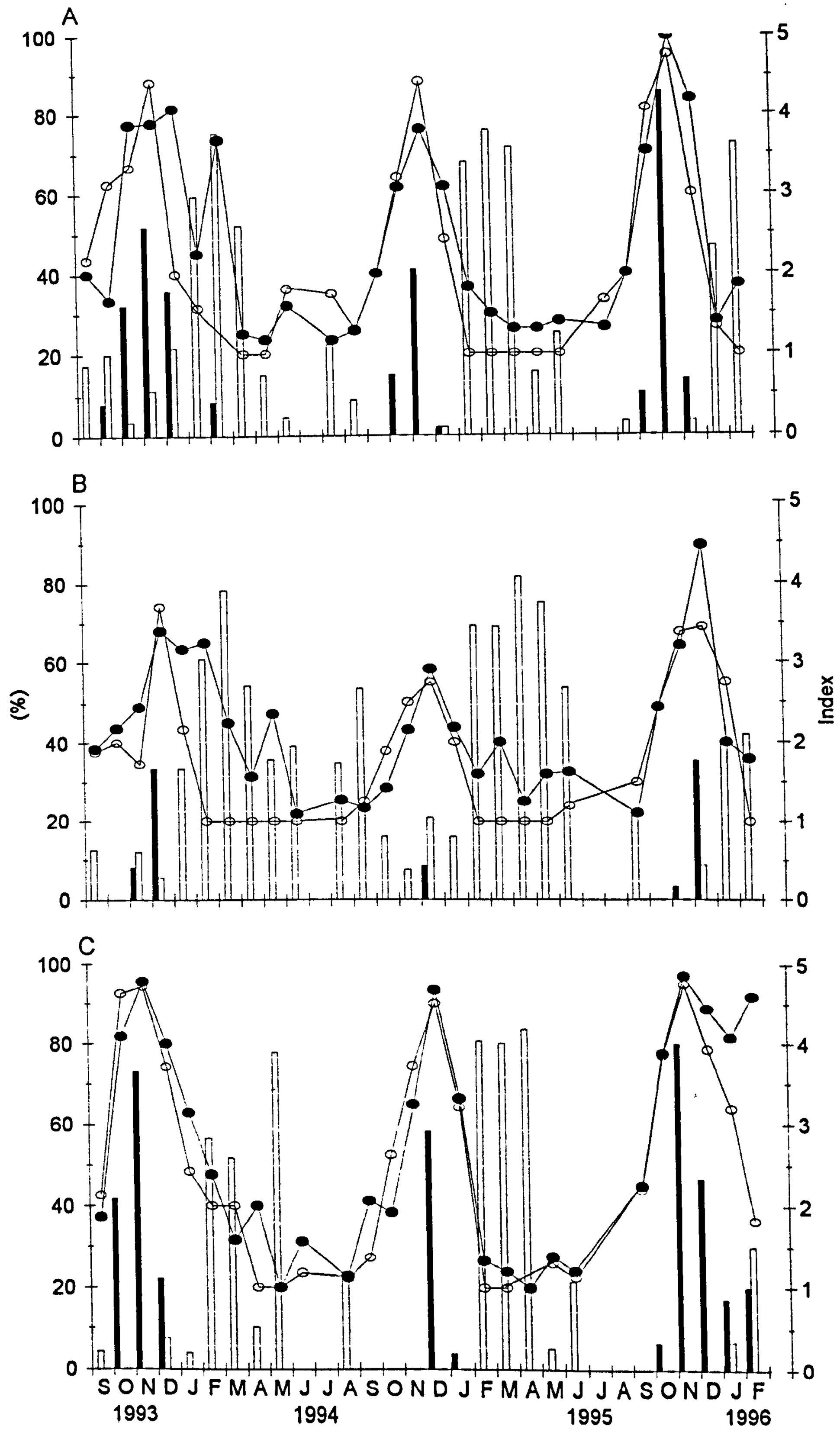
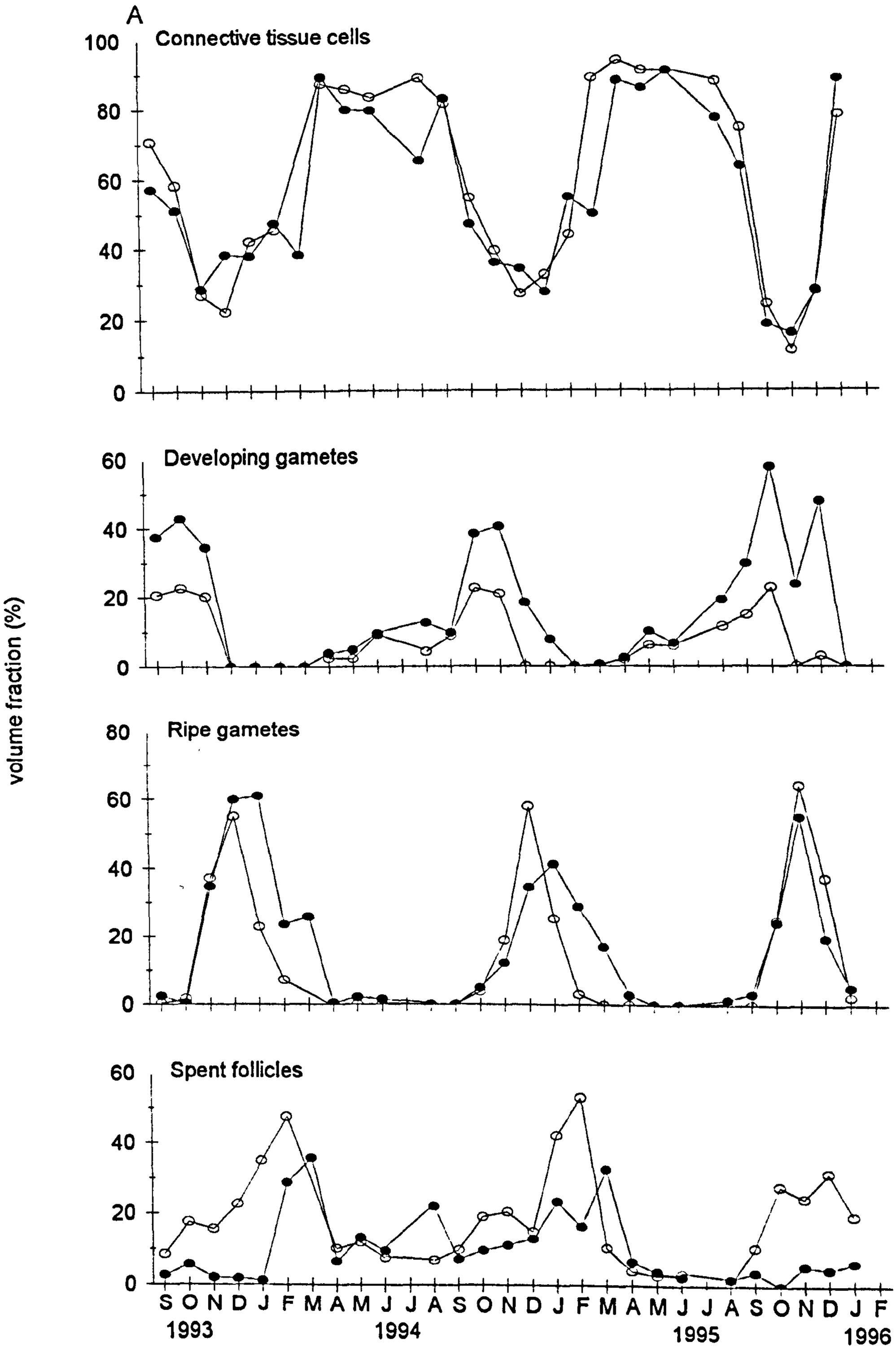

A
100
80
60
40
20
0
5
4
3
2
1
0
B
100
80
60
40
20
0
(%)
5
4
3
2
1
0
Index
C
100
80
60
40
20
0
5
4
3
2
1
0
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996

Figure 3.2 Developing gamete, ripe gamete, connective tissue and empty follicle volume fractions, for male (closed circle) and female (open circle) *Mytilus edulis chilensis* from the three study sites. Each site is on a different page.

A. Darwin

B. Camilla Creek

C. Goose Green.


A
Connective tissue cells
100
80
60
40
20
0
Developing gametes
60
40
20
0
Ripe gametes
80
60
40
20
0
Spent follicles
60
40
20
0
volume fraction (%)
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996

B

Connective tissue cells

100
80
60
40
20
0

Developing gametes

60
40
20
0

Ripe gametes

80
60
40
20
0

Spent follicles

60
40
20
0

volume fraction (%)

S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F

1993 1994 1995 1996

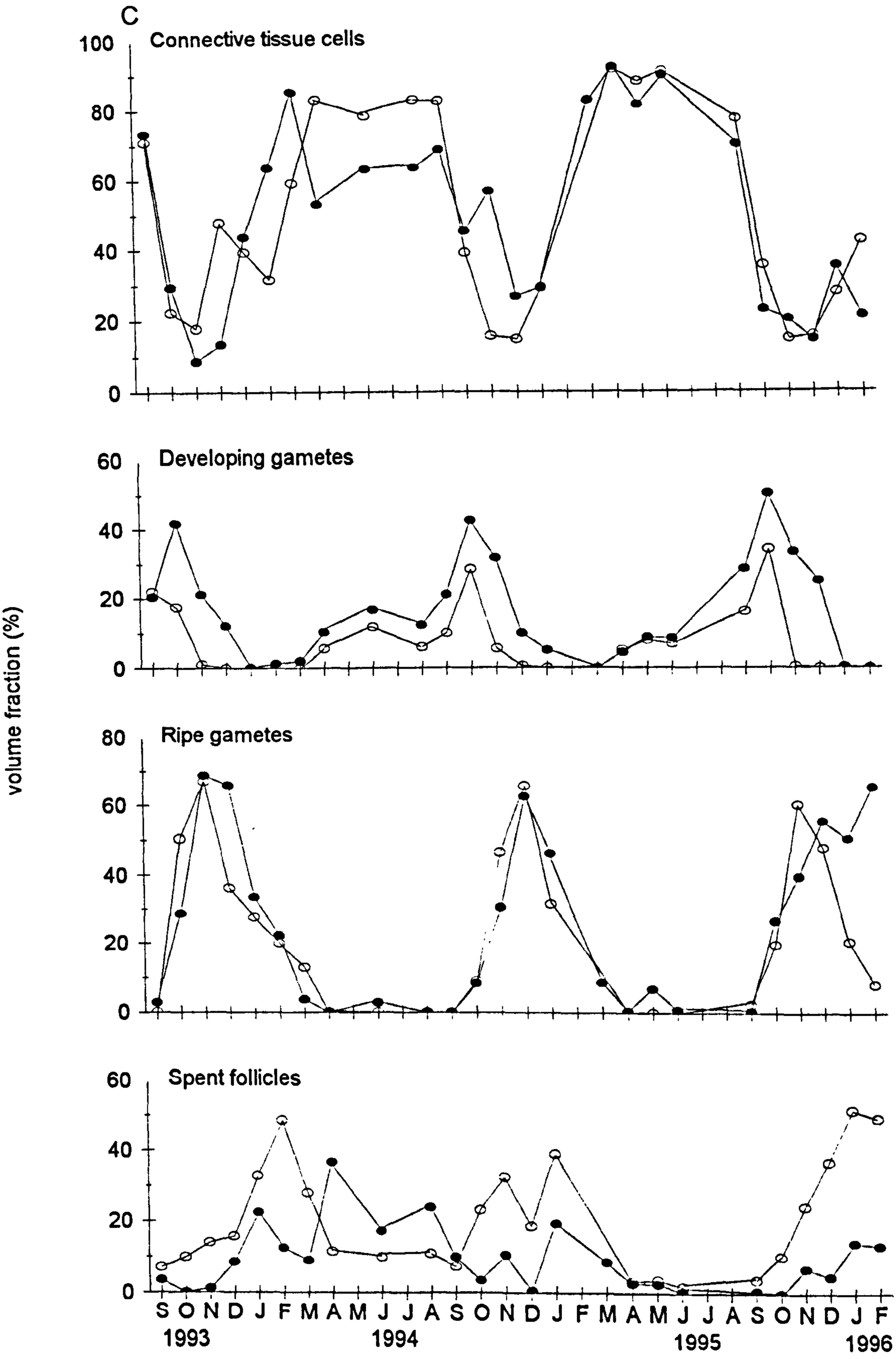

C
Connective tissue cells
100
80
60
40
20
0
Developing gametes
60
40
20
0
Ripe gametes
80
60
40
20
0
Spent follicles
60
40
20
0
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996
volume fraction (%)

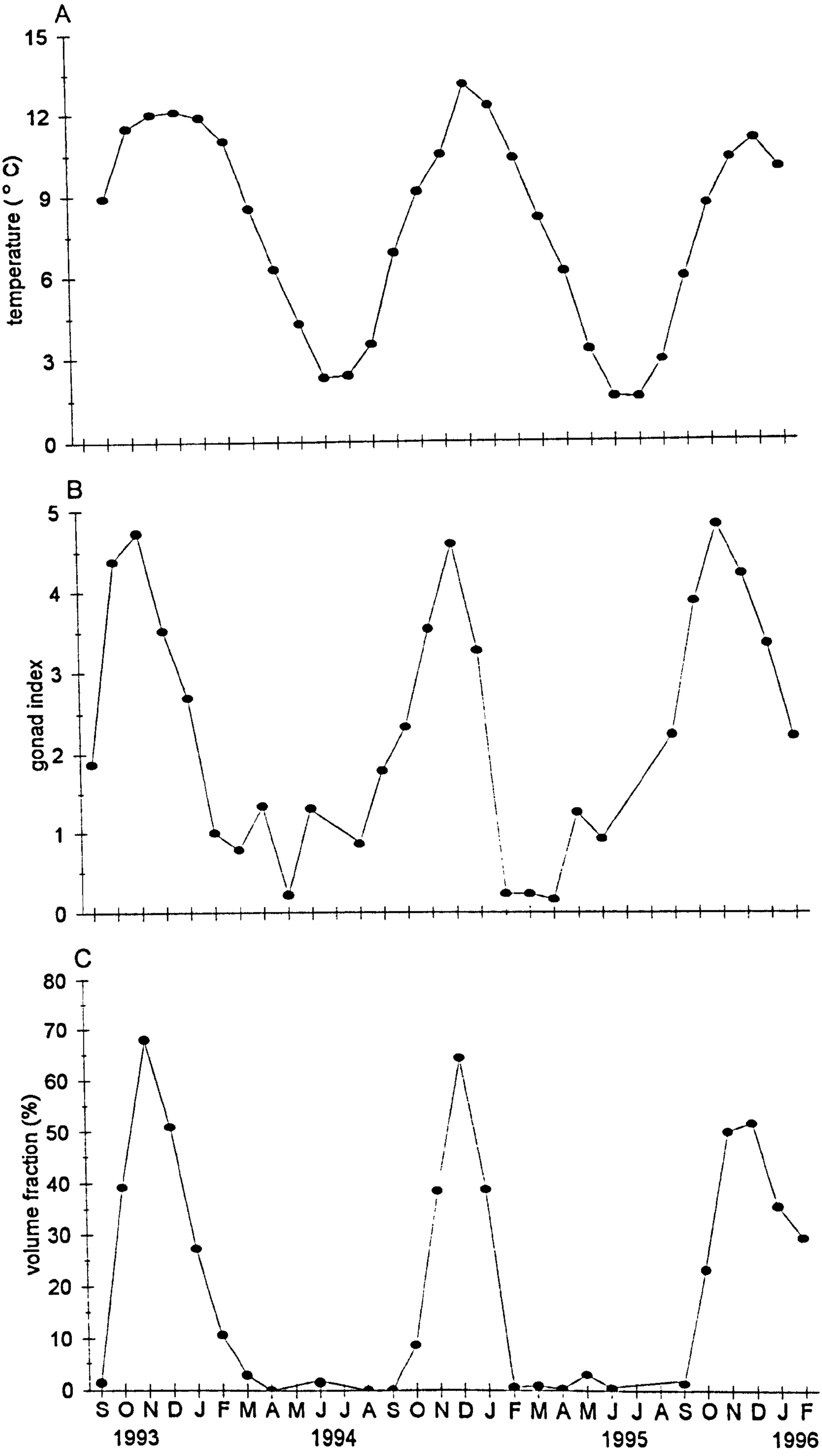
Figure 3.3

A. Mean monthly seawater temperature

B. Gonad index

C. Gamete volume fraction

of *Mytilus edulis chilensis* from Goose Green.


A
15
12
9
6
3
0
temperature (° C)
B
5
4
3
2
1
0
gonad index
C
80
70
60
50
40
30
20
10
0
volume fraction (%)
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996

temperatures reached 7 - 9°C (Figure 3.3). Peak reproductive condition was characterised by maximum gonad indices and ripe gamete volume fractions (Figure 3.1, 3.2, 3.3) which occurred in the early summer months (November - December) when temperatures were close to 10 - 12°C. A single spawning period occurred between December and March when gonad indices and gamete volume fractions decreased and empty follicles and connective tissue volume fractions increased. Populations became reproductively quiescent by early autumn (March - April) after which connective tissue volume fractions became maximal and virtually no further reproductive activity occurred until the following spring; most individuals remained in the resting or spent condition during this period. Seawater temperatures ranged from a maximum of 11 - 13°C in summer (November - February) to a minimum of 1 - 2°C during winter months (May - August), with the peak in reproductive condition broadly coinciding with the time at which mean monthly temperatures reached their maximum values (Figure 3.3).

Male and female mussels from Darwin and Camilla Creek exhibited slight uncoupling in gonad indices typically with the initiation of spawning (Figure 3.1A and B). Females appeared to release their eggs over a considerably shorter period of time, usually 2 or 3 months, whereas males did not reach minimum gonad indices for up to 6 months after spawning had started. This prolonged presence of mature male gametes was also noted when histological sections were examined stereologically (Figure 3.2A and B). In fact, the arbitrary gonad index classification and the stereological volume fraction method were in very close agreement throughout the entire gametogenic cycle. When Spearman rank order correlations were carried out on gonad indices and gamete volume fractions, the resulting correlation coefficients were found to be highly significant at all three study sites (Table 3.4). However, there was little evidence of any correlation between reproductive condition and seawater temperature despite there being evidence of broad agreement in Figure 3.3. Male and female mussels at Goose Green appear to be in synchrony virtually throughout the reproductive cycle regardless of the method of assessment.

At the time of maximum gonad maturity the population at Camilla Creek did not reach a particularly high level of reproductive condition, especially when compared to populations at the other two study sites. Maximum gonad indices were between 2.25 and 3.59 at Camilla Creek compared to 3.74 - 4.86 and 4.58 - 4.80 at Darwin and Goose Green respectively. This low reproductive condition at Camilla Creek was confirmed by a relatively low proportion (10 - 40%) of the population being in the ripe

Table 3.4 Correlation coefficients derived from Spearman Rank Order correlation analysis of gonad index (G.I.), gamete volume fraction (GVF), condition index (C.I.), dry tissue weight and temperature (T°C) at A.Darwin, B.Camilla Creek and C.Goose Green.

A.

	Dry tissue wt	C.I.	G.I.	GVF
C.I.	0.954*			
G.I.	-0.039	0.036		
GVF	-0.083	0.002	0.942*	
T°C	0.105	0.102	0.031	0.149

B.

	Dry tissue wt	C.I.	G.I.	GVF
C.I.	0.505*			
G.I.	0.116	0.305		
GVF	0.017	0.230	0.963*	
T°C	0.110	0.408*	0.349	0.308

C.

	Dry tissue wt	C.I.	G.I.	GVF
C.I.	0.842*			
G.I.	0.502*	0.443*		
GVF	0.496*	0.428*	0.972*	
T°C	0.376	0.275	0.204	0.141

* significant at p = 0.05, d.f. = 23, r_s crit = 0.400

condition at the time of peak sexual maturity compared to 40 - 85% and 60 - 80% at Darwin and Goose Green respectively (Figure 3.1). Volume fractions of ripe gametes, despite reaching up to 60% for males in 1994, were also typically low with overall maxima of 12 to 48% (Appendix 2) at the time of peak condition.

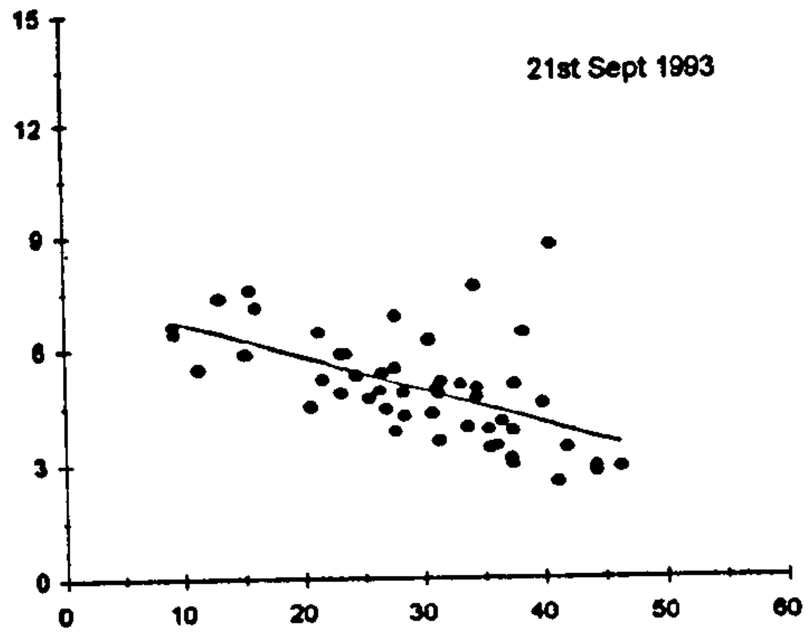
Peak reproductive condition (gonad index and gamete volume fraction) of *M.e.chilensis* differed slightly between years. At Darwin gonad indices increased with time, maximum values being observed in the summer of 1995/6. Gamete volume fraction data, on the other hand, revealed minimum values during 1994/5, with slightly higher values in 1993/4 and 1995/6. At Camilla Creek and Goose Green both gonad index and gamete volume fraction were low in 1994/5 compared to those in 1993/4 and 1995/6.

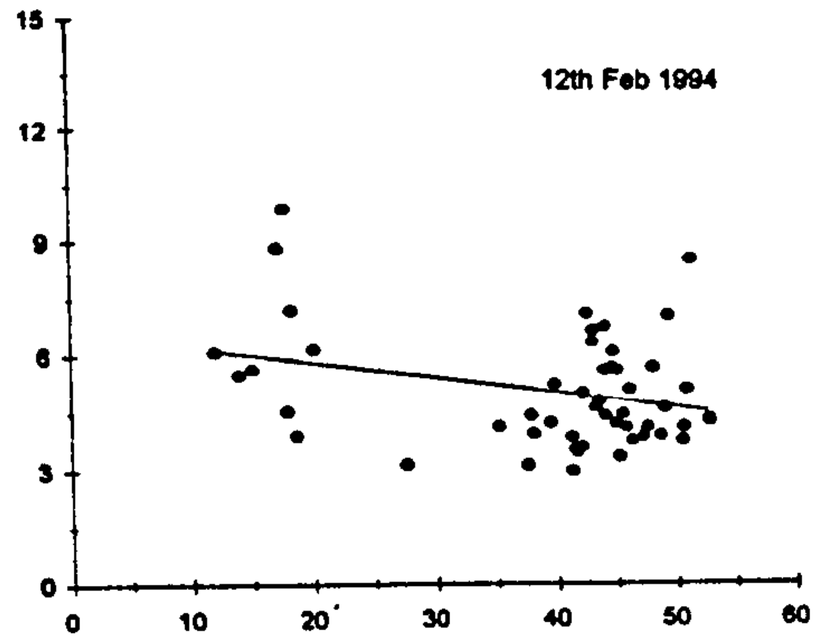
3.3.3. Condition index and dry tissue weight

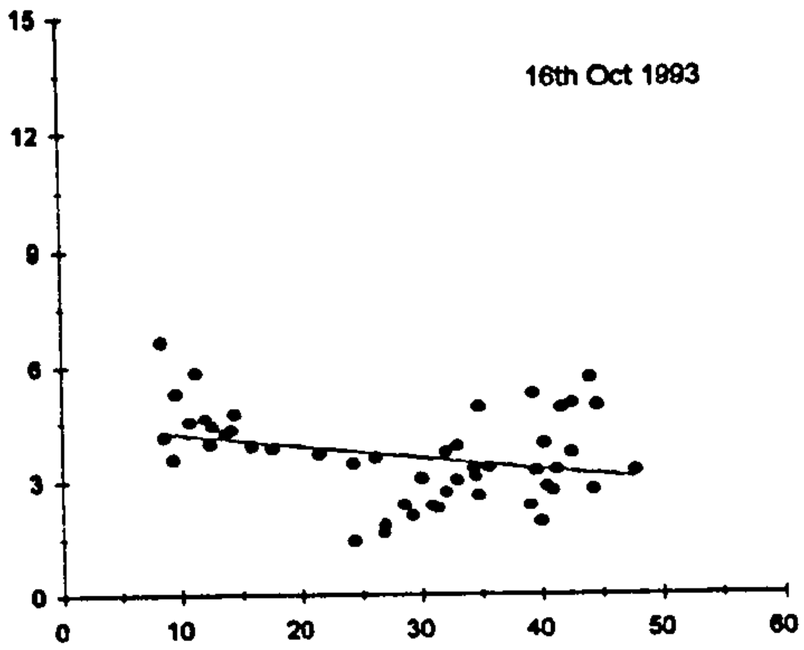
In order to observe whether there was any seasonal variation in mussel population condition, monthly plots of condition index and dry tissue weight on shell length are presented for the three study sites (Figures 3.4, 3.5, 3.6 and Figures 3.7, 3.8, 3.9, respectively). Regression constants and F-values from least squares linear regression are provided in Tables 3.5, 3.6, 3.7. Very low, and in most cases non significant, F-values indicate that the least squares linear regression is not particularly well suited for studying the relationship between condition index and shell length. Seasonal plots of the data show there is insufficient spread of data over the independent shell length axis, and in some cases size ranges between months may differ considerably. However, monthly samples of log transformed dry tissue weight on shell length exhibit a significant linear relationship at all three study sites when least squares linear regression is applied.

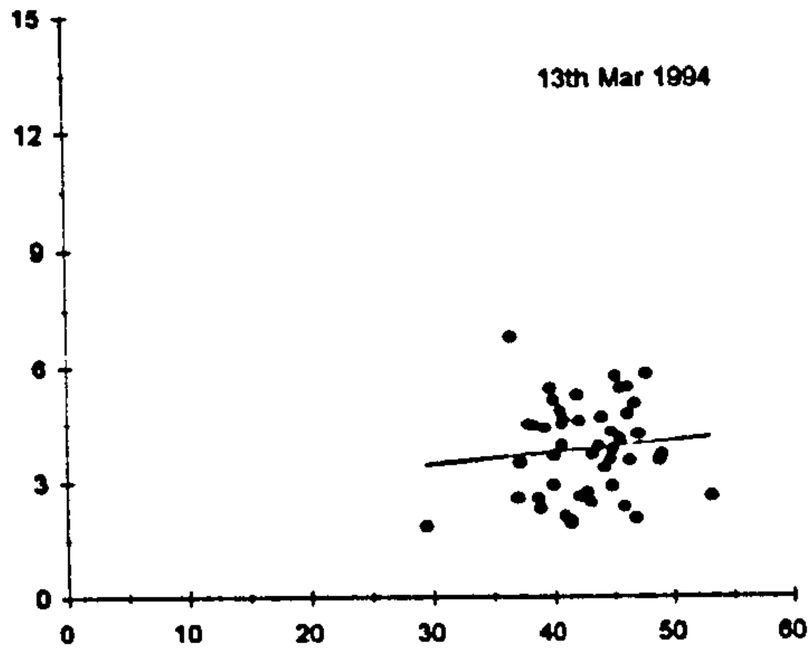
In order to examine whether there was any seasonal relationship between a) condition index and shell length and b) dry tissue weight and shell length, a combination of two methods of analysis was used whereby variations in the condition of a standard sized mussel was followed throughout the study period. A standard size of 40 mm was used for all sites as this was close to the average size of mussels from any of the three study populations (38.4 mm, 39.2 mm and 41.1 mm at Darwin, Camilla Creek and Goose Green, respectively). Any variation between months was identified by applying the general linear model with a single covariate (Minitab) to those monthly samples

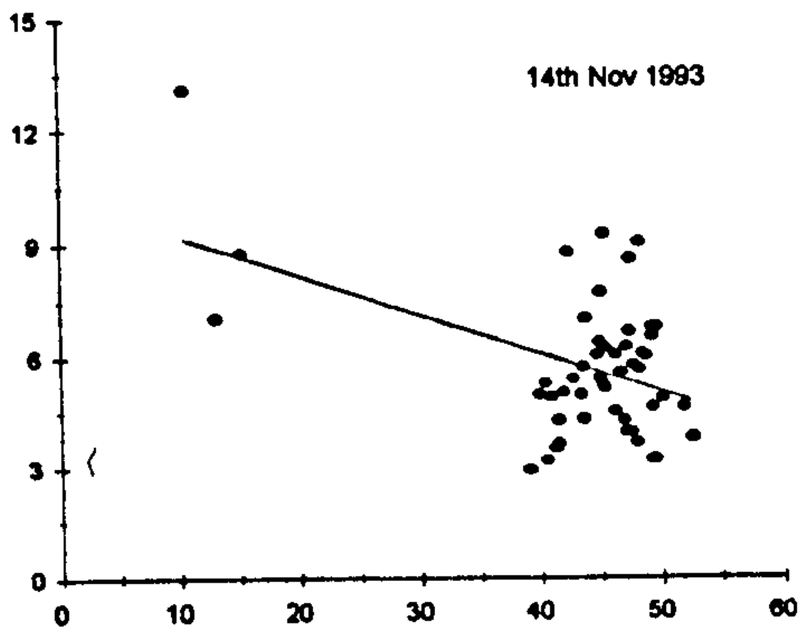
Figure 3.4 The relationship between condition index and shell length in monthly samples of *Mytilus edulis chilensis* from Darwin. Lines fitted by least squares linear regression (on 3 consecutive pages).

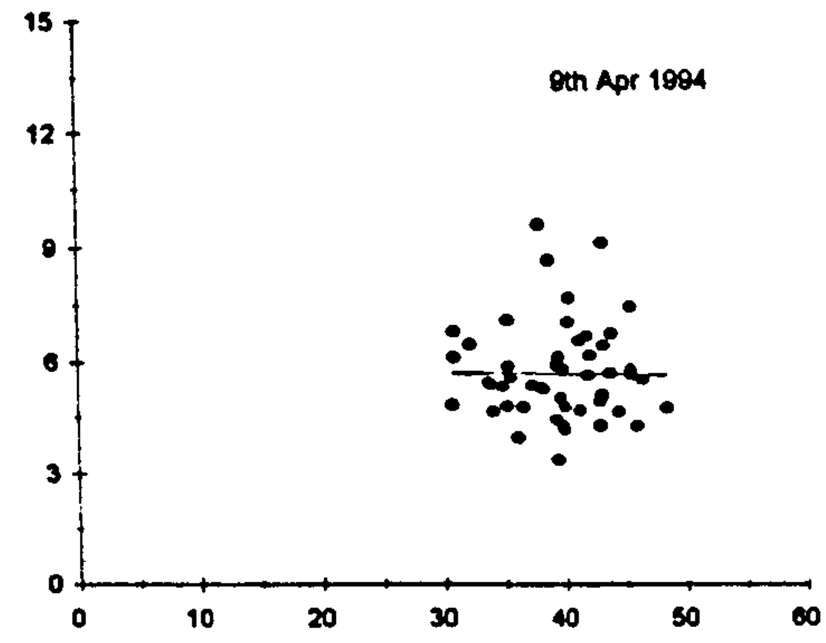


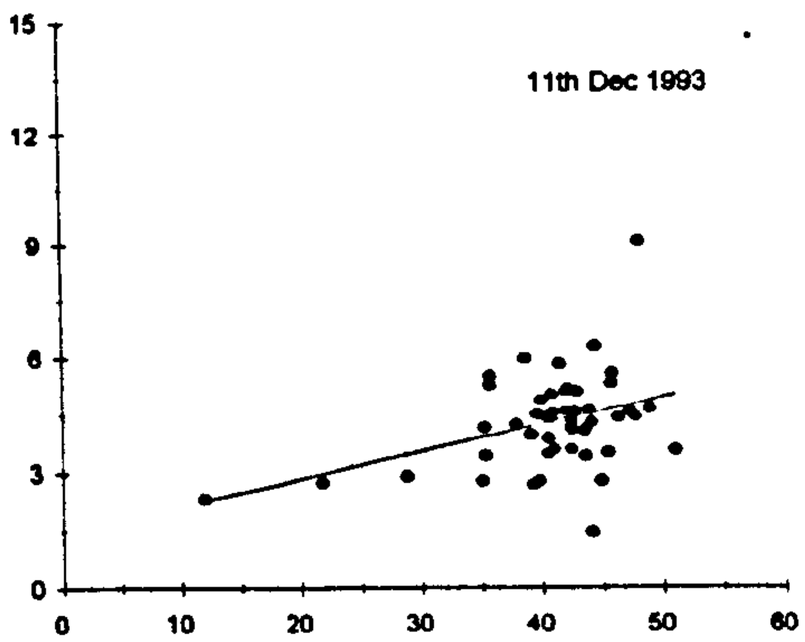


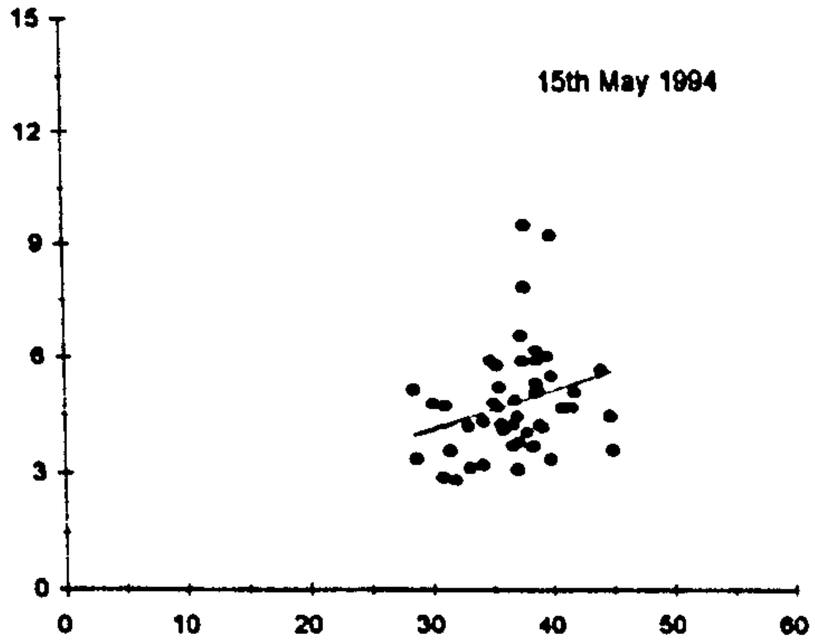


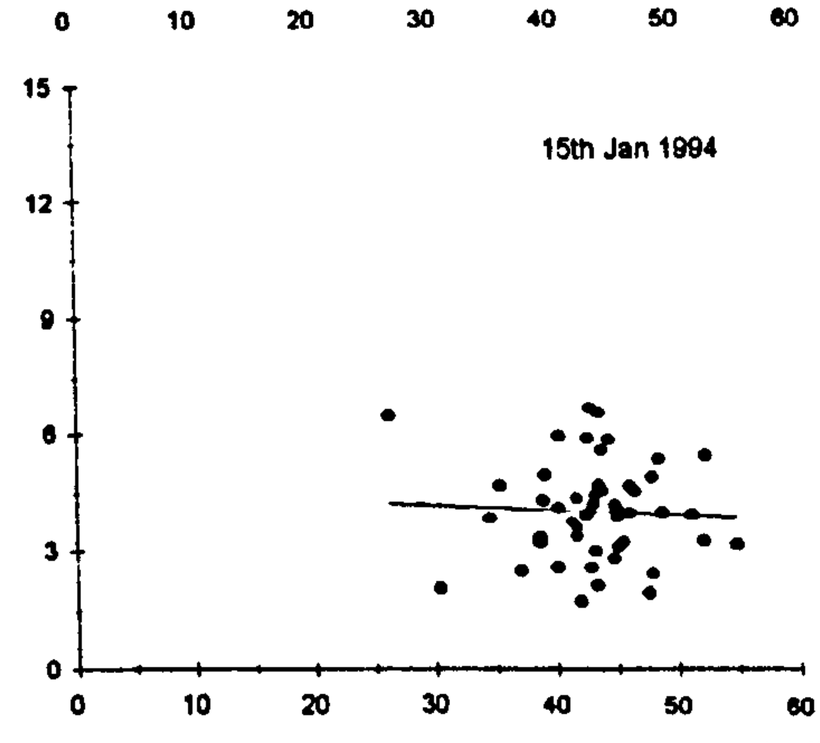


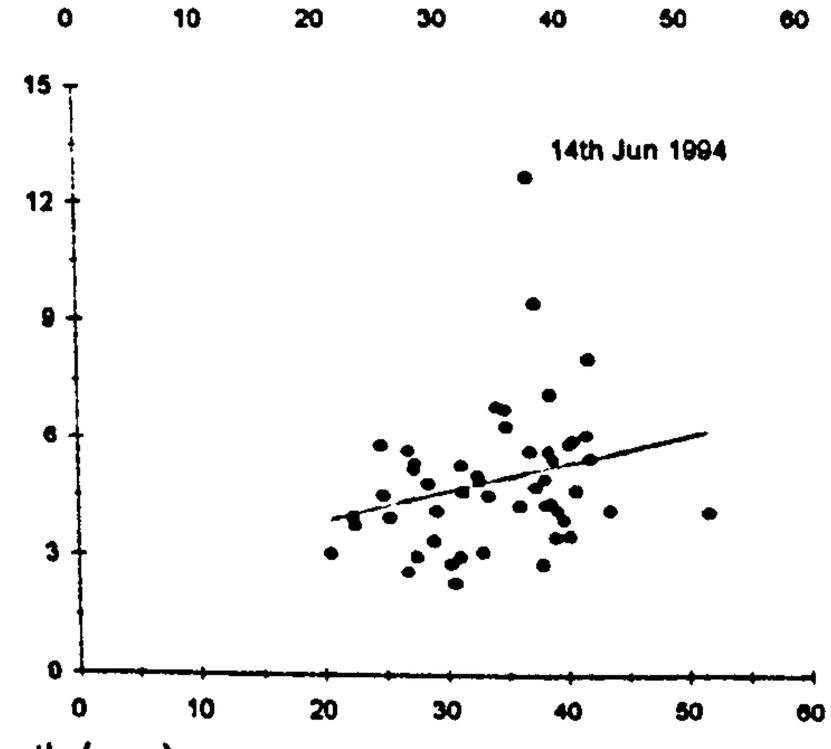








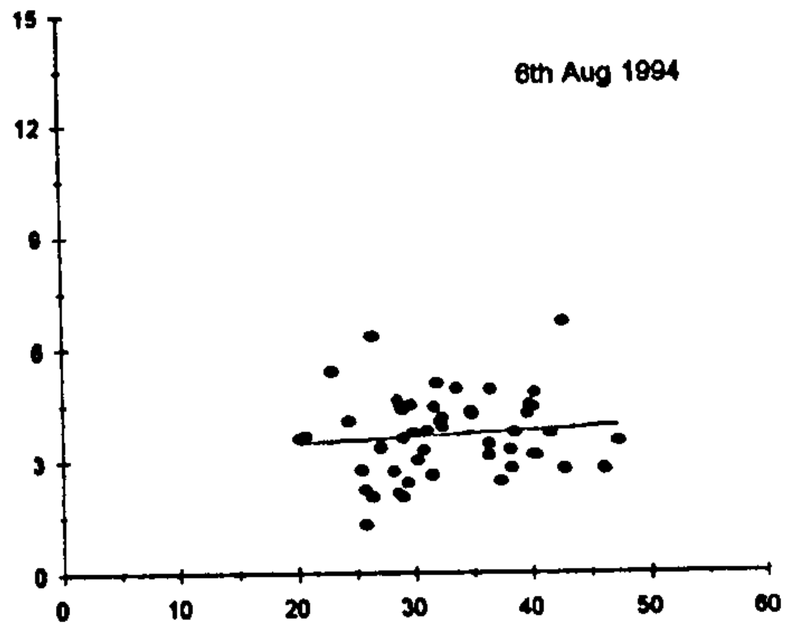




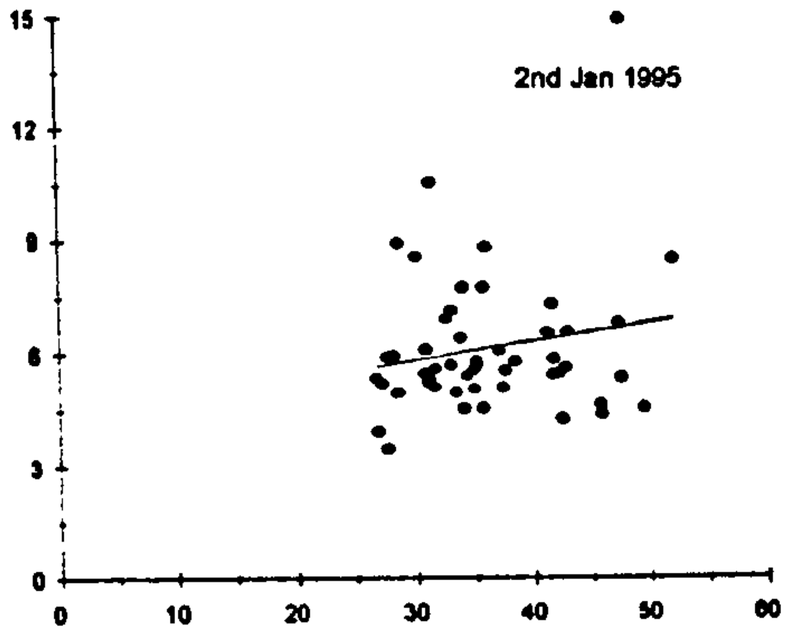
condition index (%)

shell length (mm)

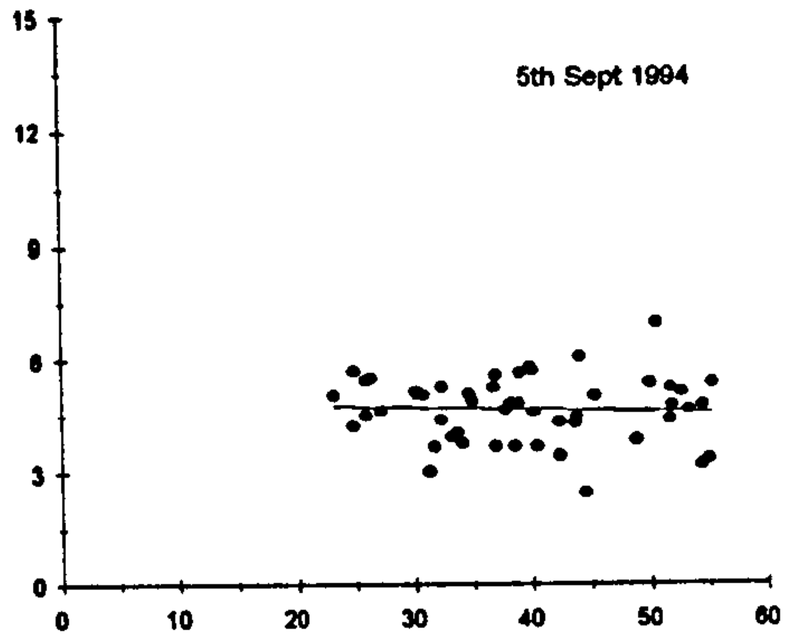
6th Aug 1994



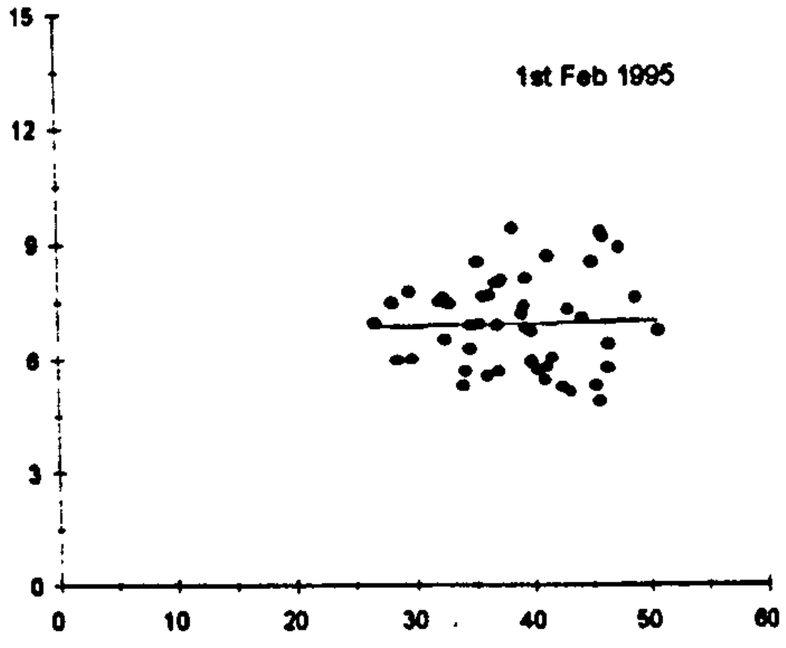
2nd Jan 1995



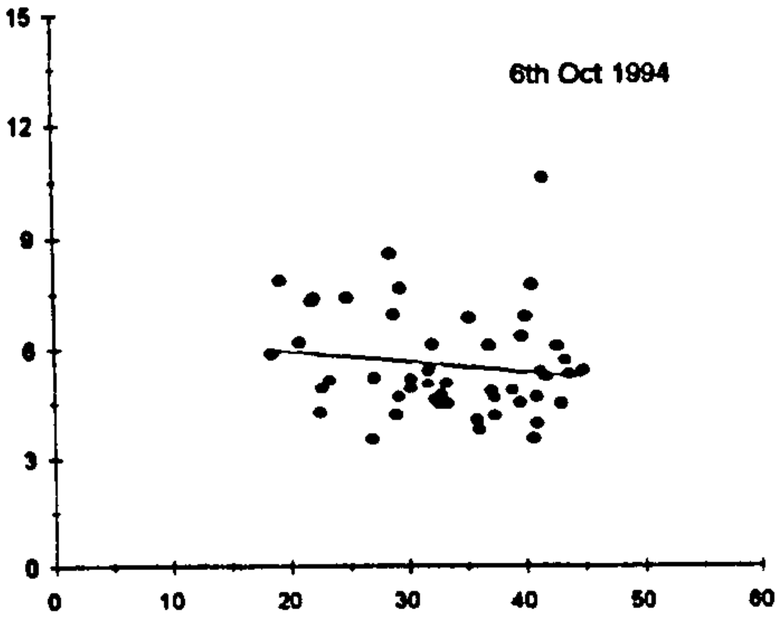
5th Sept 1994



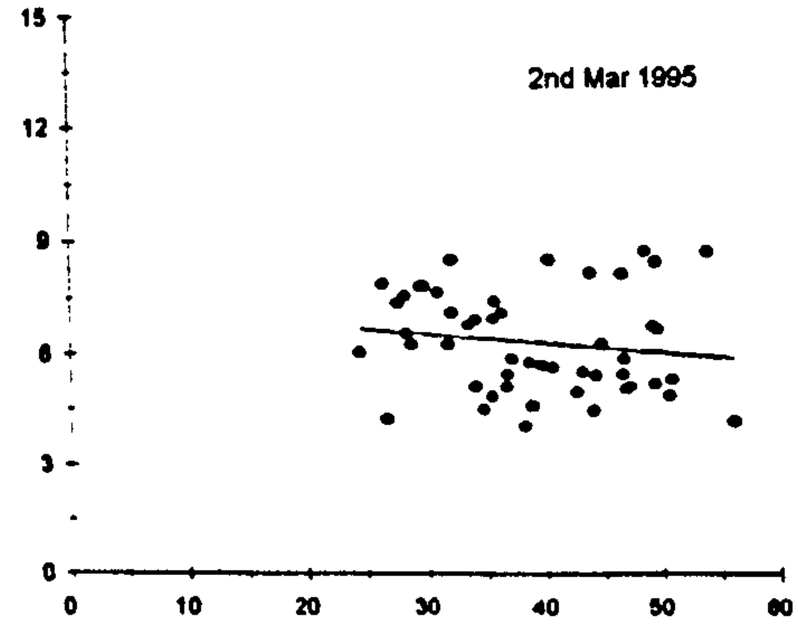
1st Feb 1995



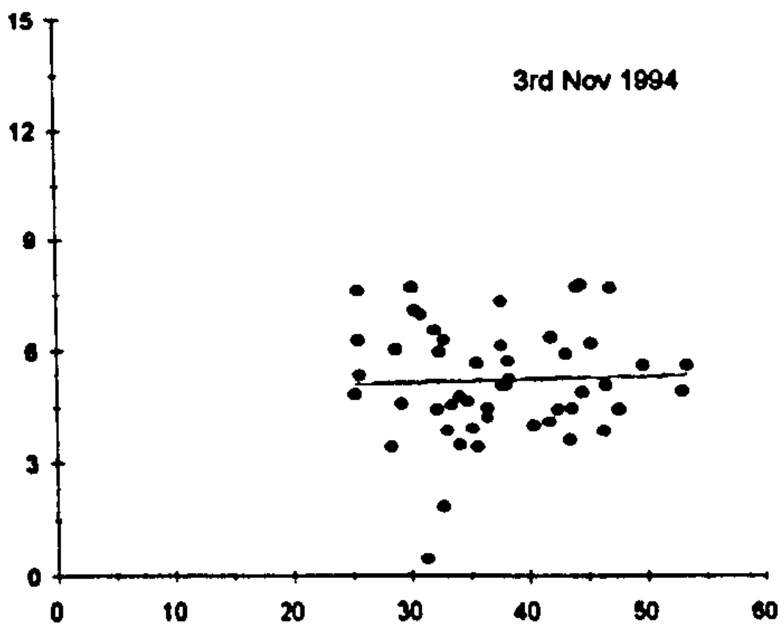
6th Oct 1994



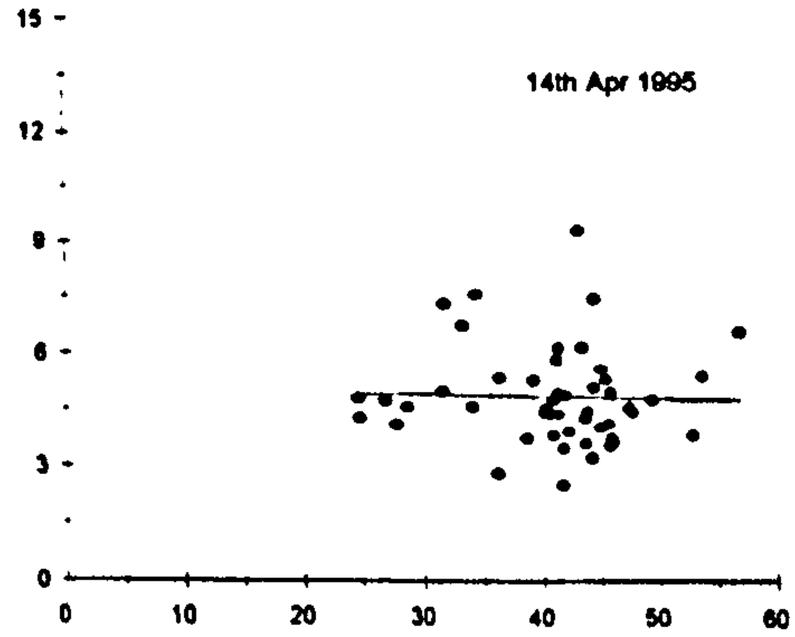
2nd Mar 1995



3rd Nov 1994



14th Apr 1995

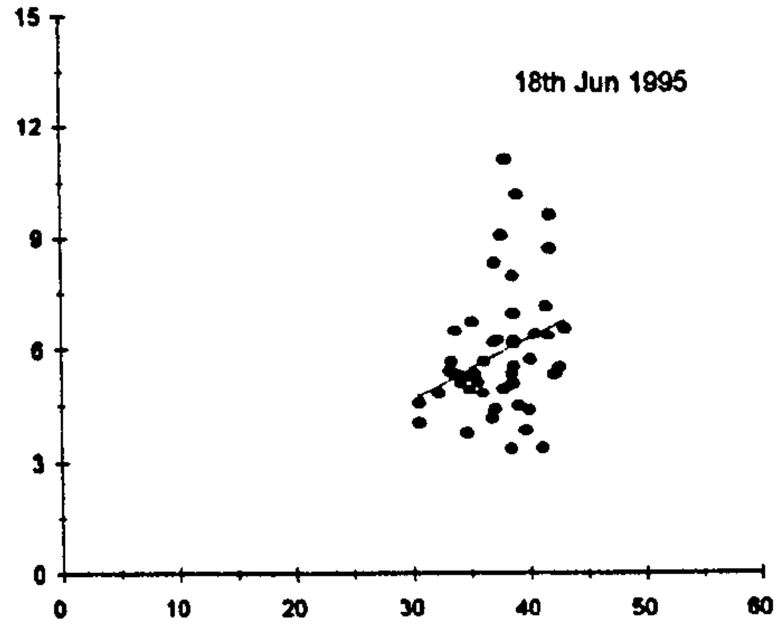


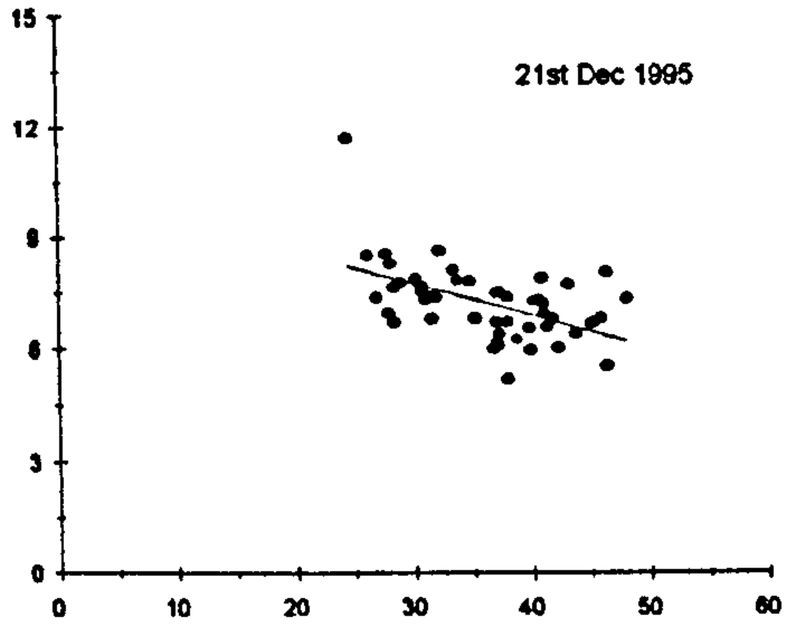
8th Dec 1994

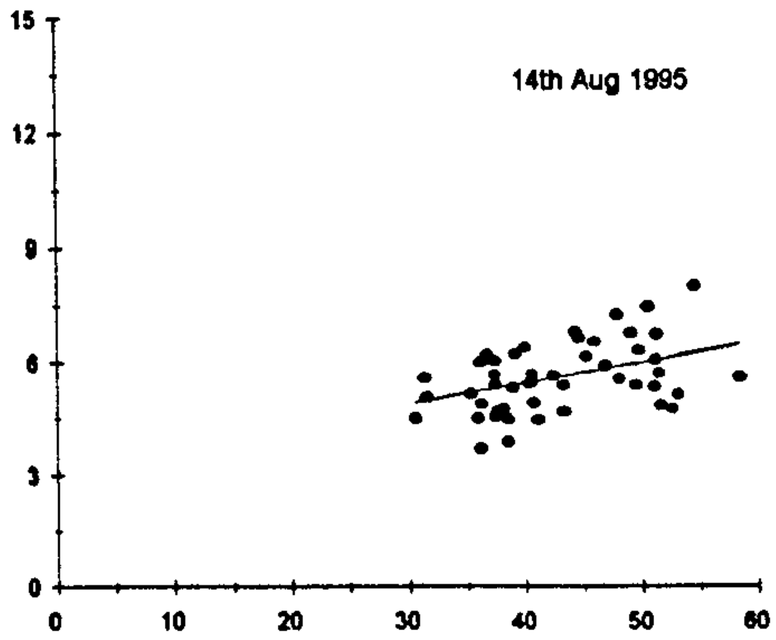
13th May 1995

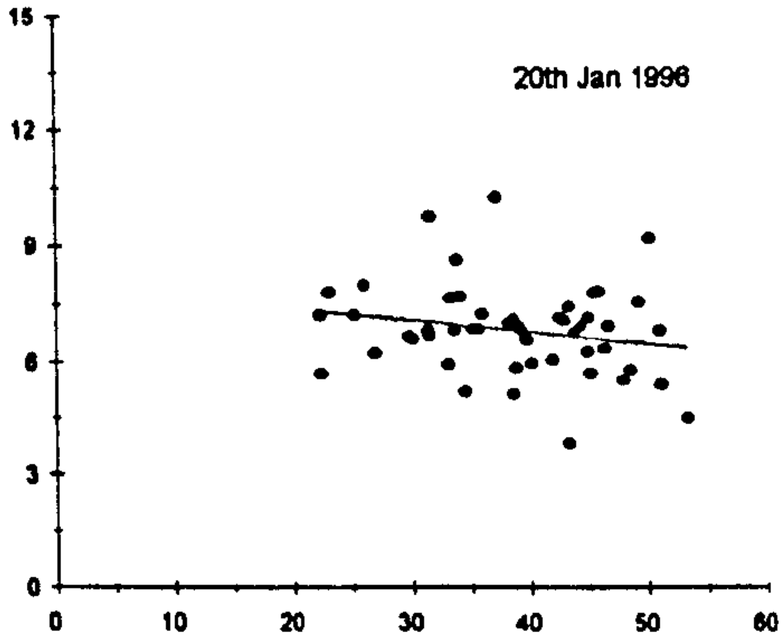
condition index (%)

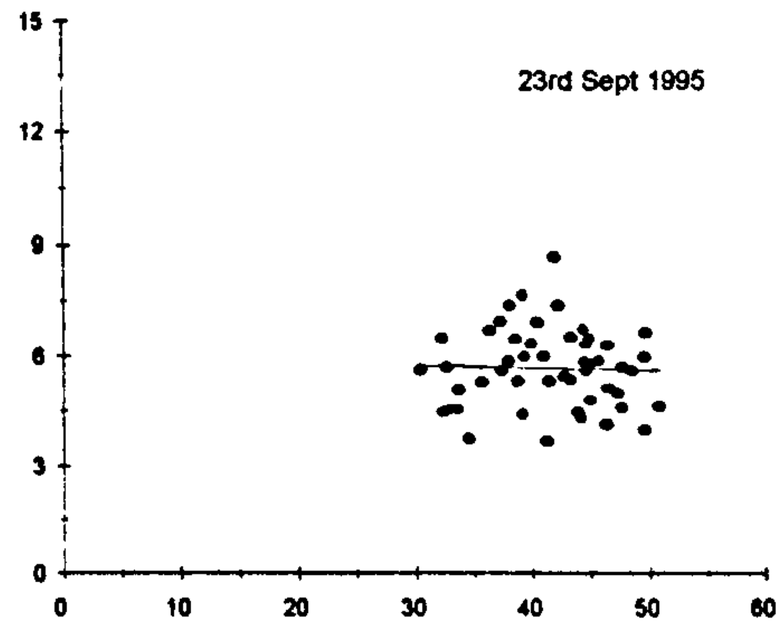
shell length (mm)

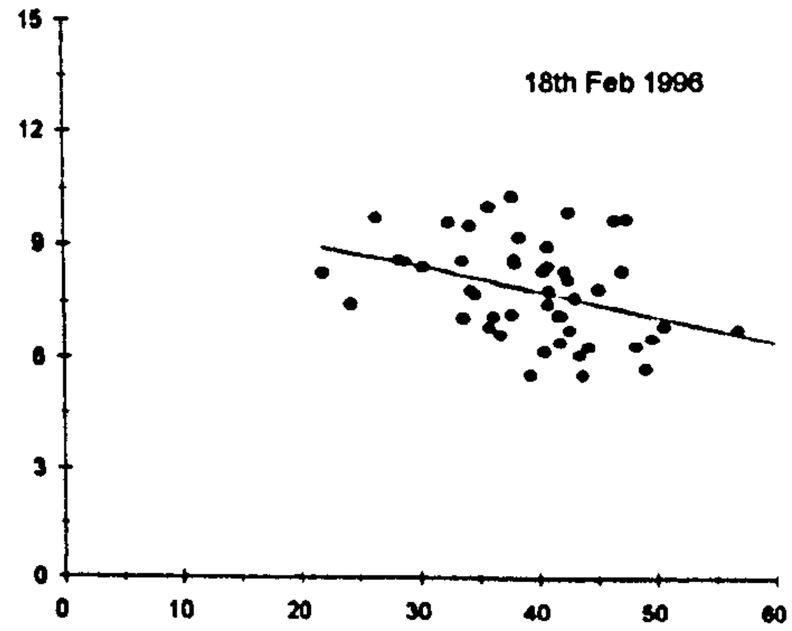

18th Jun 1995

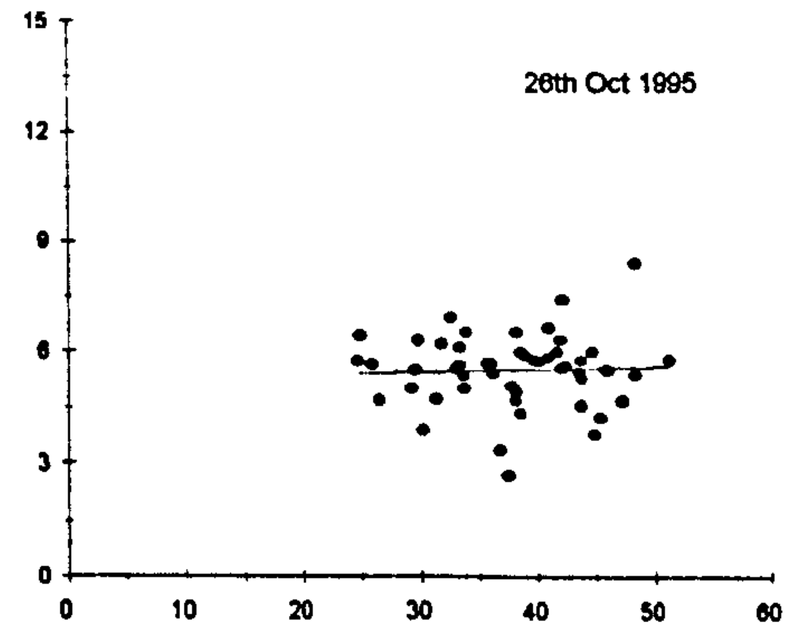

21st Dec 1995


14th Aug 1995


20th Jan 1996

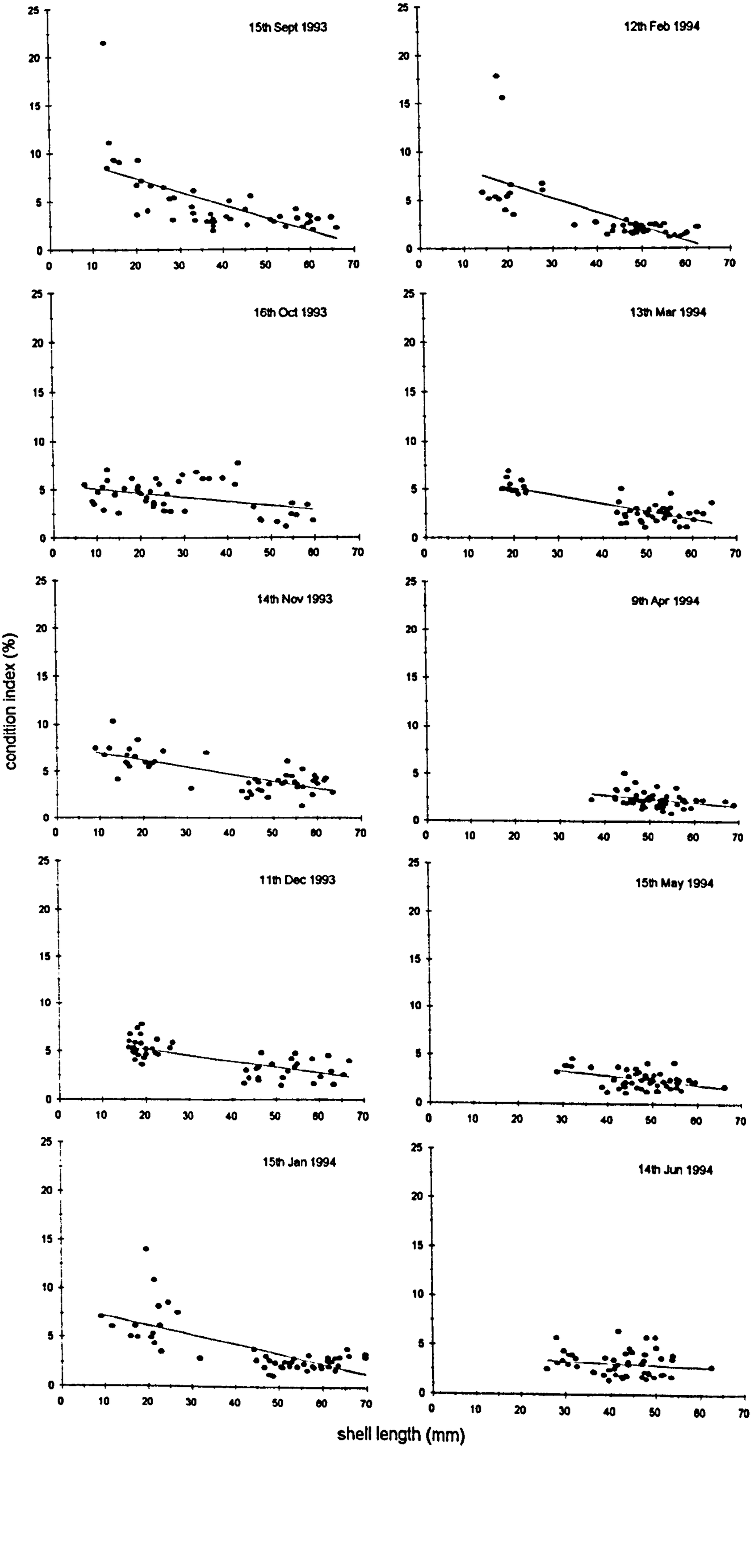

23rd Sept 1995

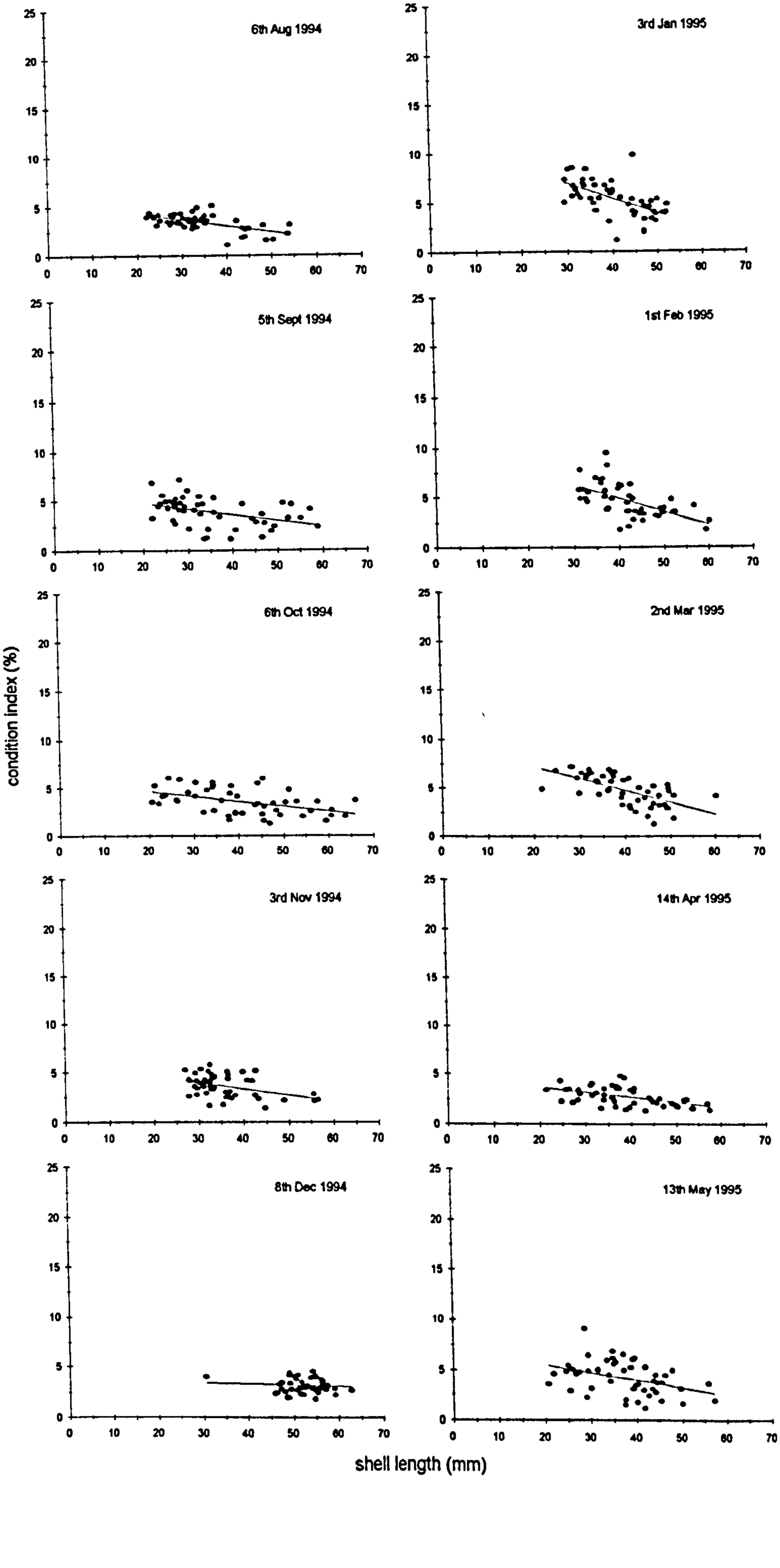

18th Feb 1996

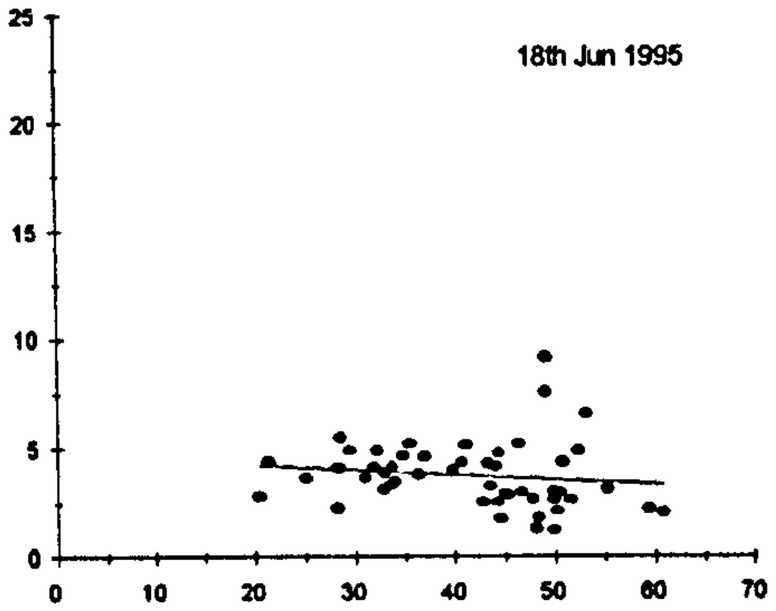

26th Oct 1995

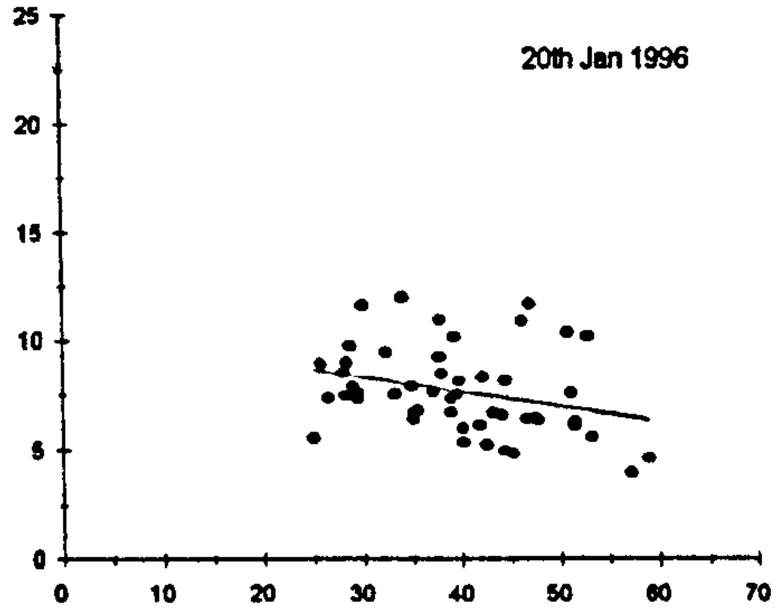
24th Nov 1995
condition index (%)
shell length (mm)

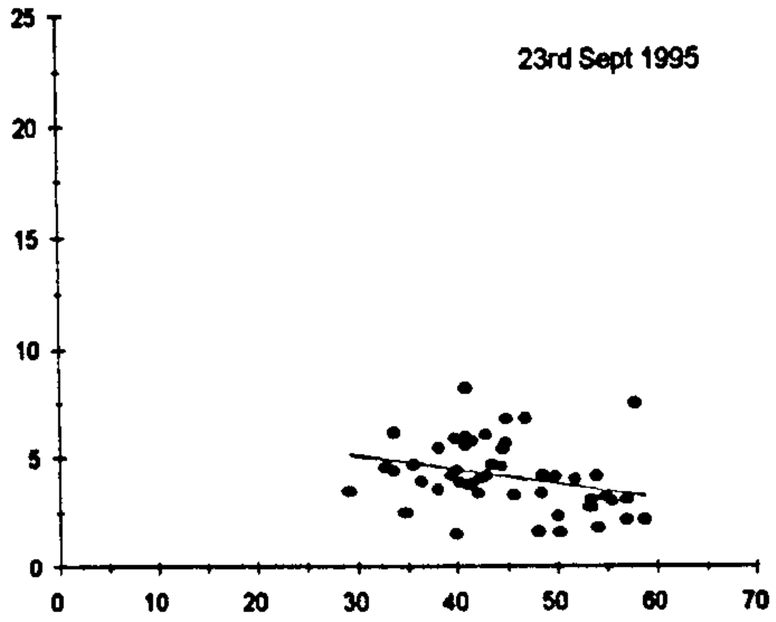
Figure 3.5 The relationship between condition index and shell length in monthly samples of *Mytilus edulis chilensis* from Camilla Creek. Lines fitted by least squares linear regression (on 3 consecutive pages).

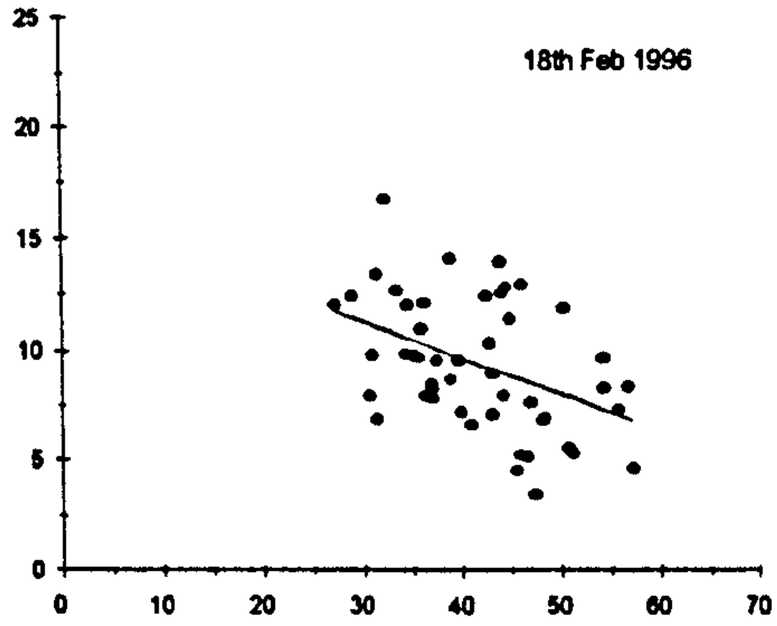

15th Sept 1993
12th Feb 1994
16th Oct 1993
13th Mar 1994
14th Nov 1993
9th Apr 1994
11th Dec 1993
15th May 1994
15th Jan 1994
14th Jun 1994
condition index (%)
shell length (mm)
0
5
10
15
20
25
0
10
20
30
40
50
60
70

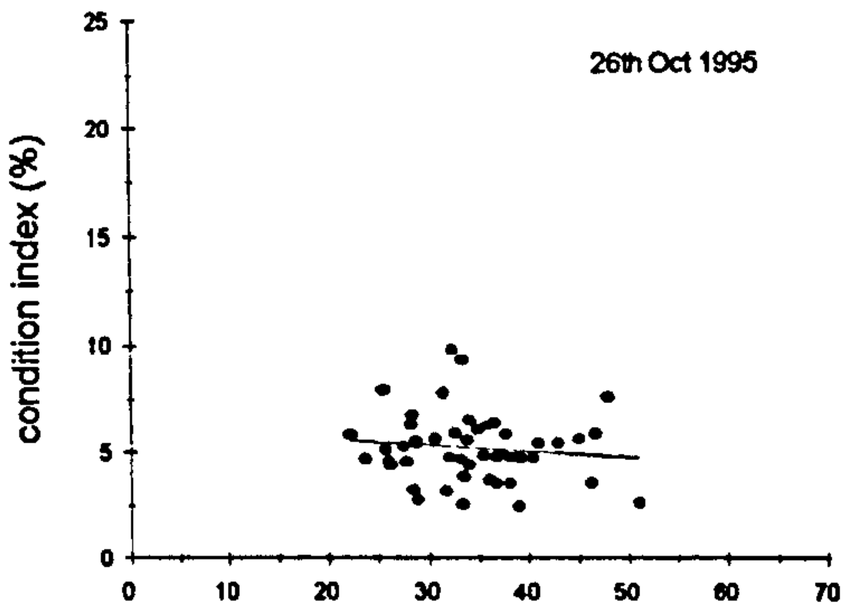

6th Aug 1994
3rd Jan 1995
5th Sept 1994
1st Feb 1995
6th Oct 1994
2nd Mar 1995
3rd Nov 1994
14th Apr 1995
8th Dec 1994
13th May 1995
condition index (%)
shell length (mm)
0
5
10
15
20
25
0
10
20
30
40
50
60
70

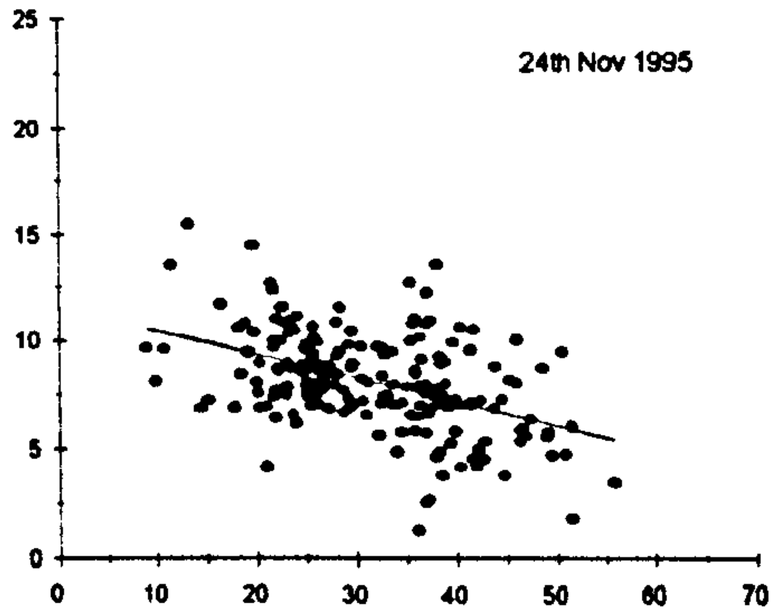

18th Jun 1995


20th Jan 1996


23rd Sept 1995


18th Feb 1996


26th Oct 1995


24th Nov 1995

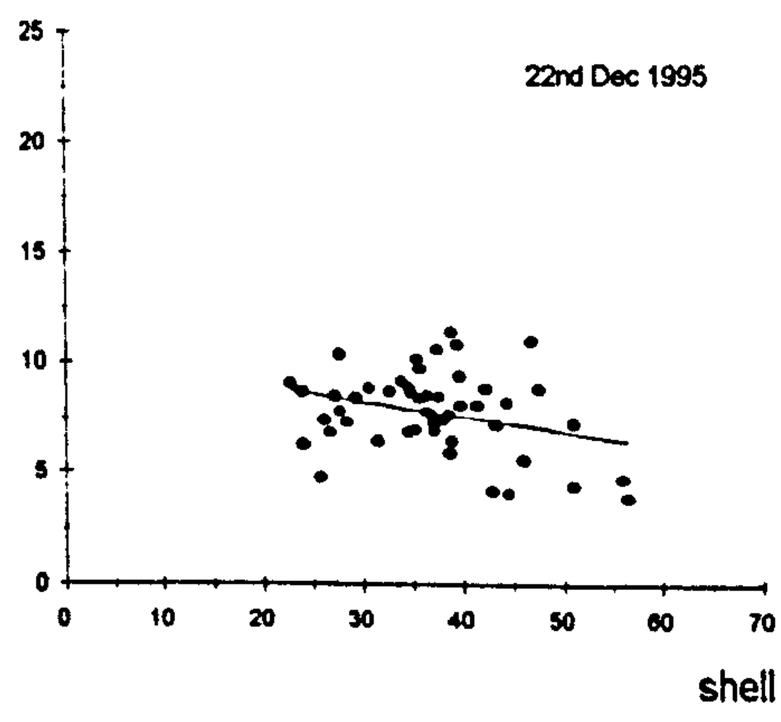
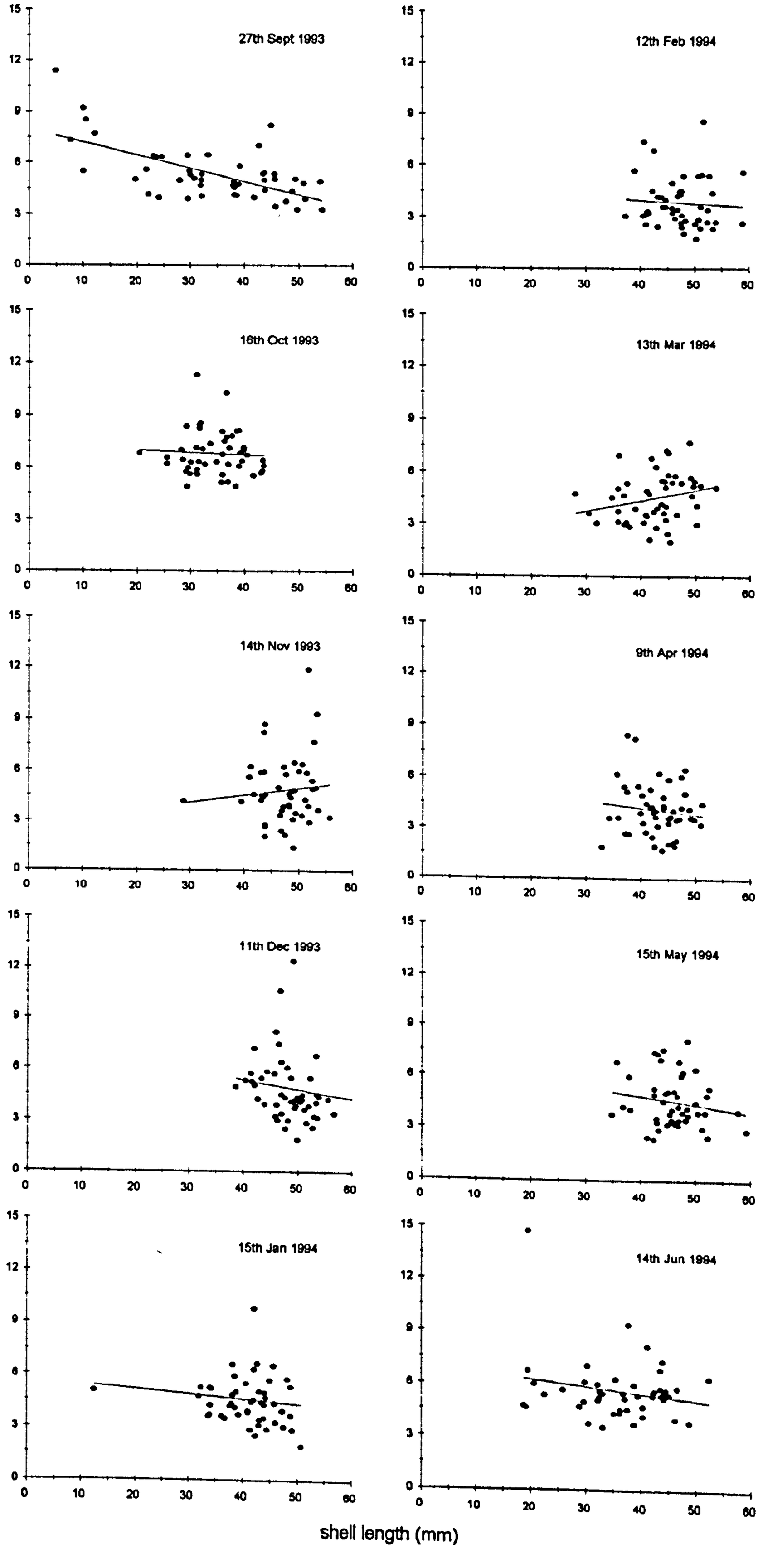
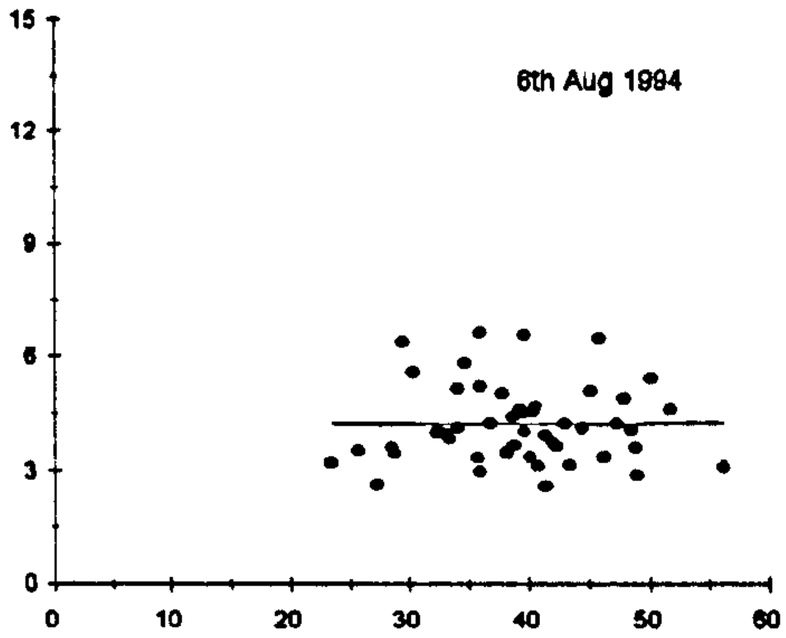
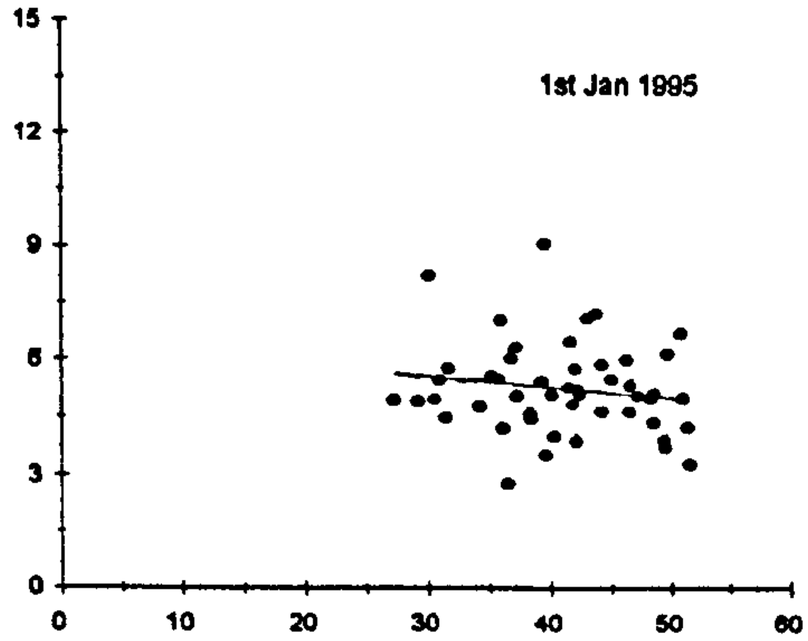
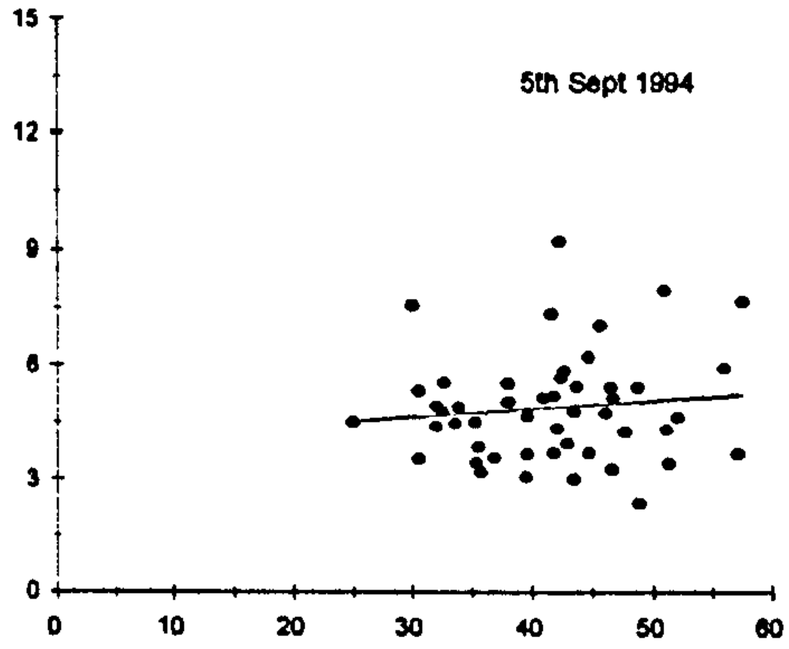

22nd Dec 1995
condition index (%)
shell length (mm)

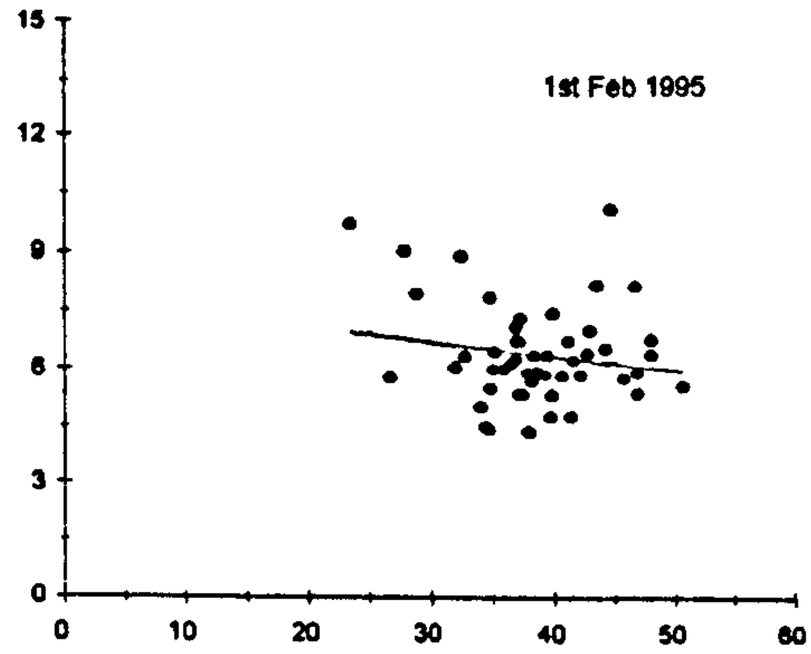
Figure 3.6 The relationship between condition index and shell length in monthly samples of *Mytilus edulis chilensis* from Goose Green. Lines fitted by least squares linear regression (on 3 consecutive pages).

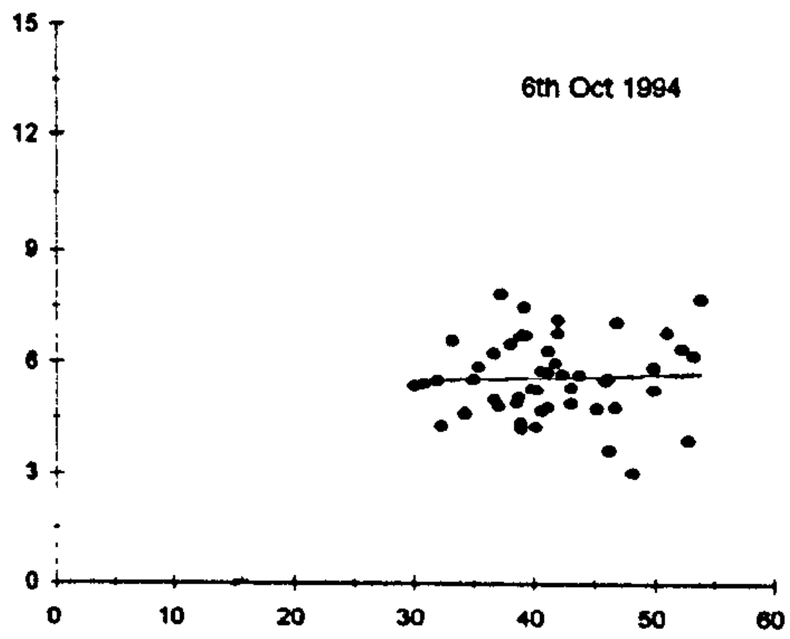

27th Sept 1993
12th Feb 1994
16th Oct 1993
13th Mar 1994
14th Nov 1993
9th Apr 1994
11th Dec 1993
15th May 1994
15th Jan 1994
14th Jun 1994
condition index (%)
shell length (mm)
0
3
6
9
12
15
0
10
20
30
40
50
60

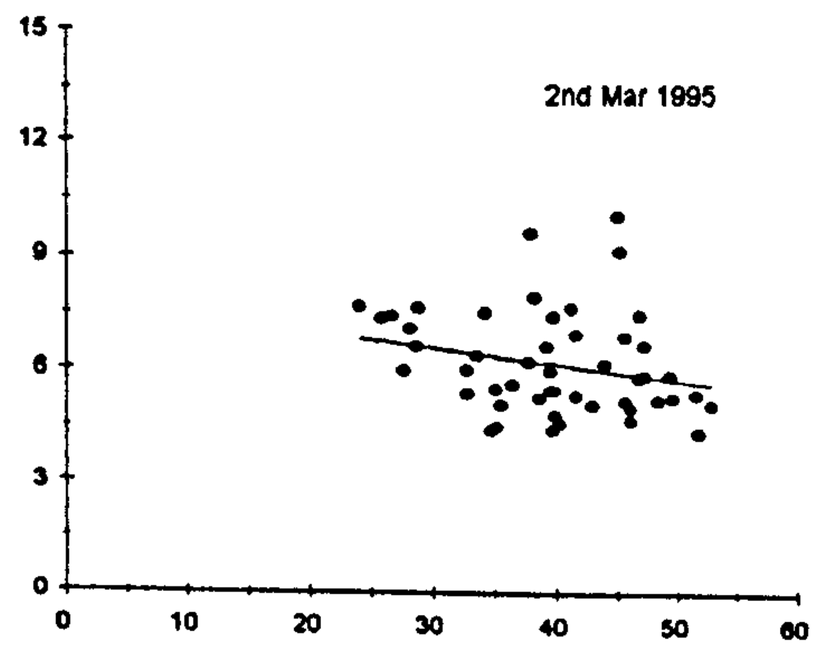


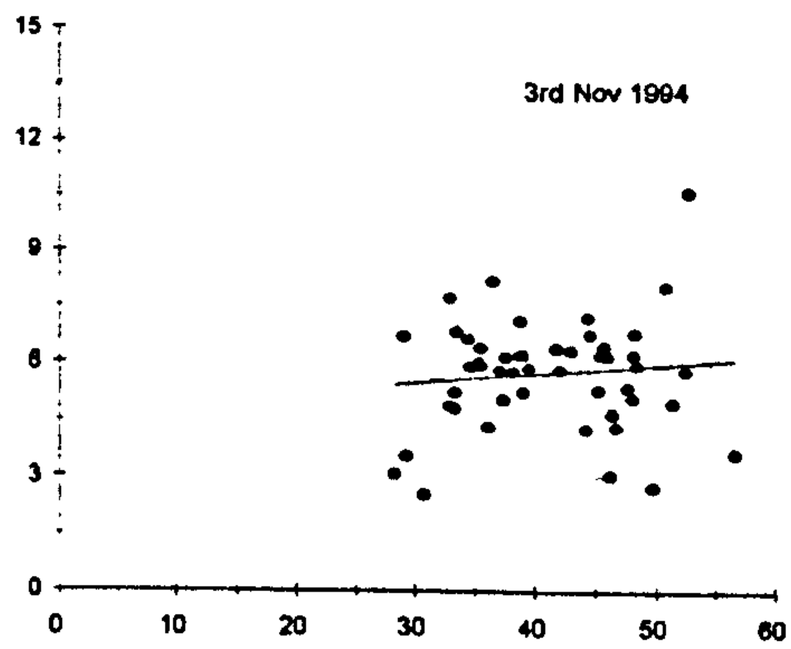


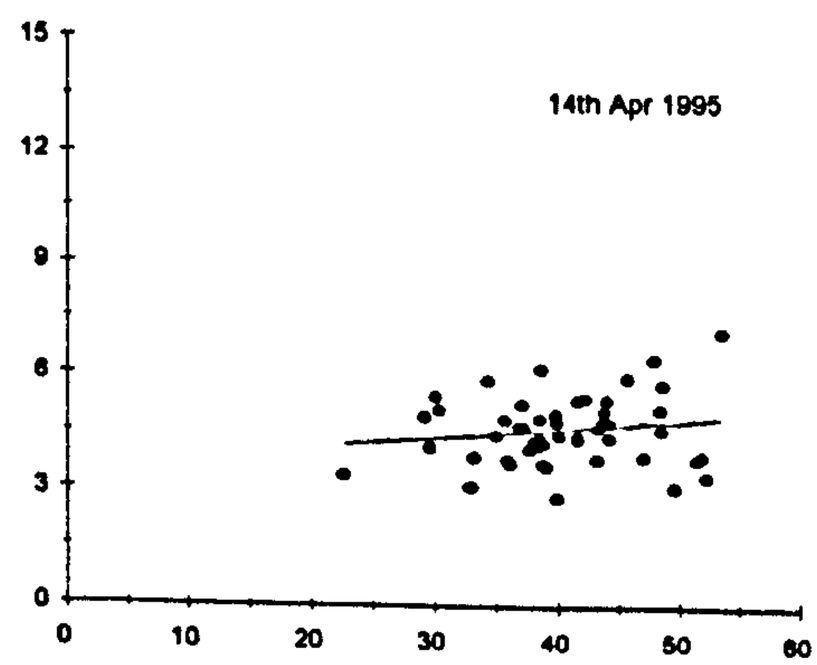


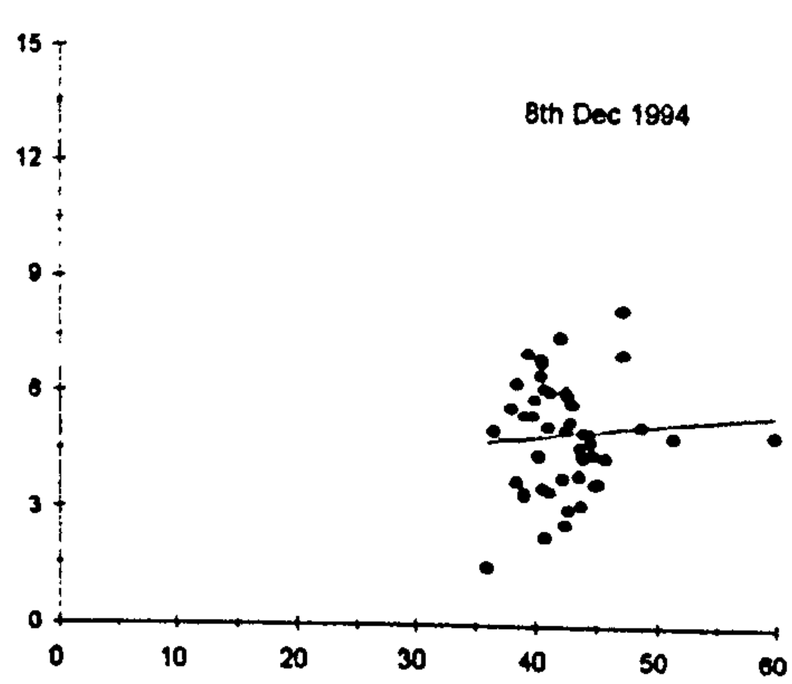


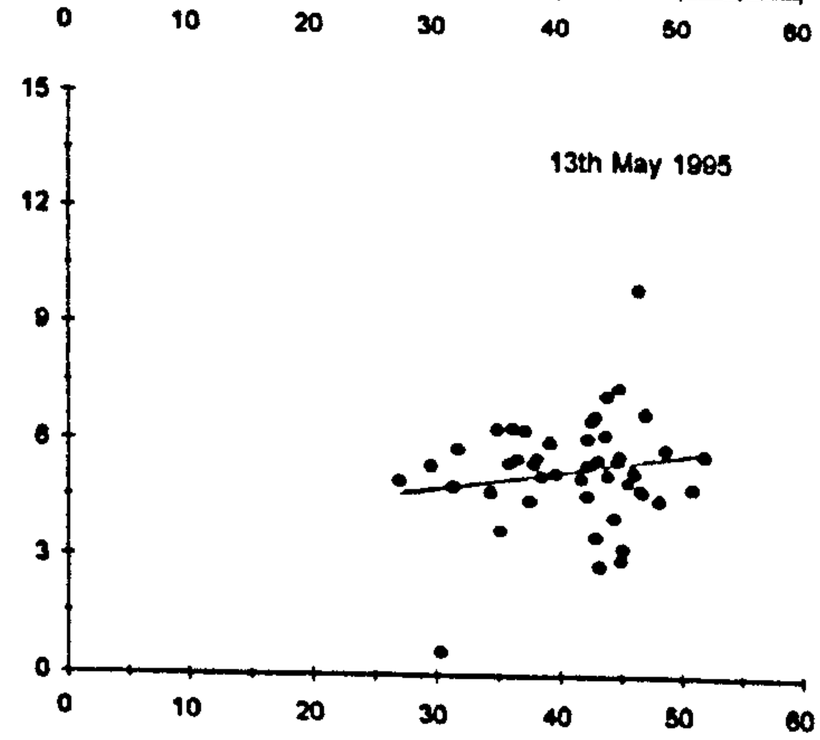






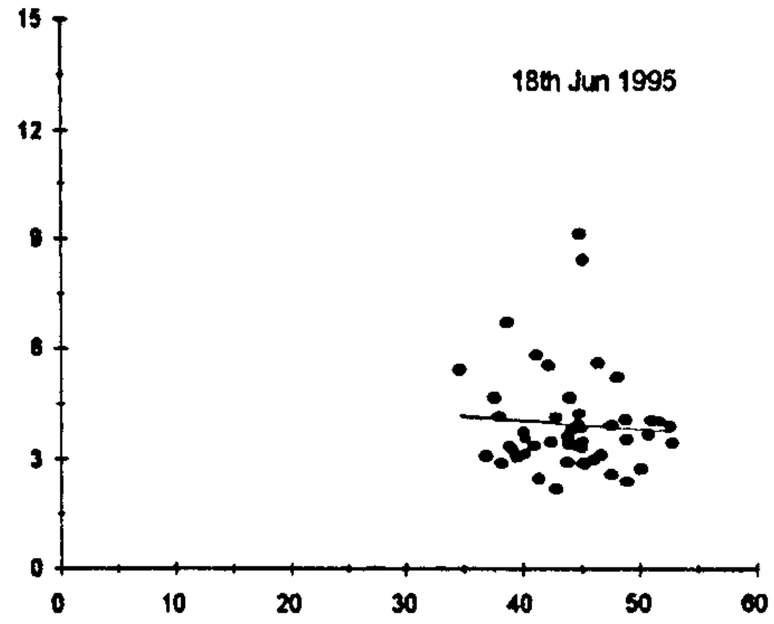


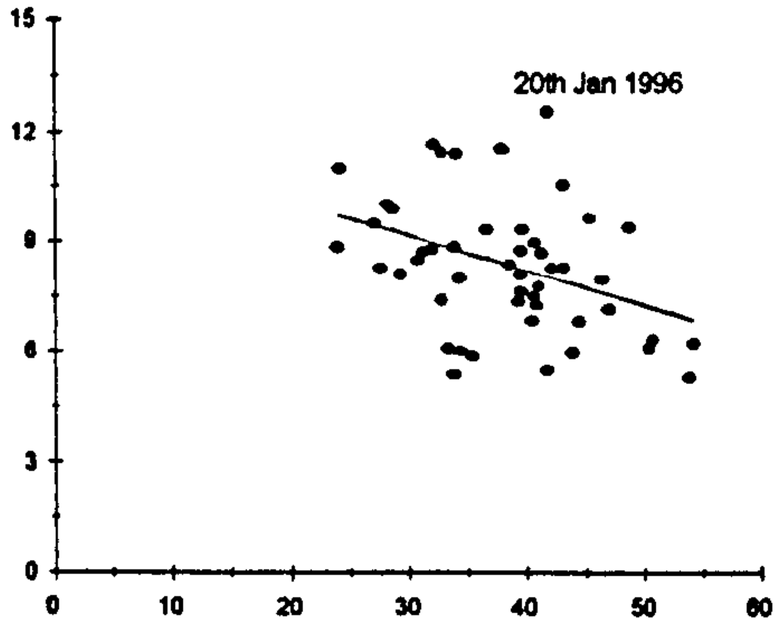


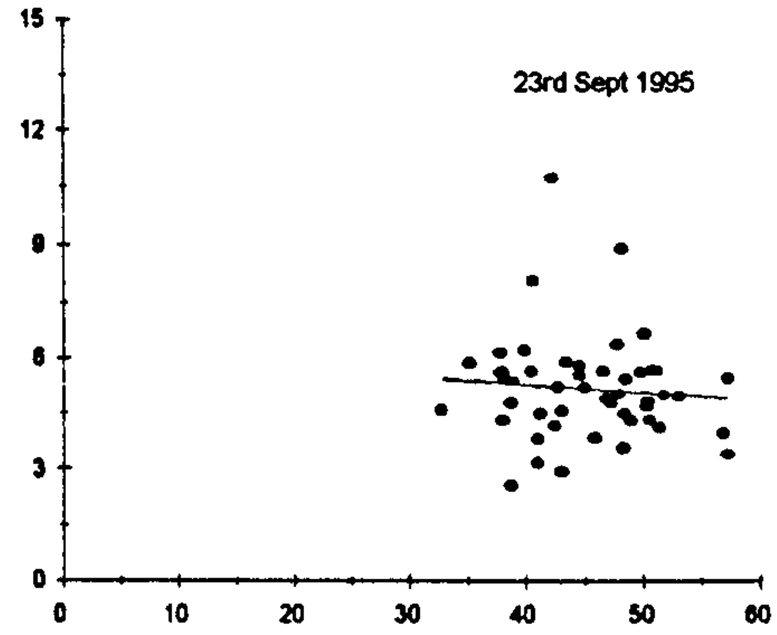


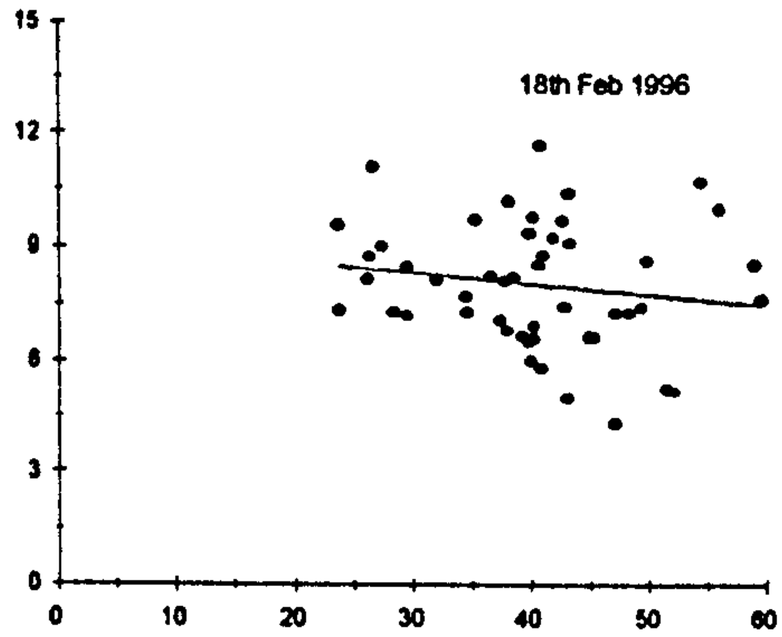
condition index (%)

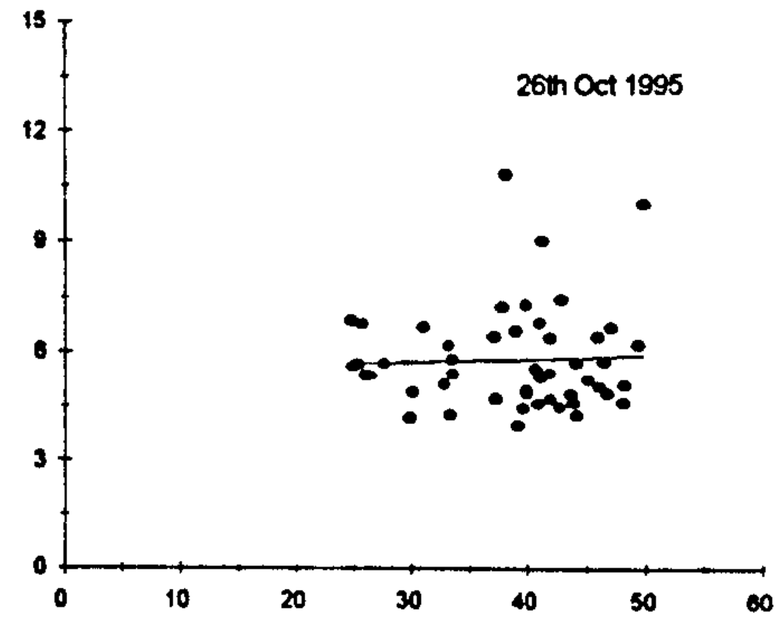
shell length (mm)

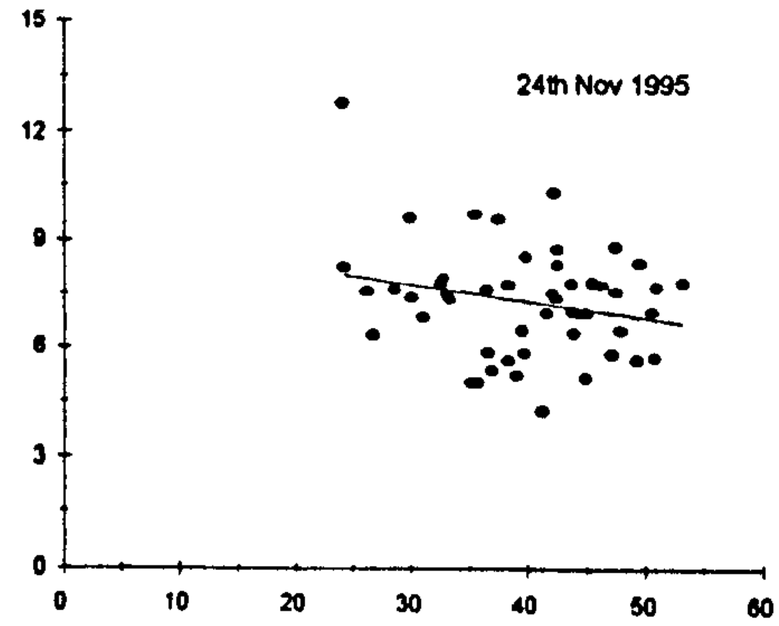


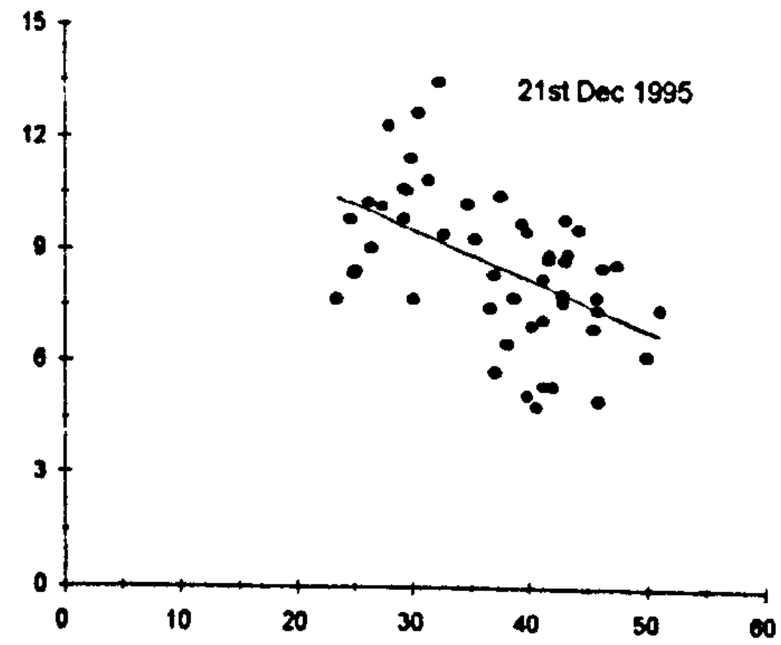








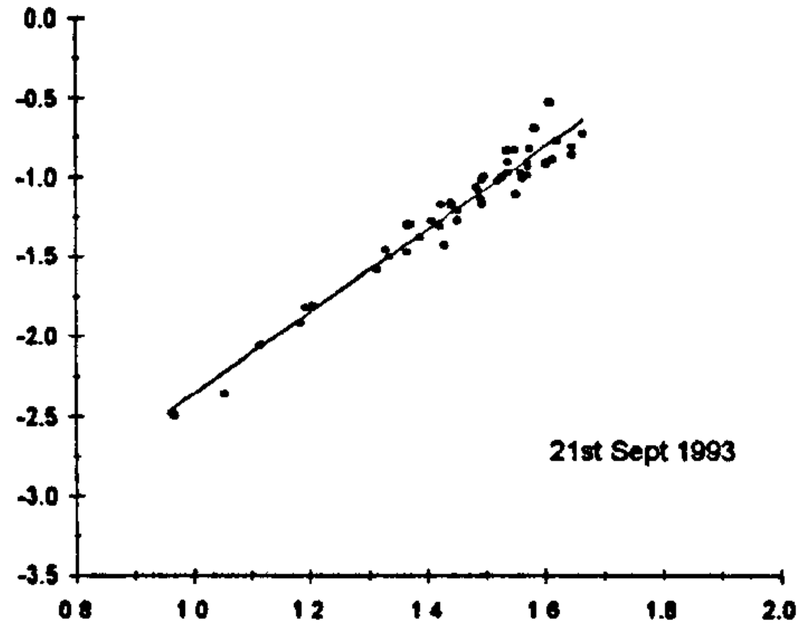


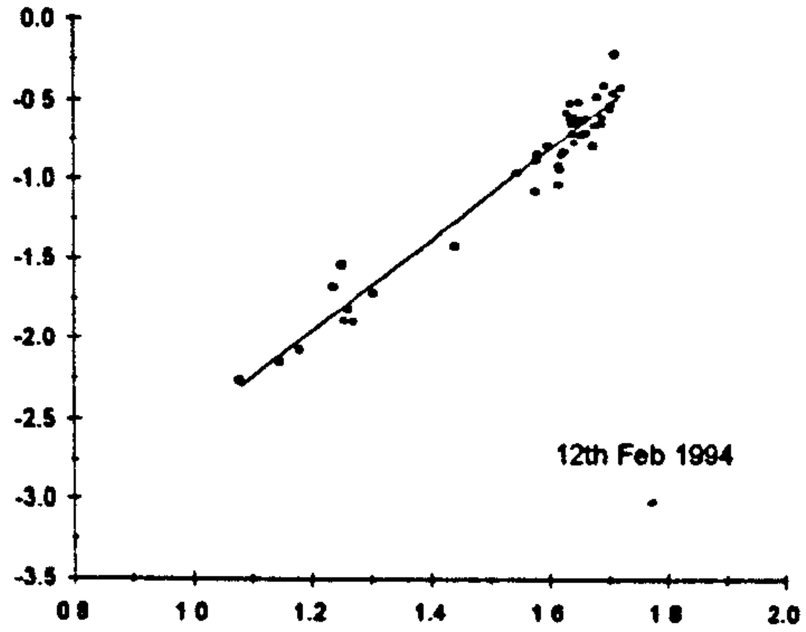


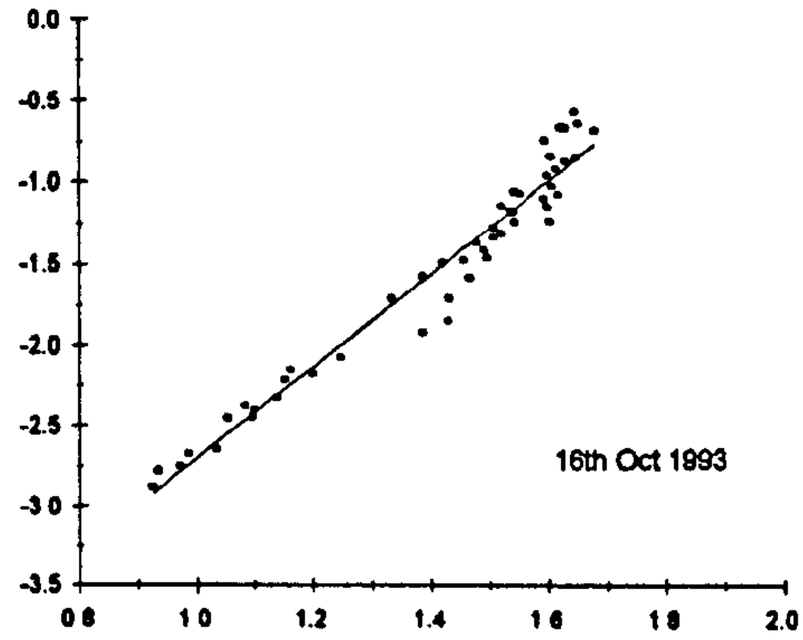
condition index (%)

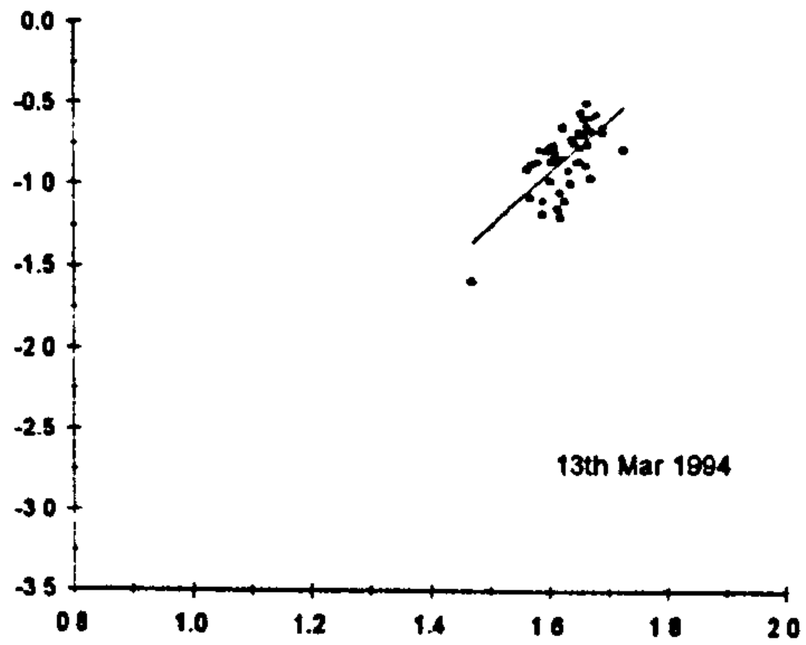
shell length (mm)

Figure 3.7 The relationship between log transformed dry tissue weight and shell length data in monthly samples of *Mytilus edulis chilensis* from Darwin. Lines fitted by least squares linear regression (on 3 consecutive pages).

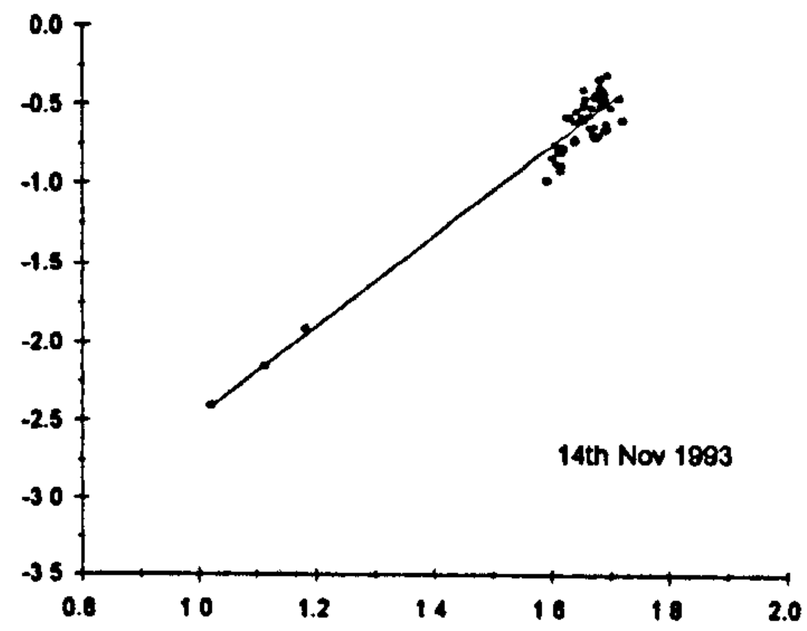


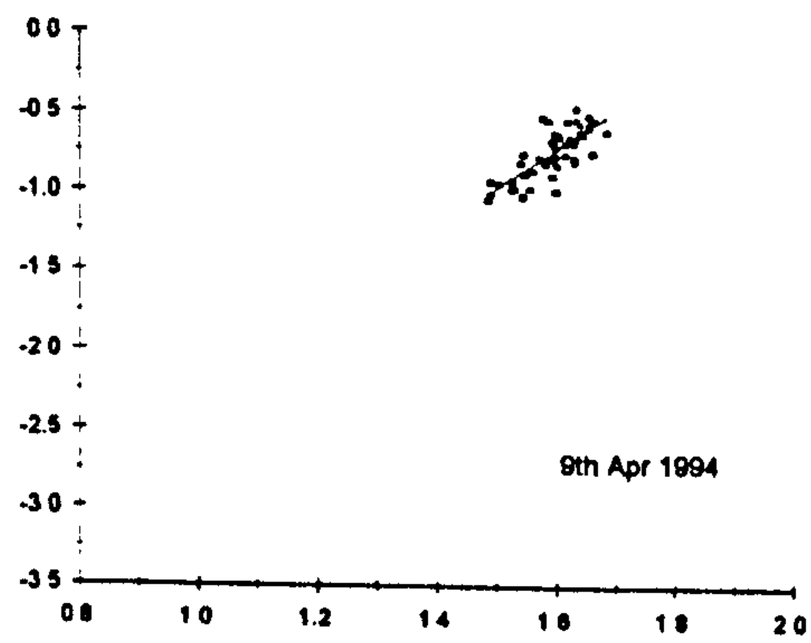


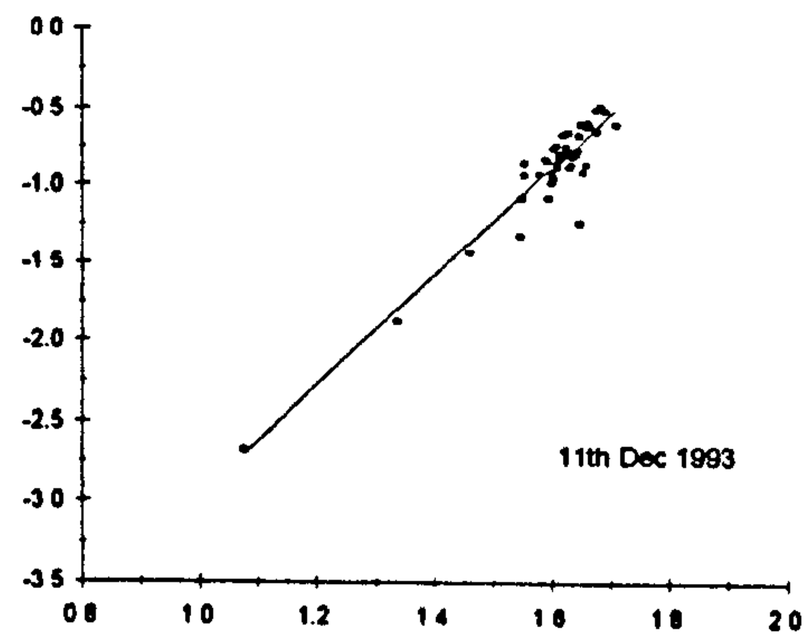


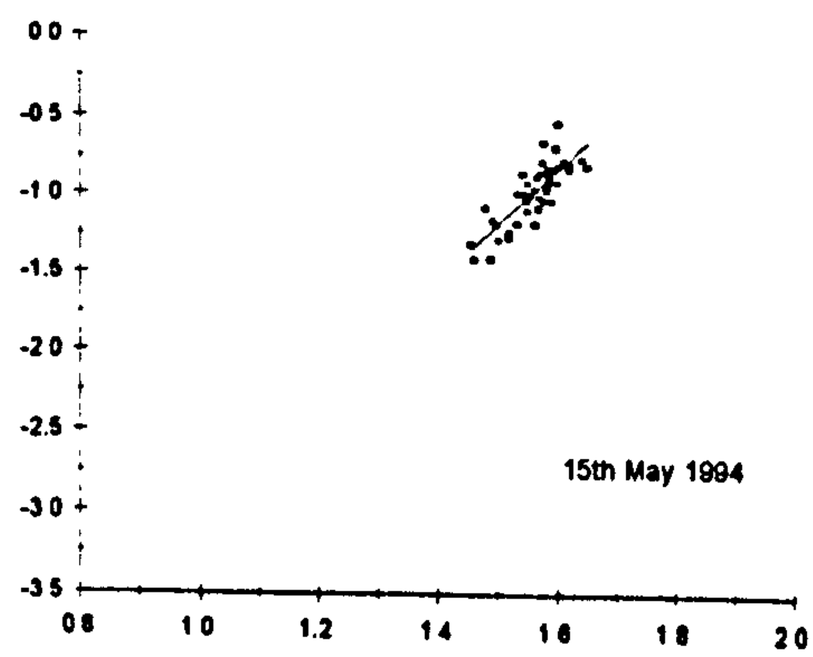


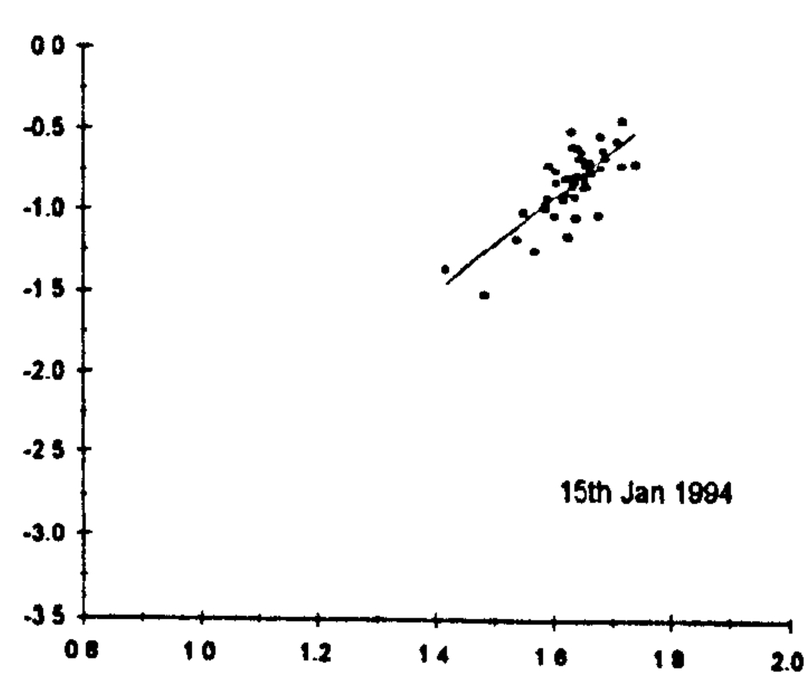
dry tissue weight (g)

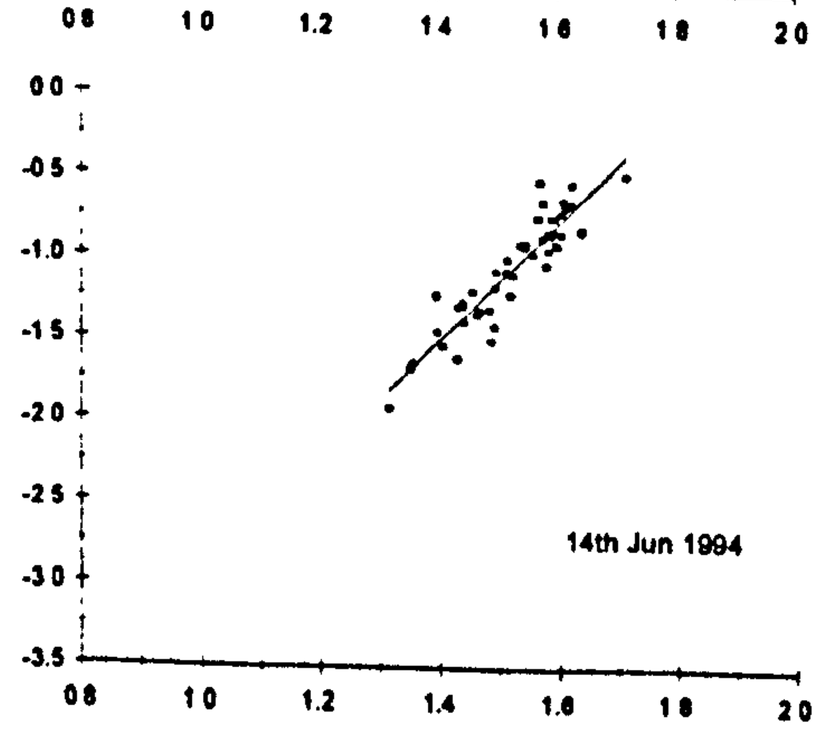




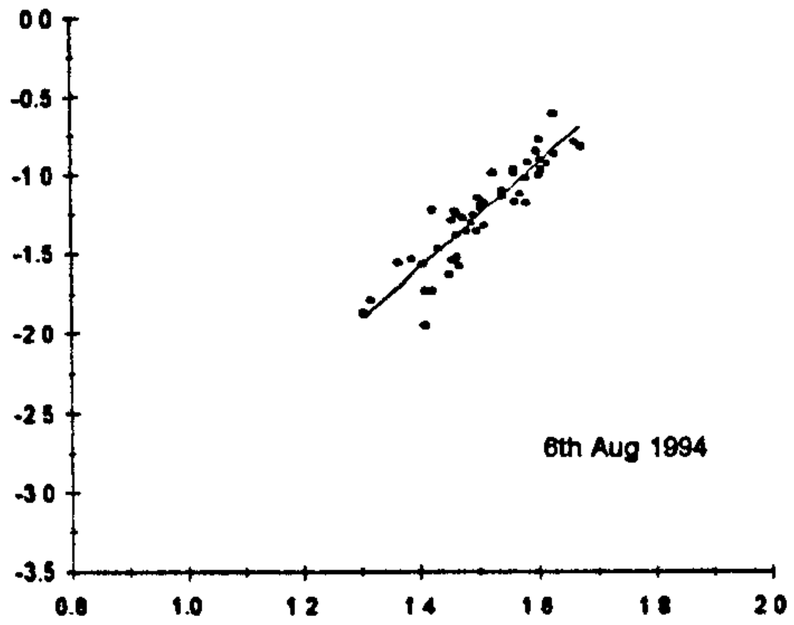


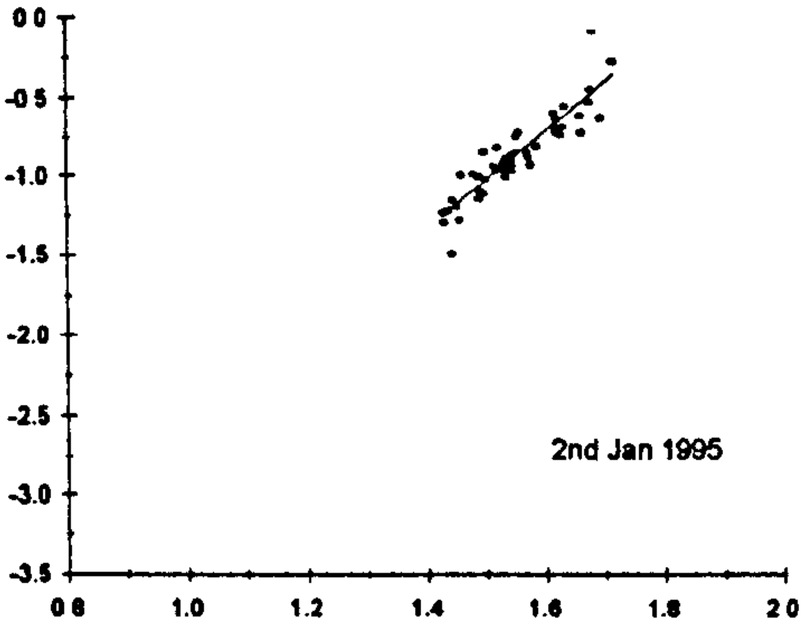


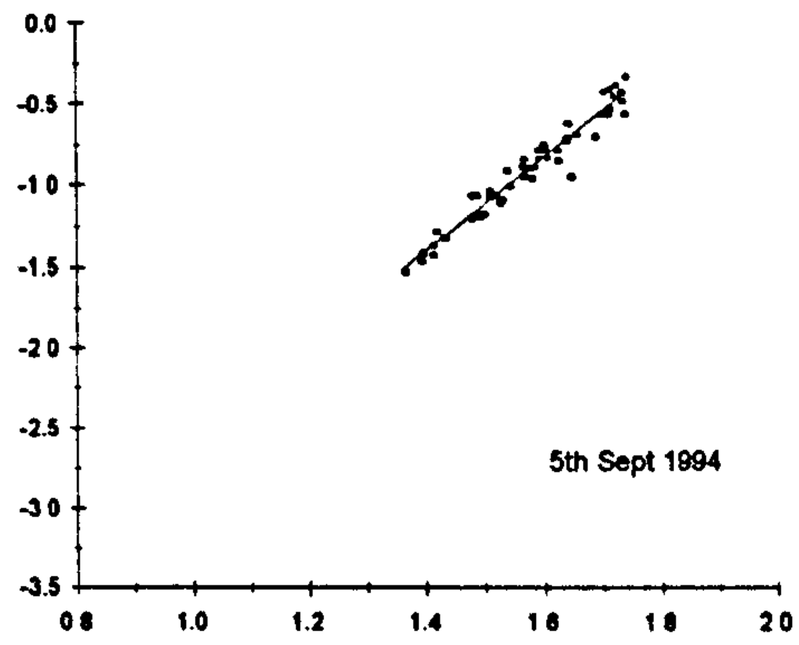


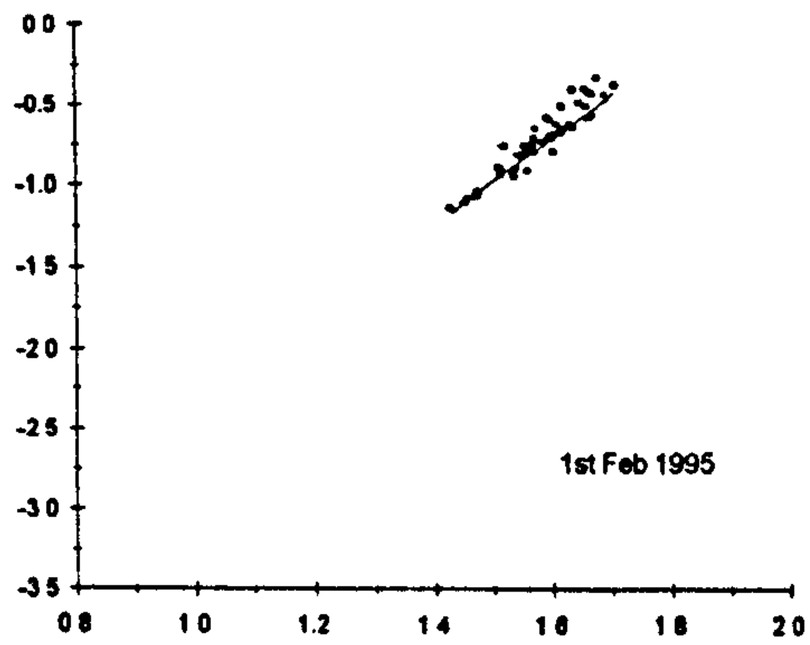


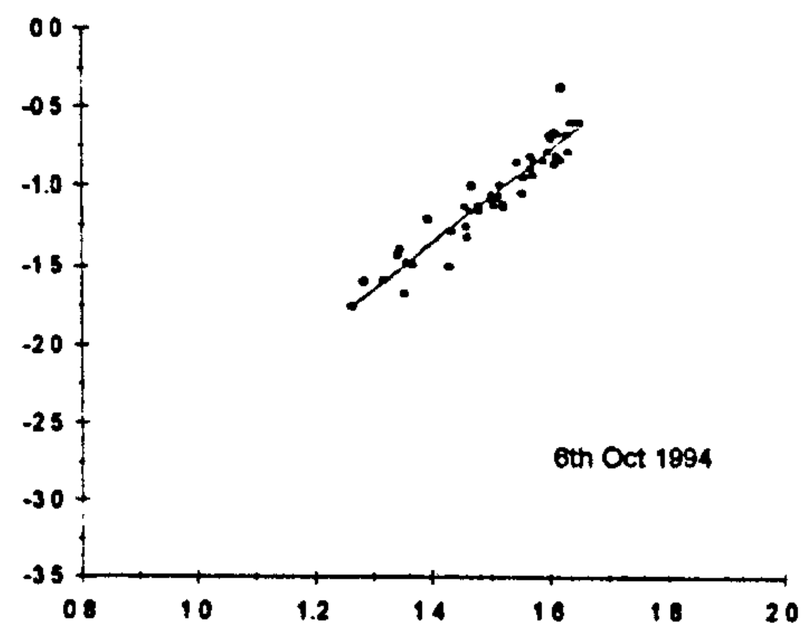
shell length (mm)

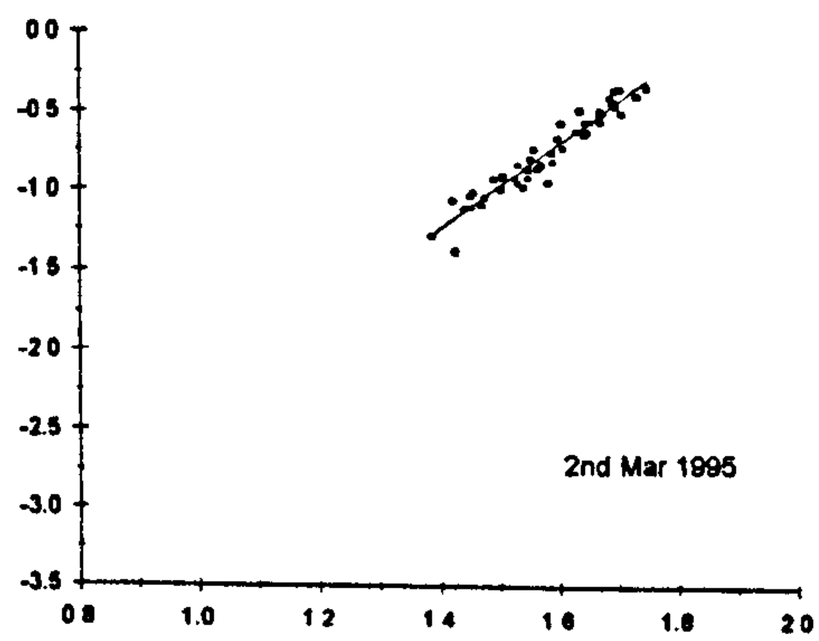


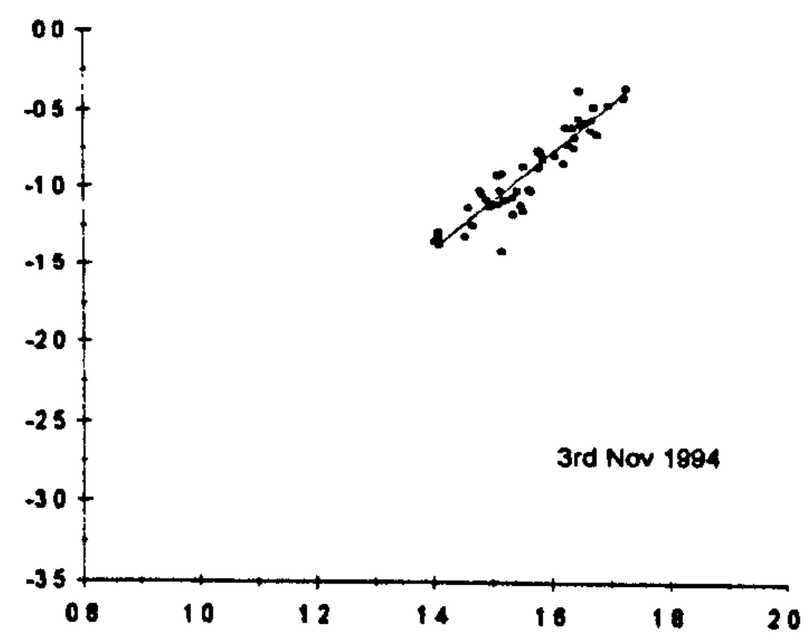


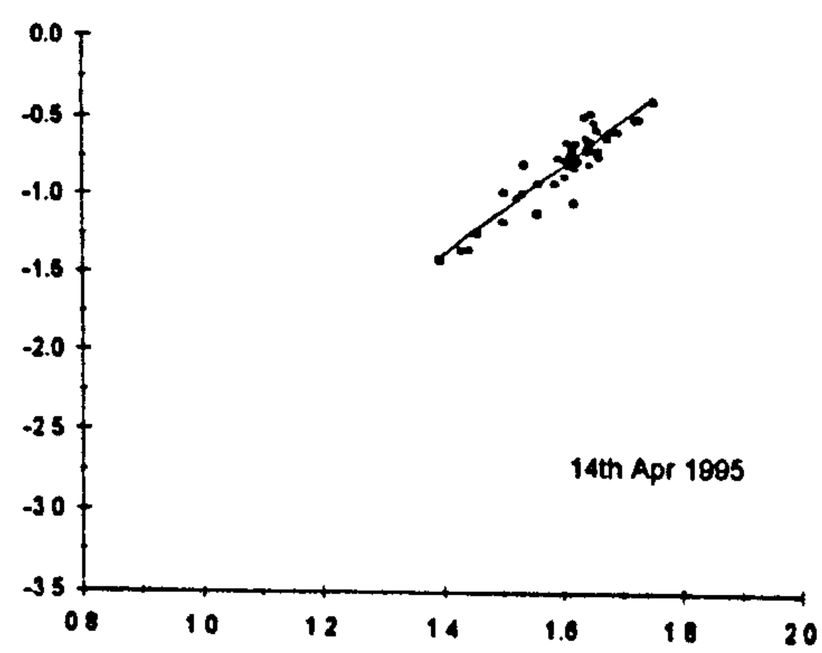


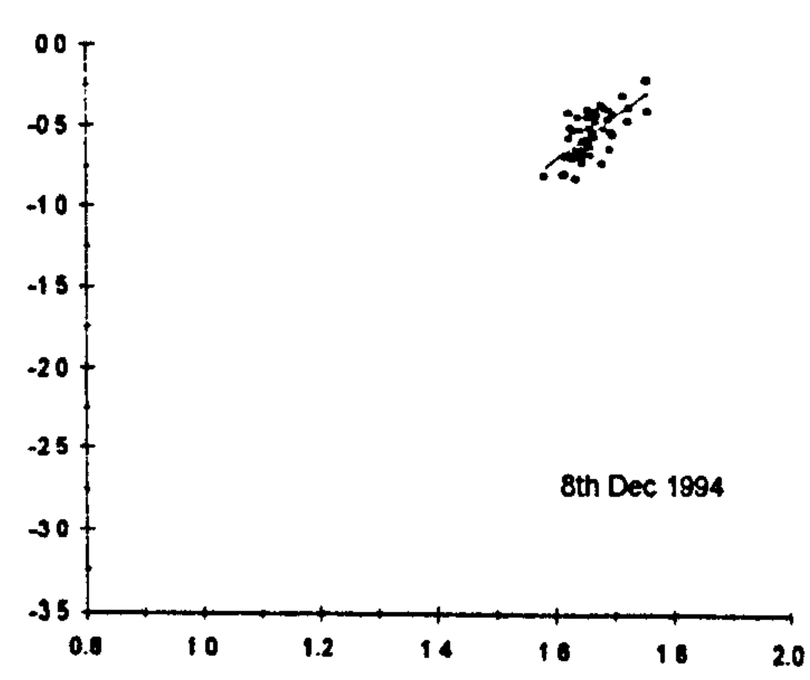


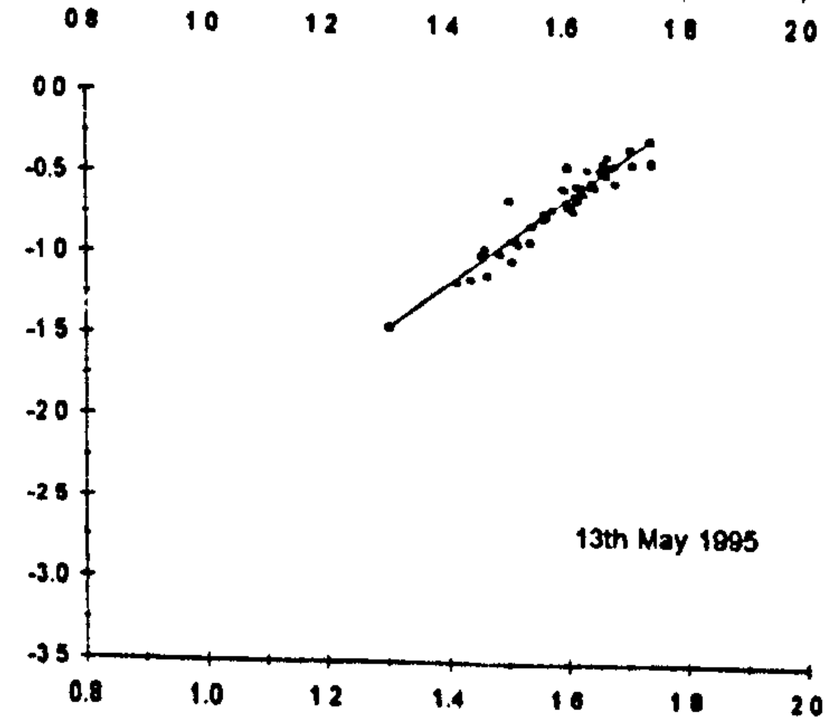






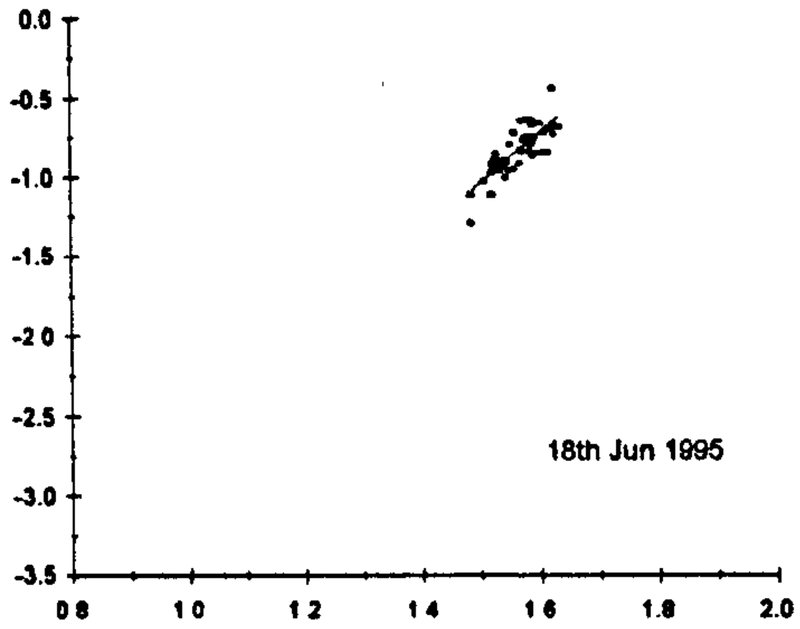


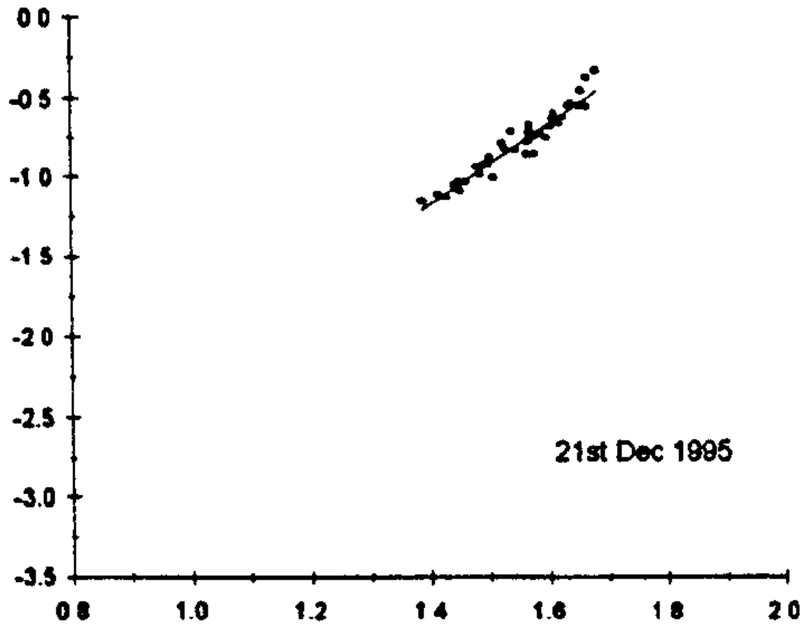


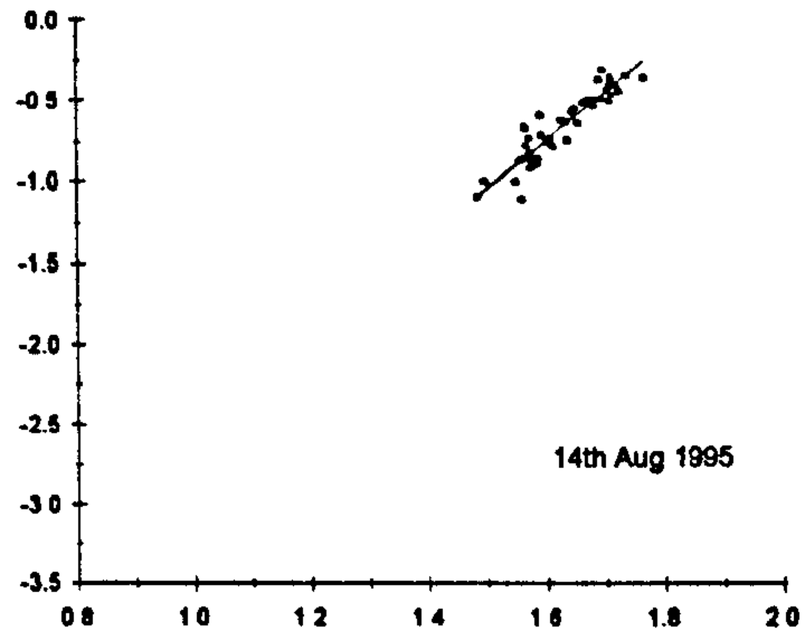


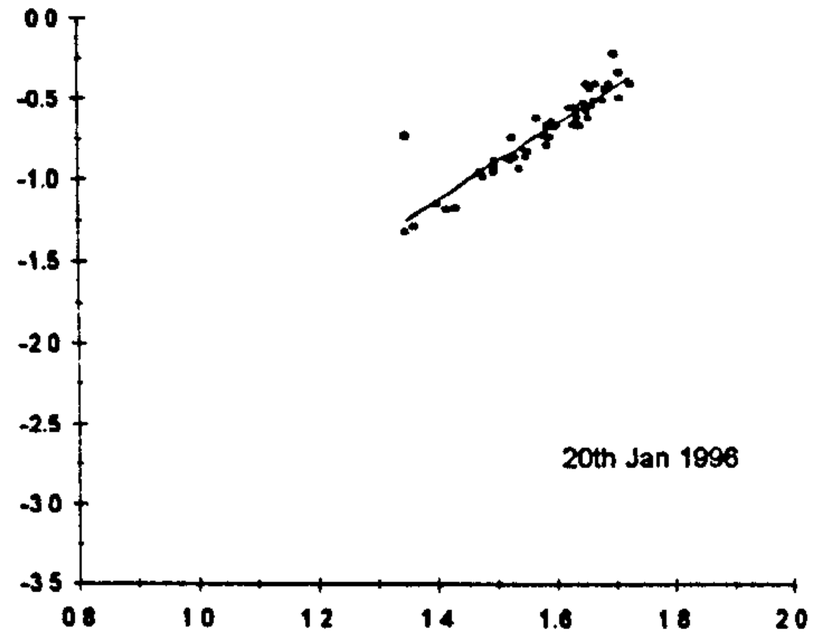
dry tissue weight (g)

shell length (mm)

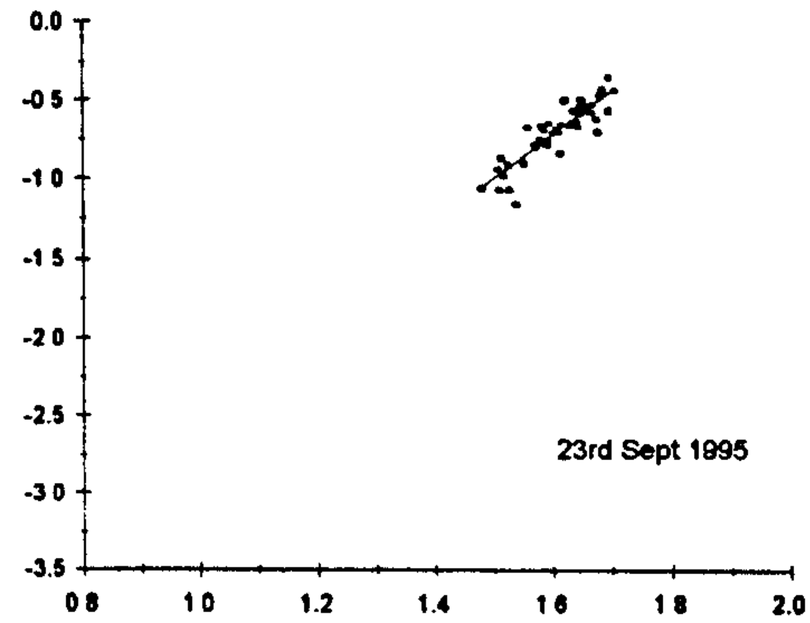


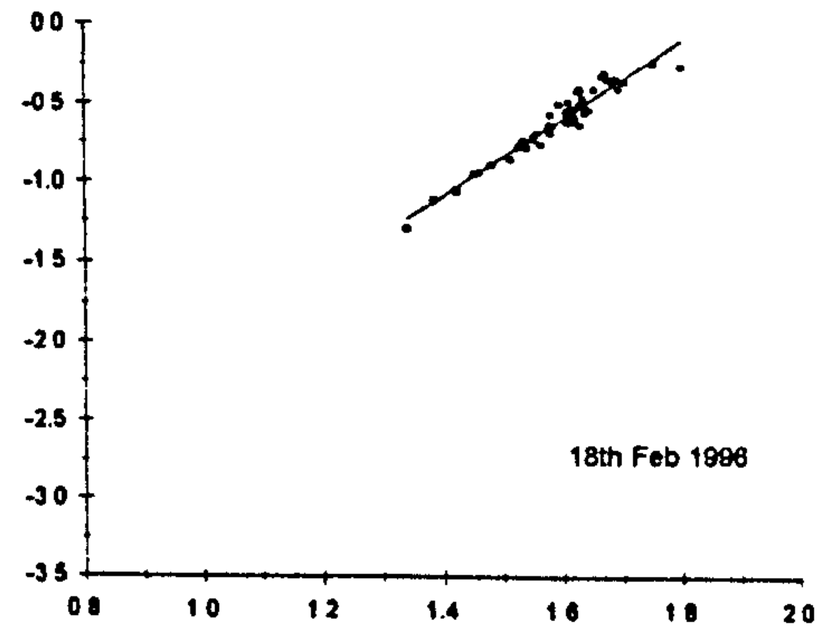


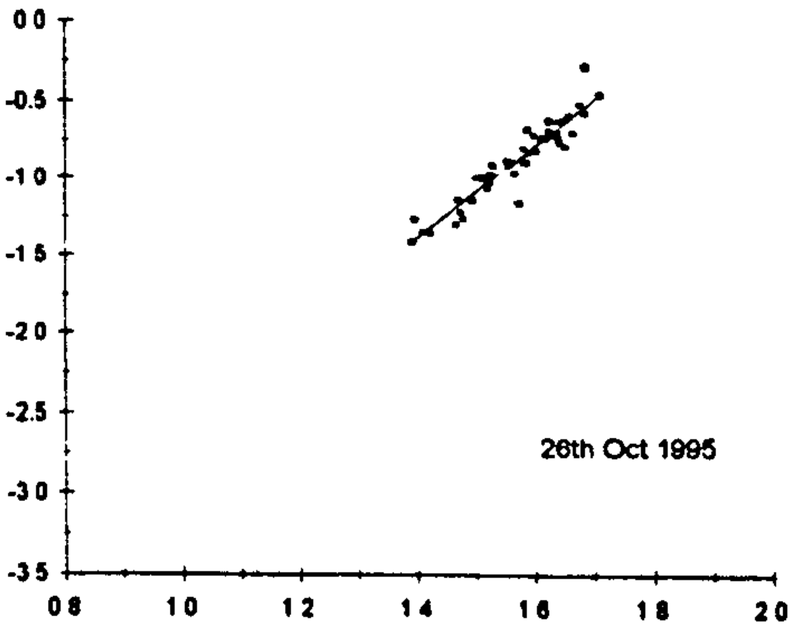


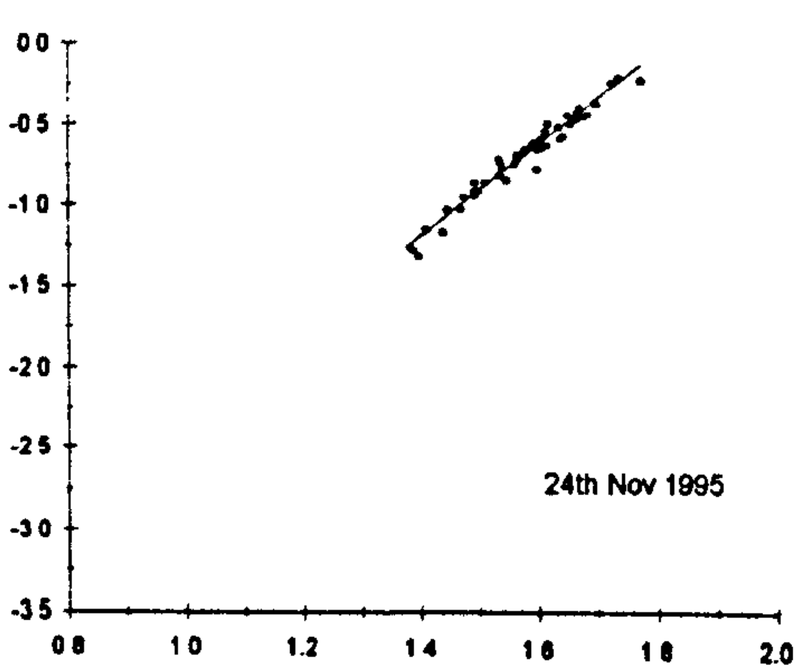


dry tissue weight (g)



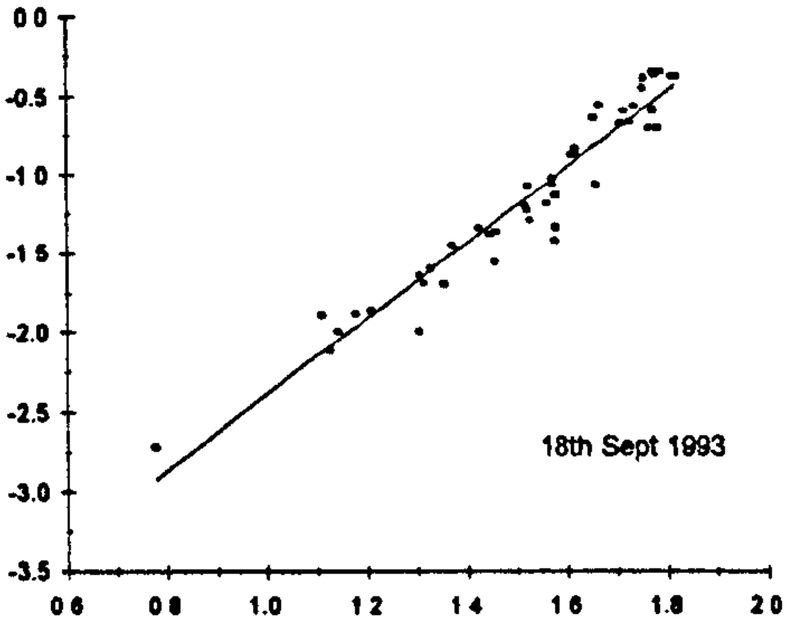


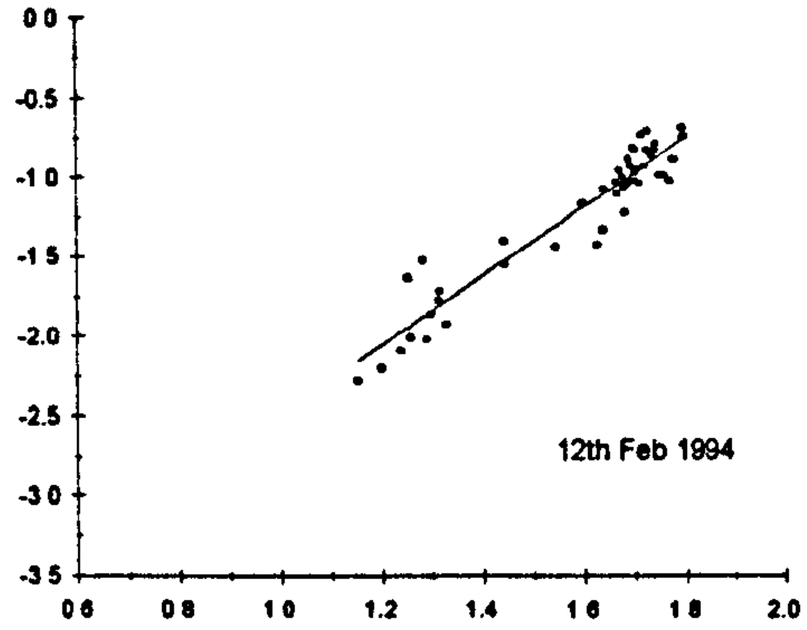


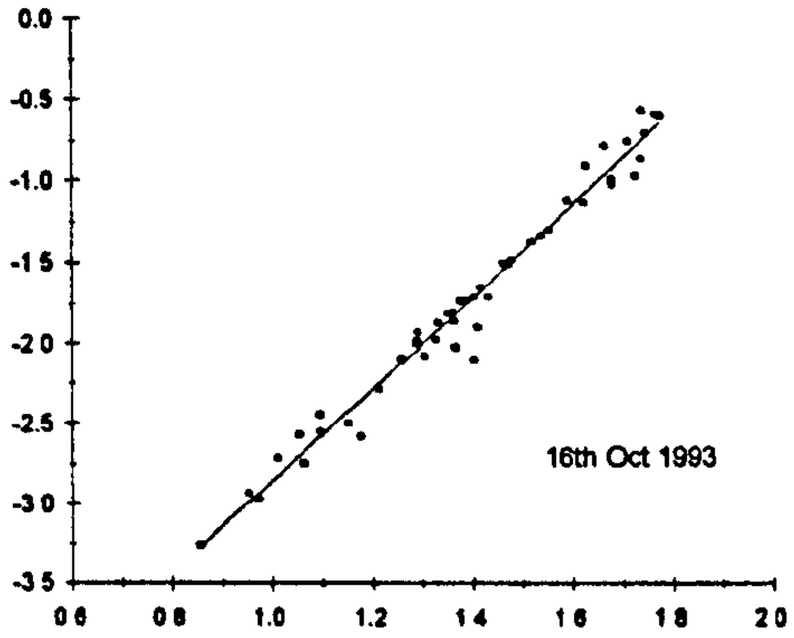


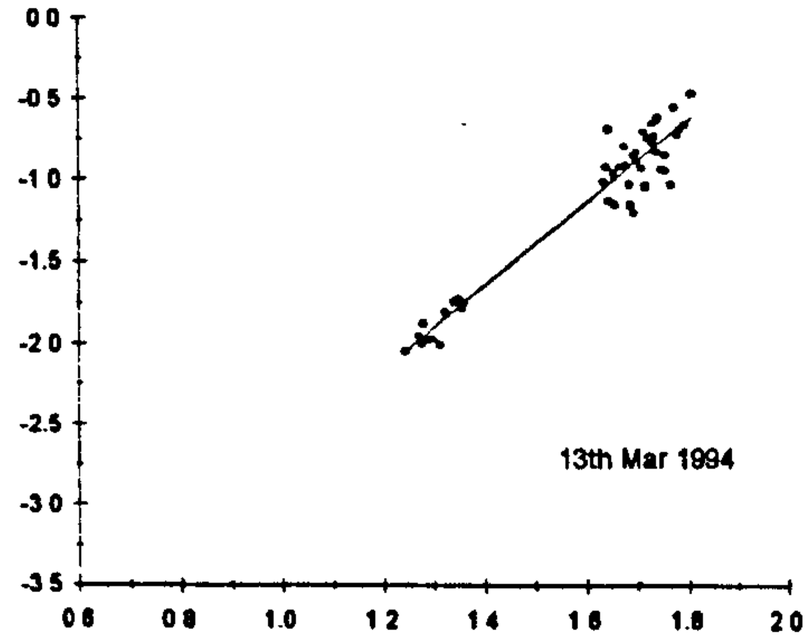
shell length (mm)

Figure 3.8 The relationship between log transformed dry tissue weight and shell length data in monthly samples of *Mytilus edulis chilensis* from Camilla Creek. Lines fitted by least squares linear regression (on 3 consecutive pages).

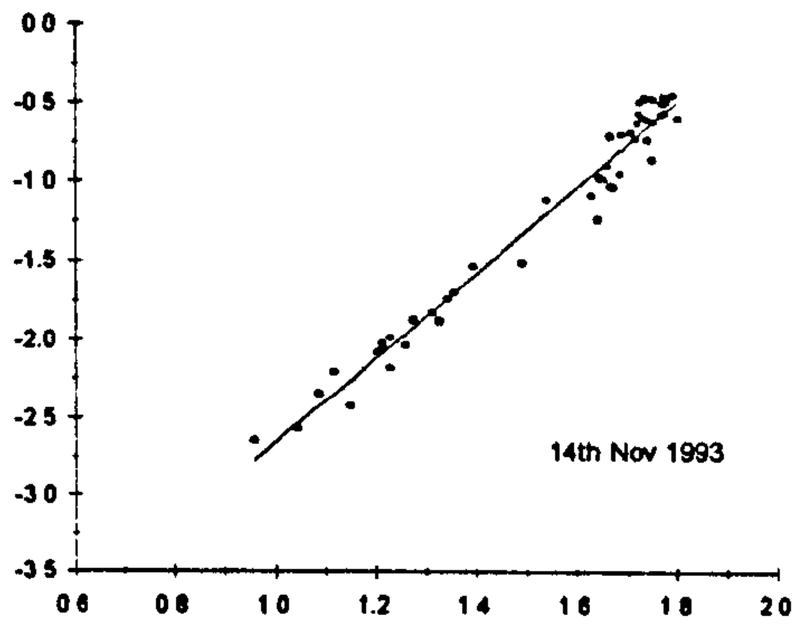


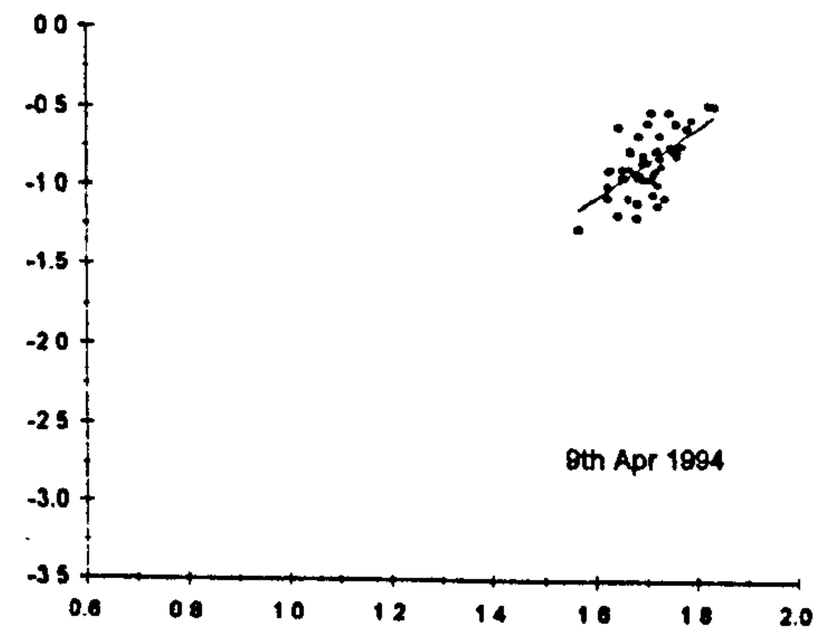


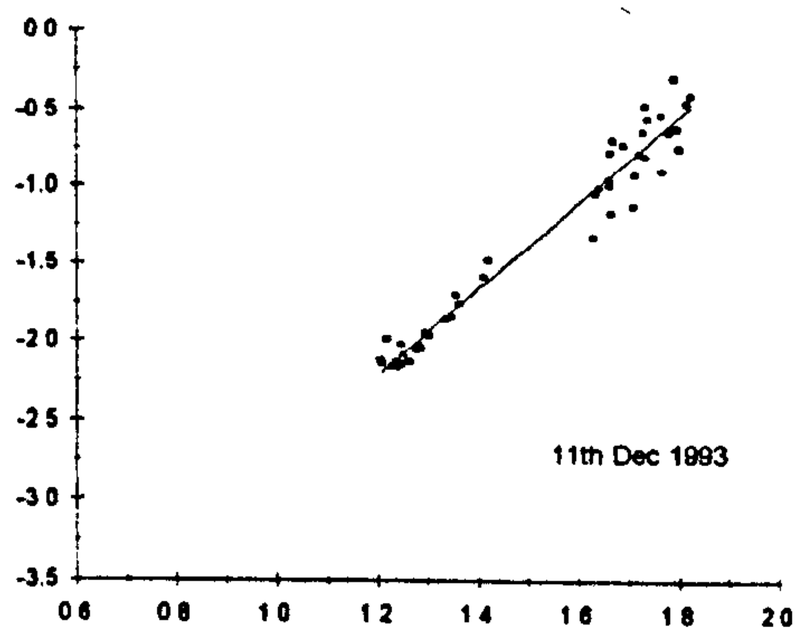


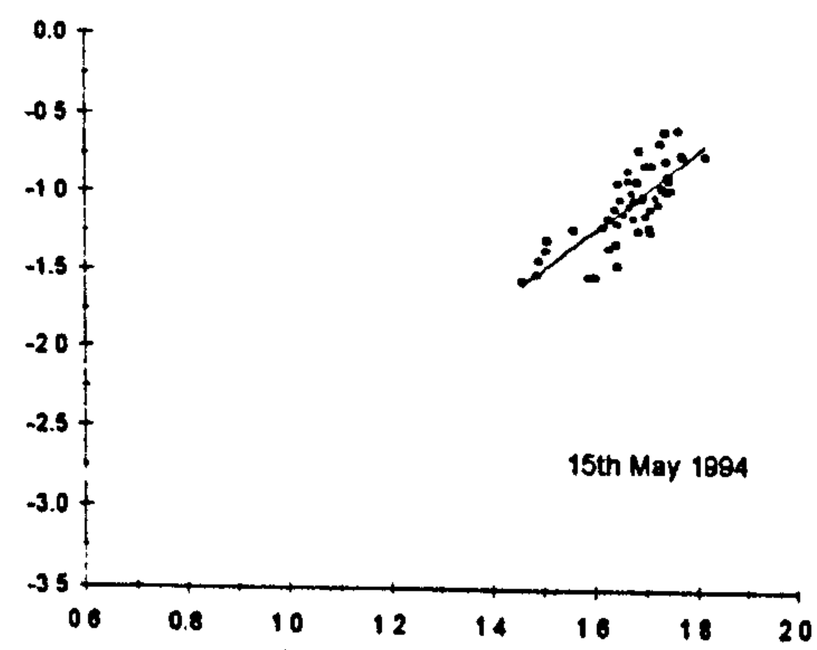


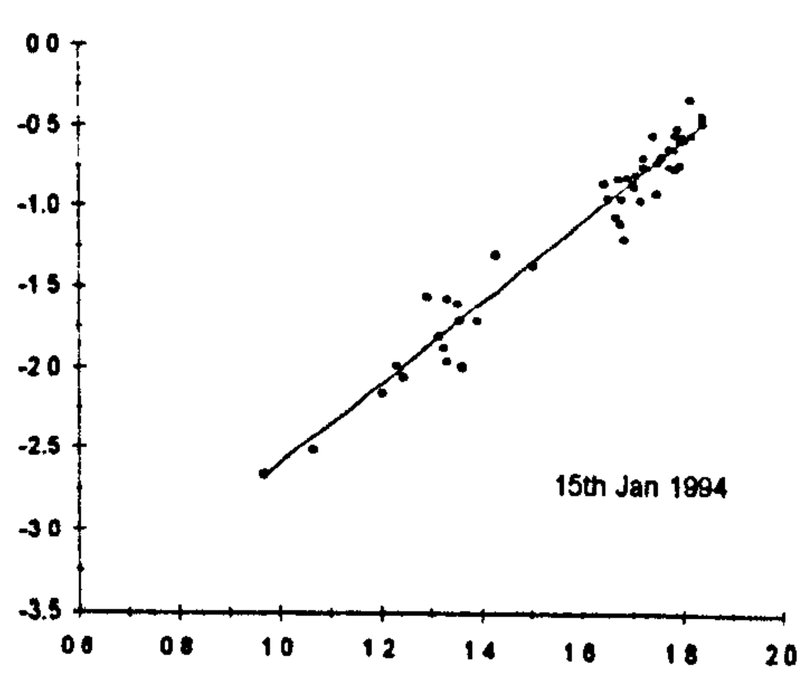
dry tissue weight (g)

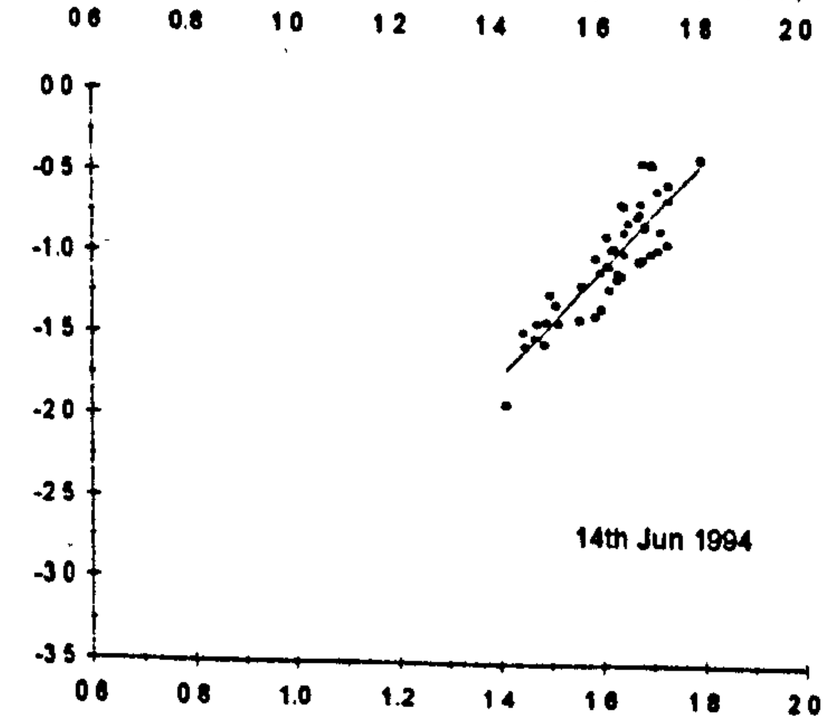




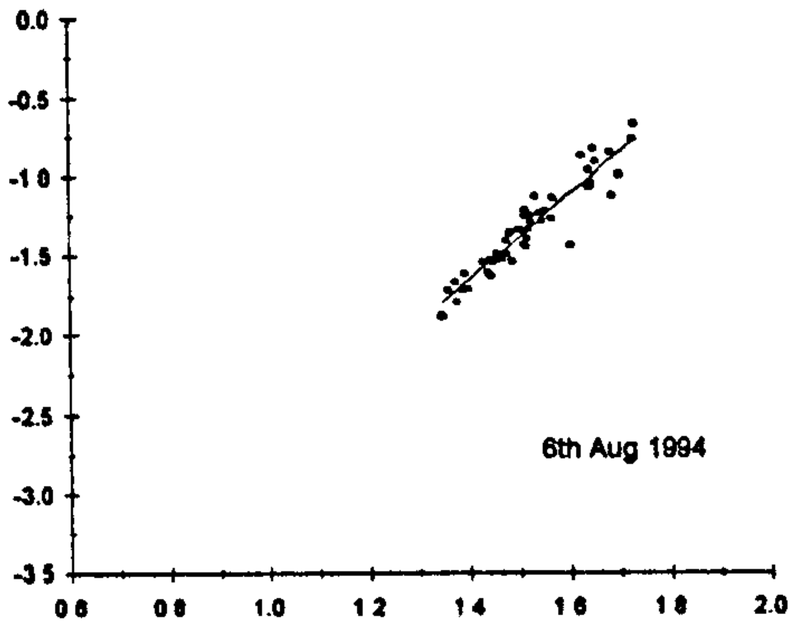


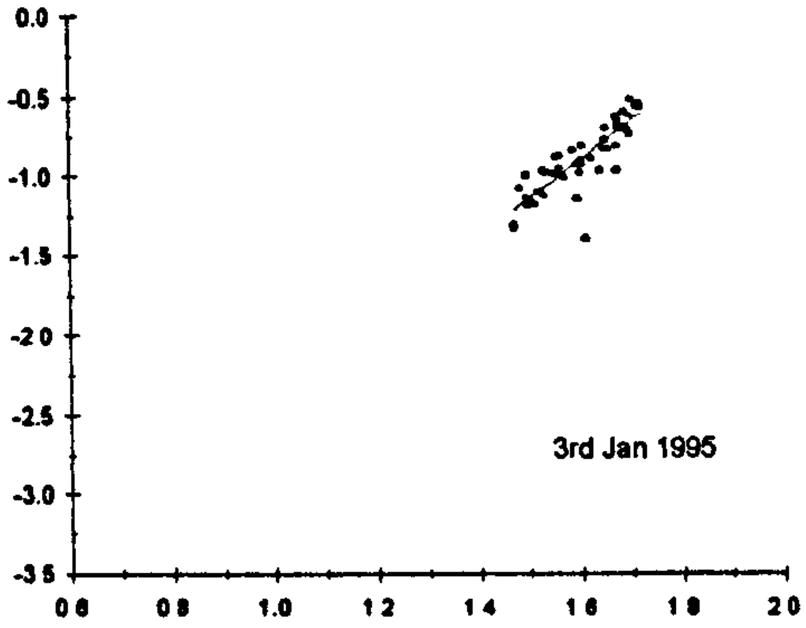


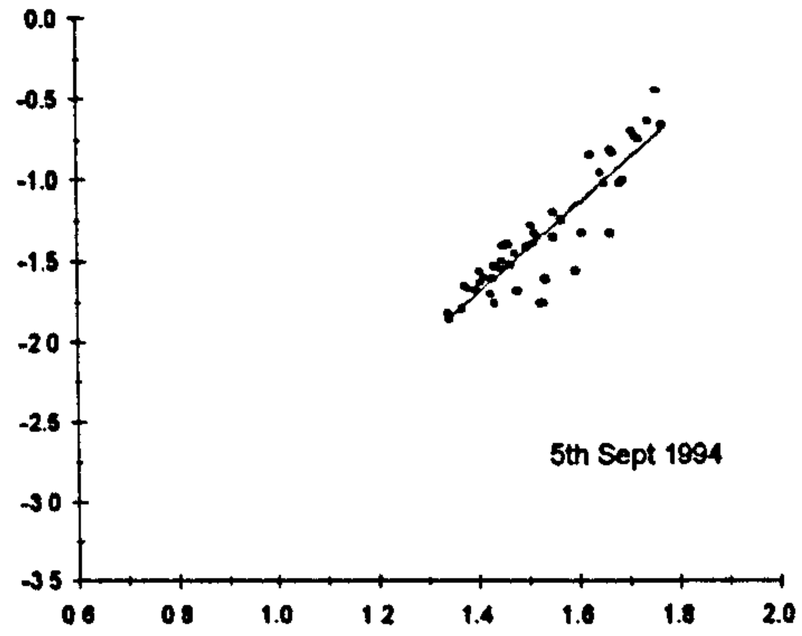


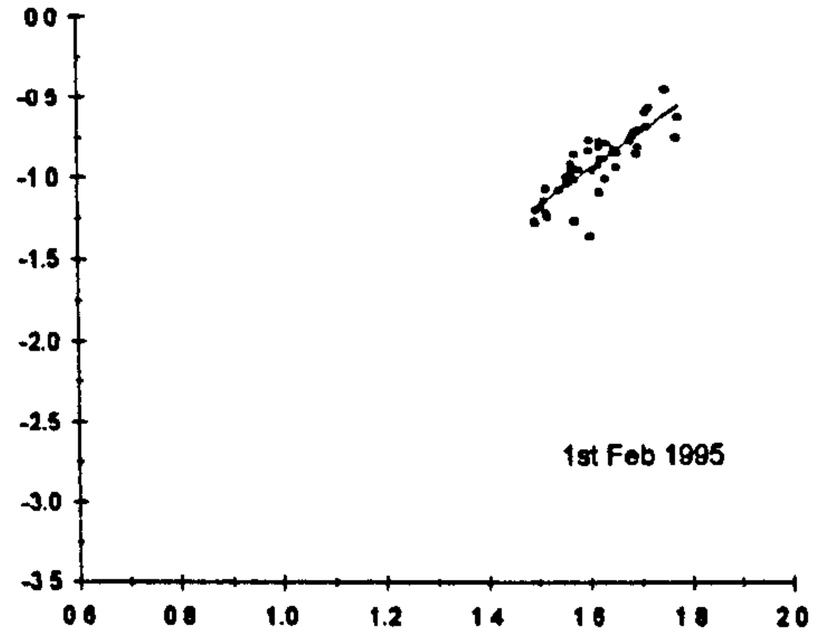


shell length (mm)

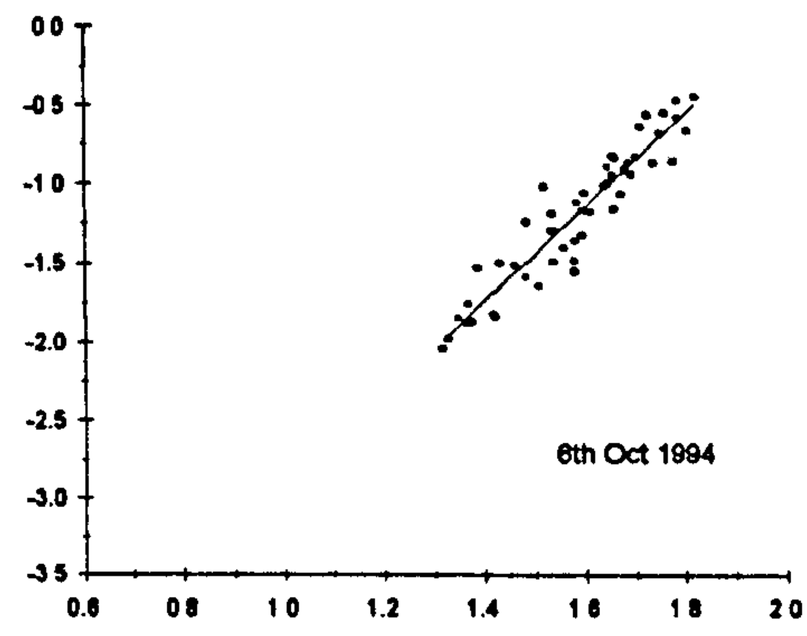


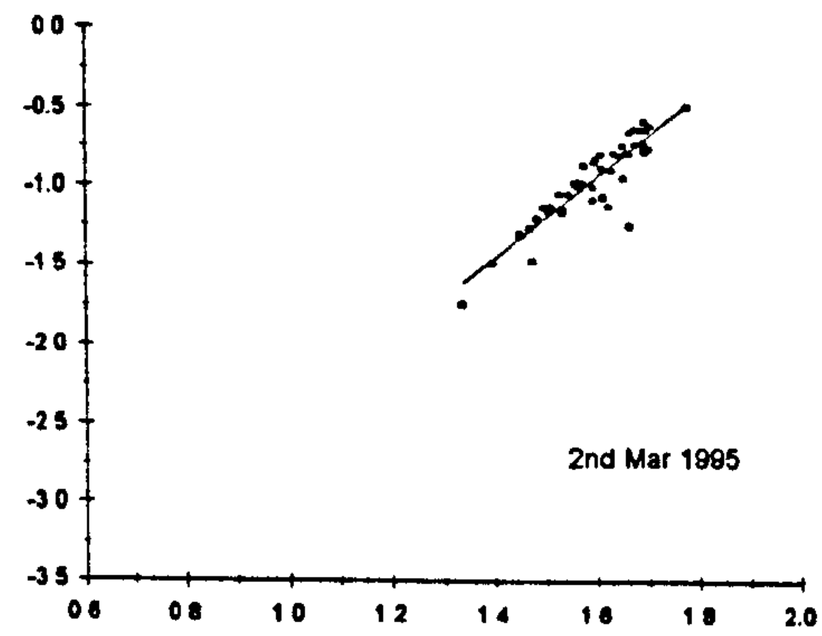


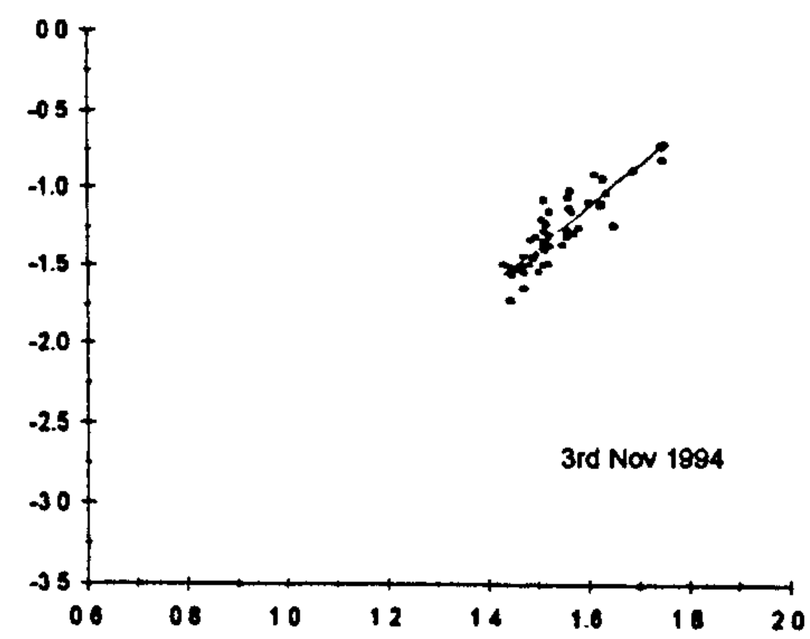


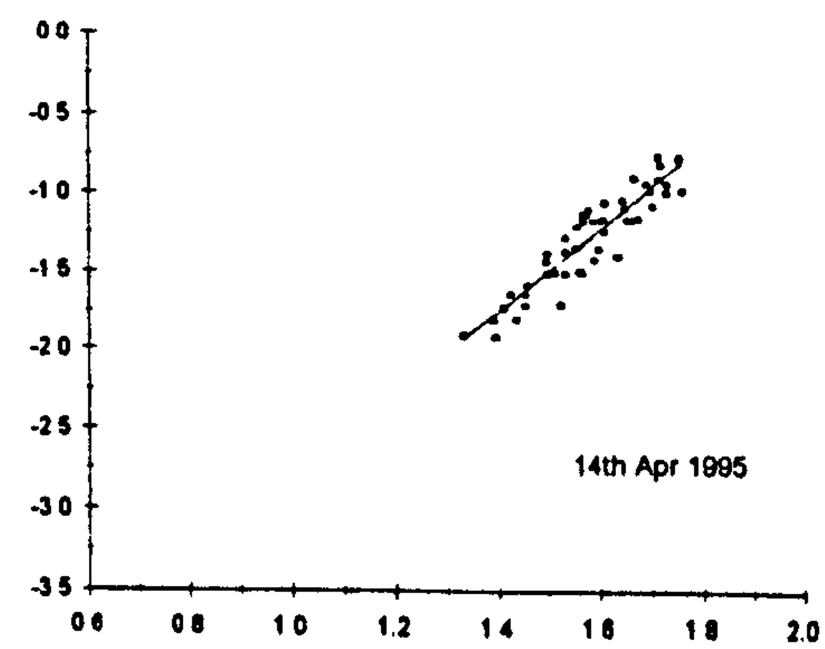


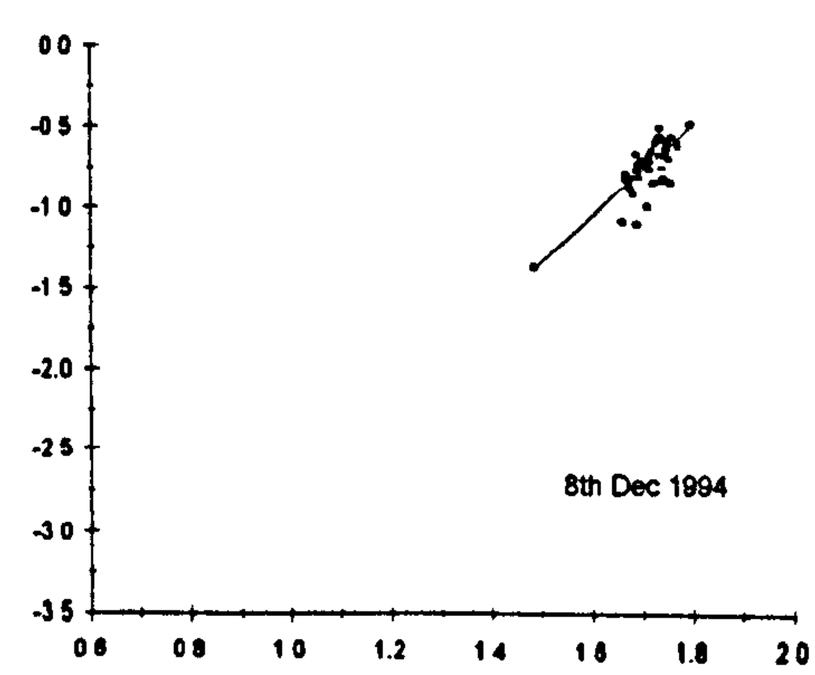
dry tissue weight (g)

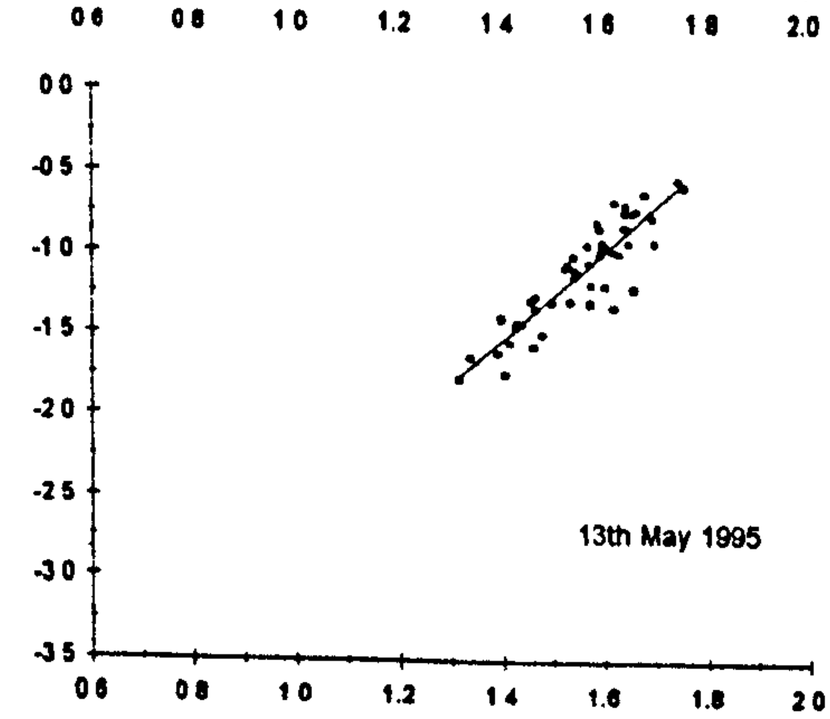




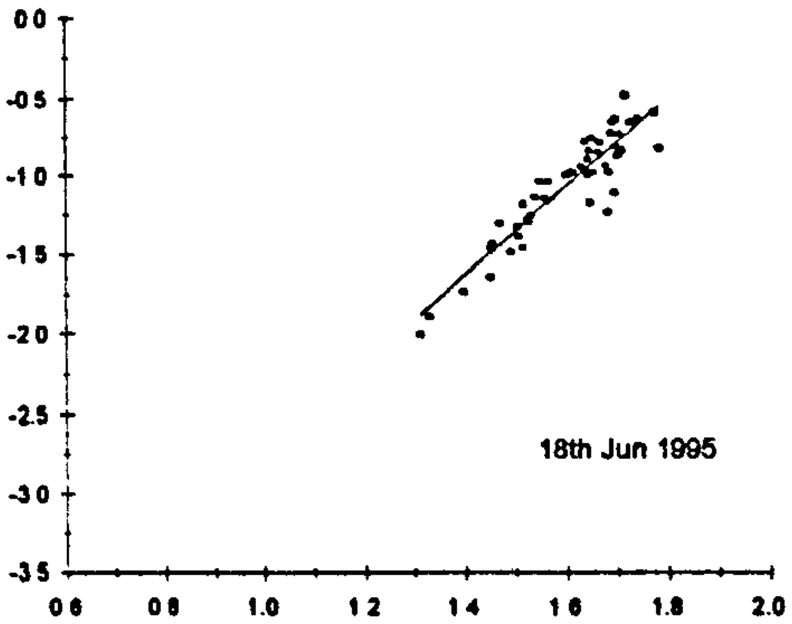


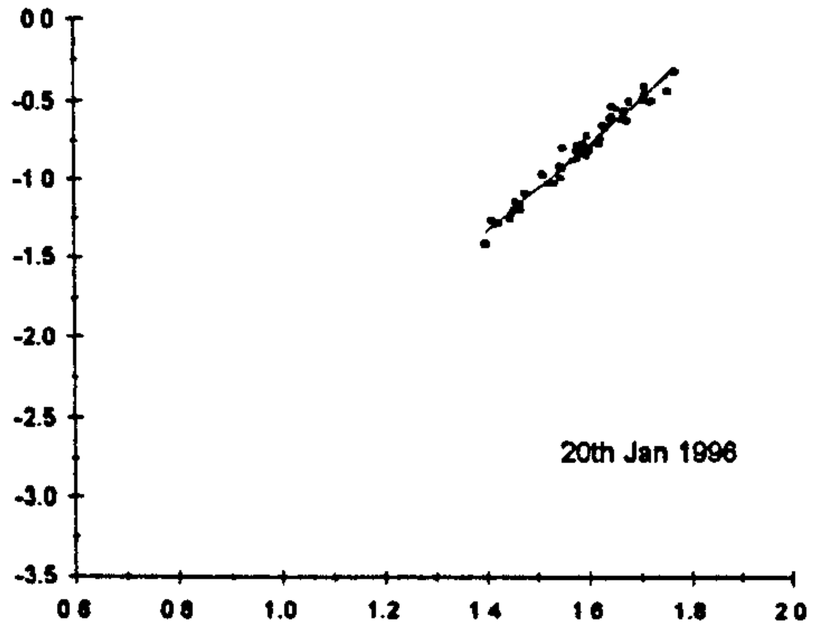


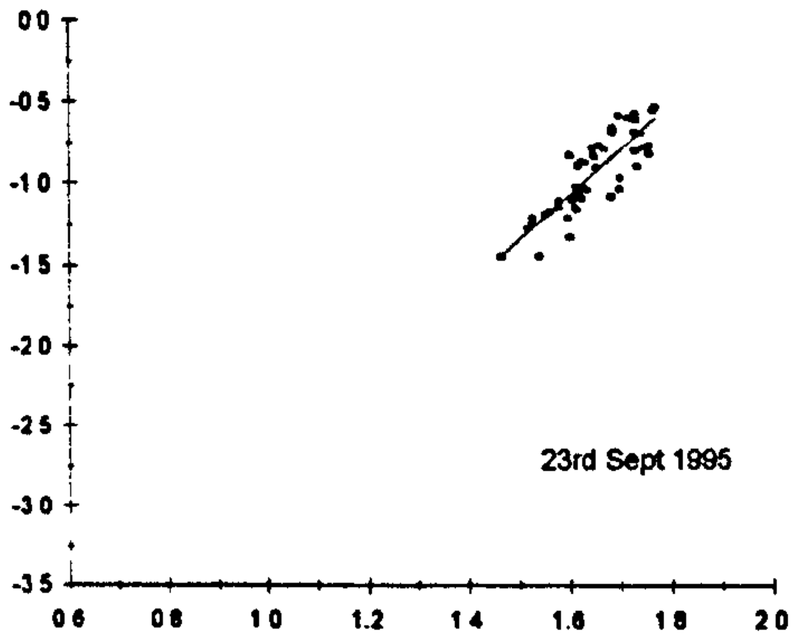


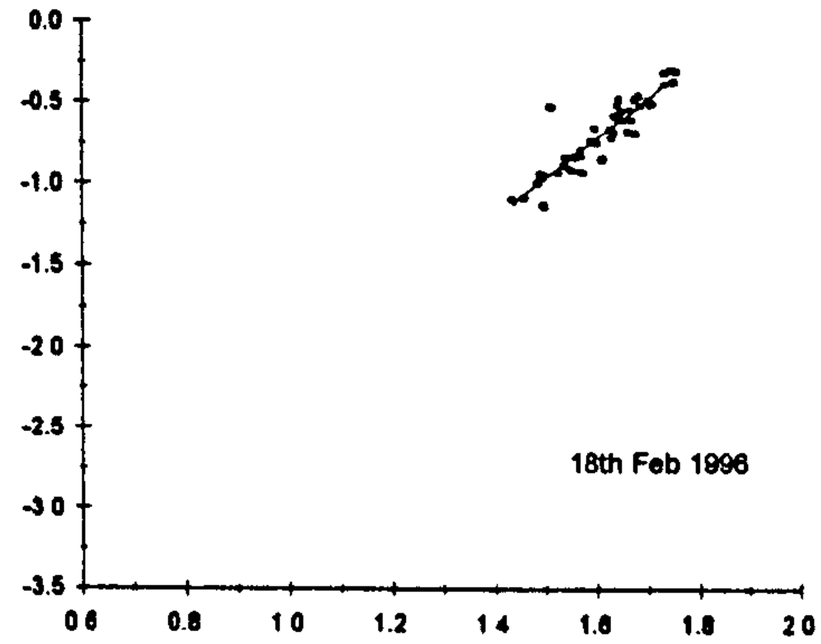


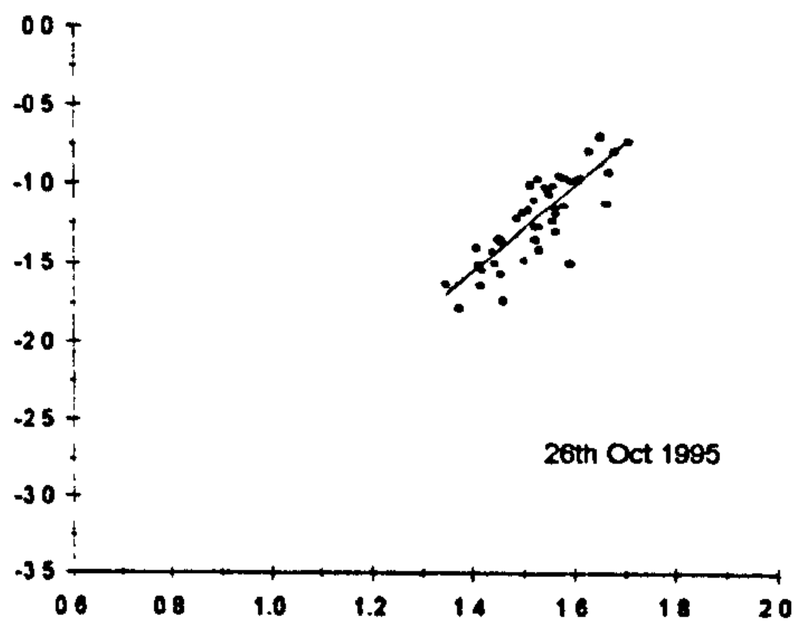
shell length (mm)

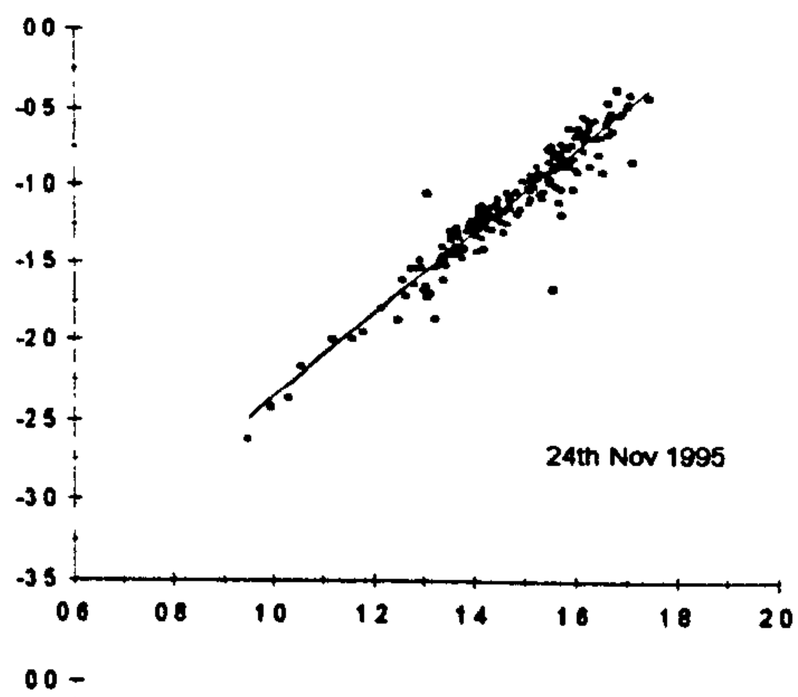


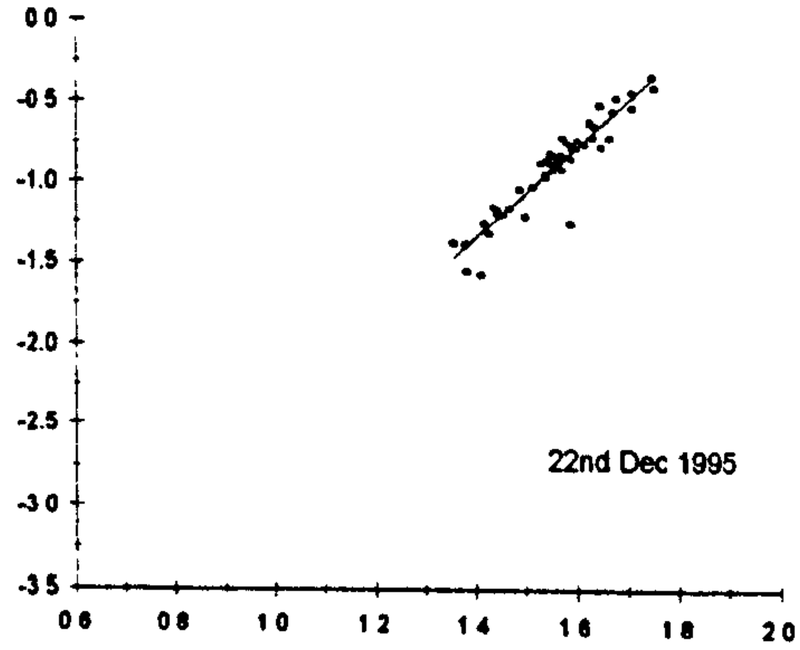








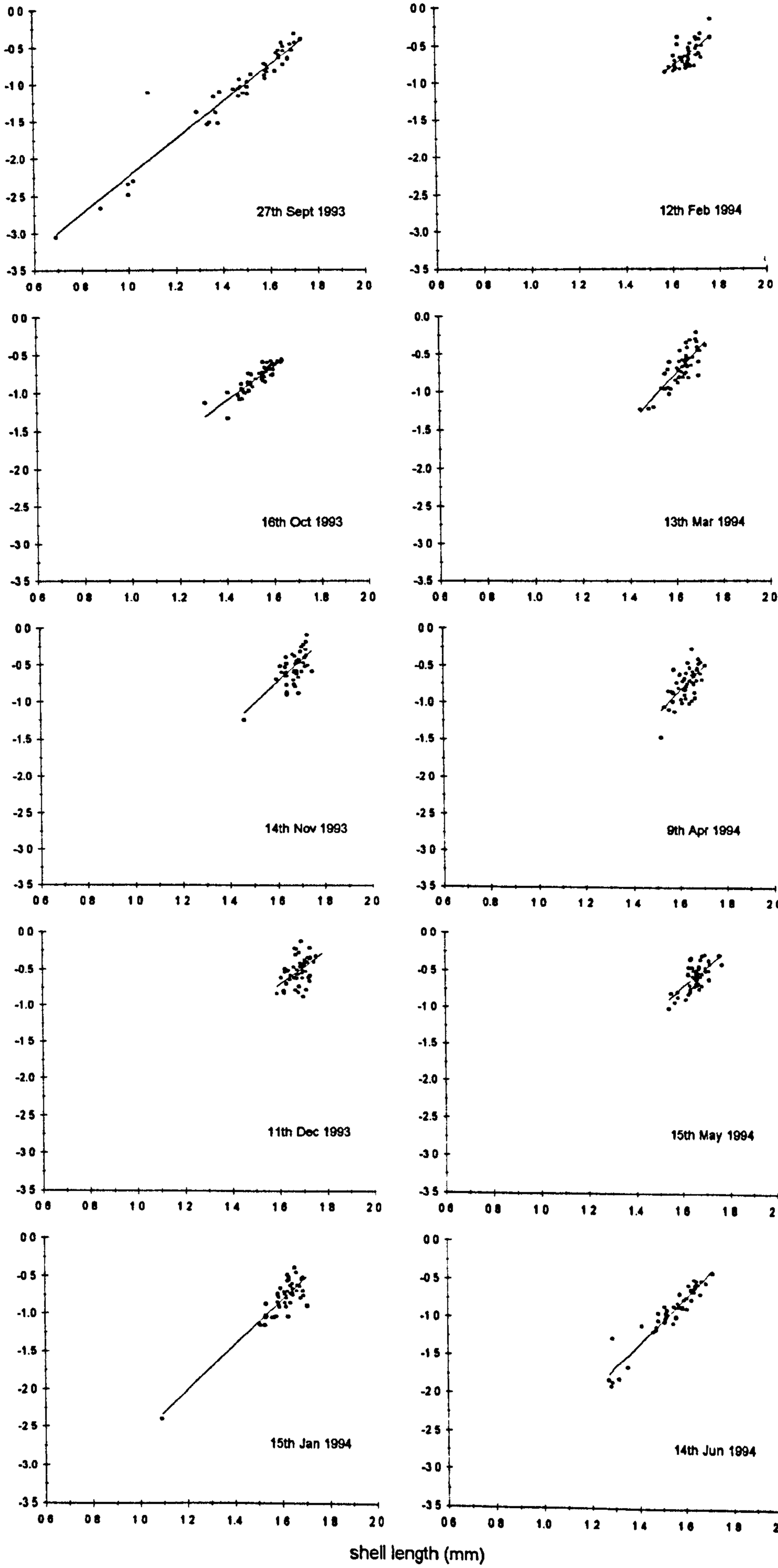


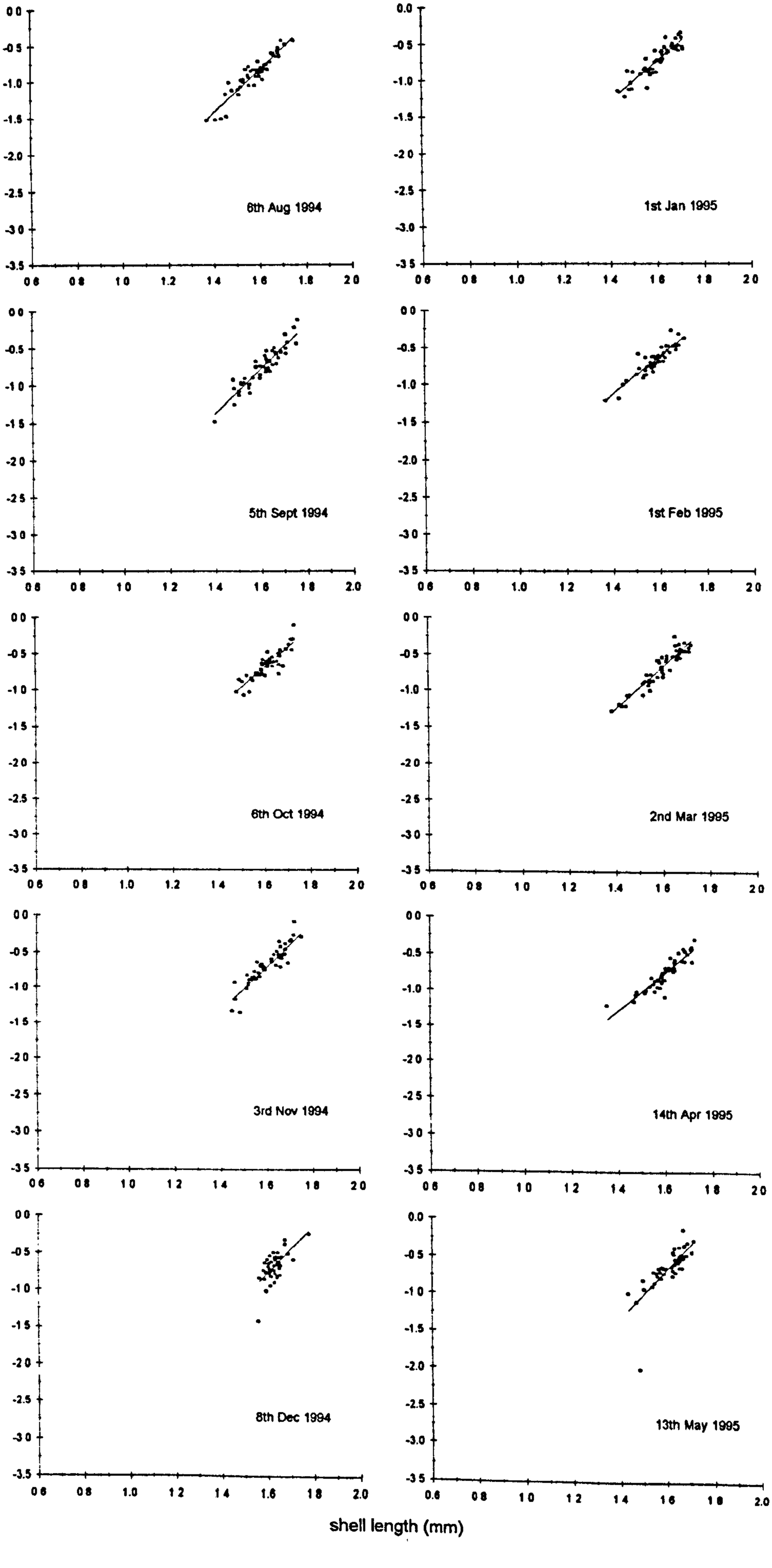


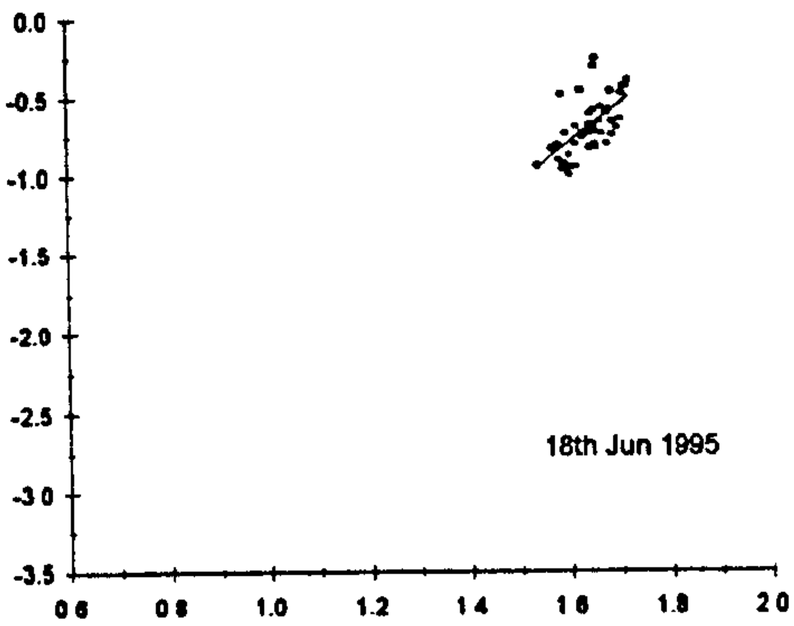
dry tissue weight (g)

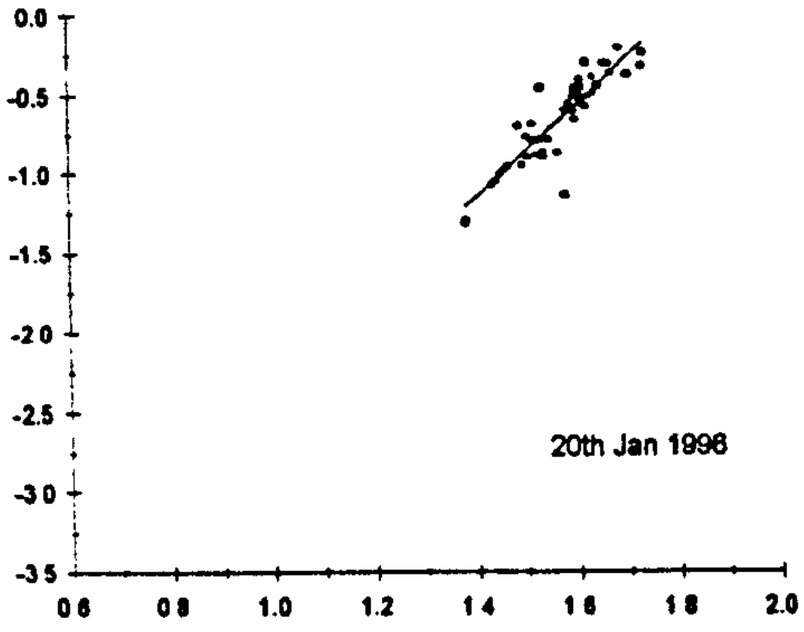
shell length (mm)

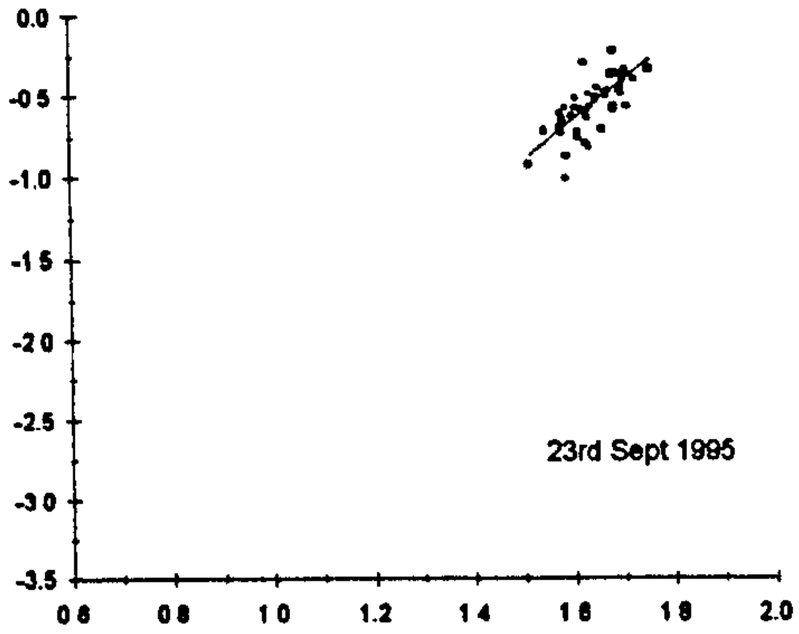
Figure 3.9 The relationship between log transformed dry tissue weight and shell length data in monthly samples of *Mytilus edulis chilensis* from Goose Green. Lines fitted by least squares linear regression (on 3 consecutive pages).

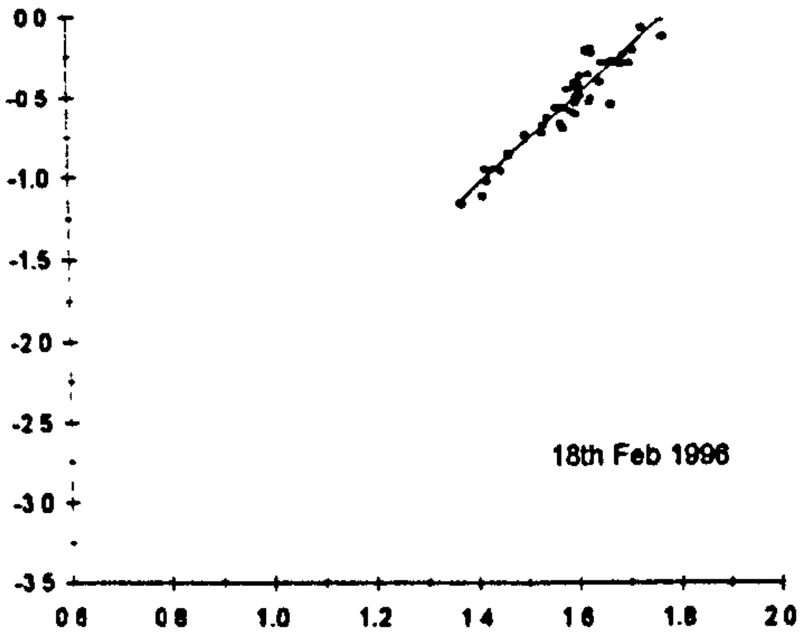

dry tissue weight (g)
shell length (mm)
27th Sept 1993
12th Feb 1994
16th Oct 1993
13th Mar 1994
14th Nov 1993
9th Apr 1994
11th Dec 1993
15th May 1994
15th Jan 1994
14th Jun 1994

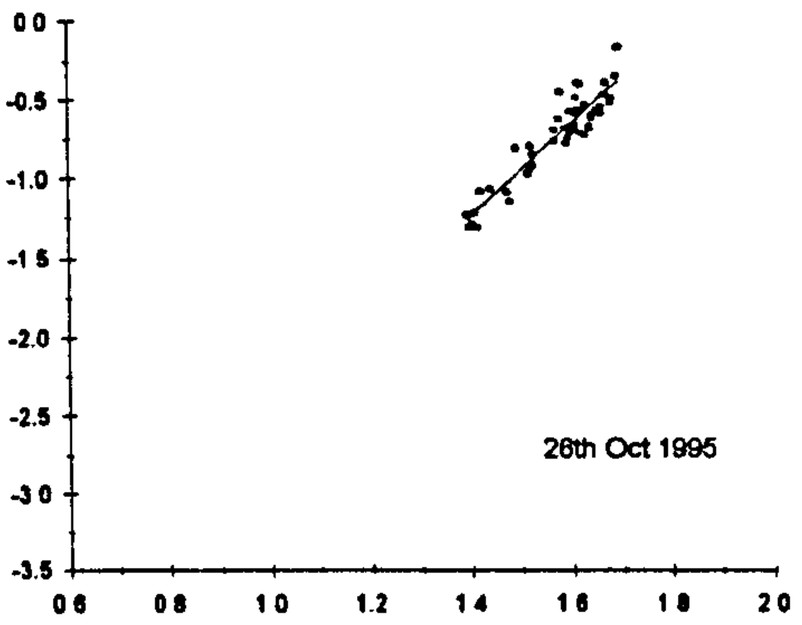

dry tissue weight (g)
6th Aug 1994
1st Jan 1995
5th Sept 1994
1st Feb 1995
6th Oct 1994
2nd Mar 1995
3rd Nov 1994
14th Apr 1995
8th Dec 1994
13th May 1995
shell length (mm)

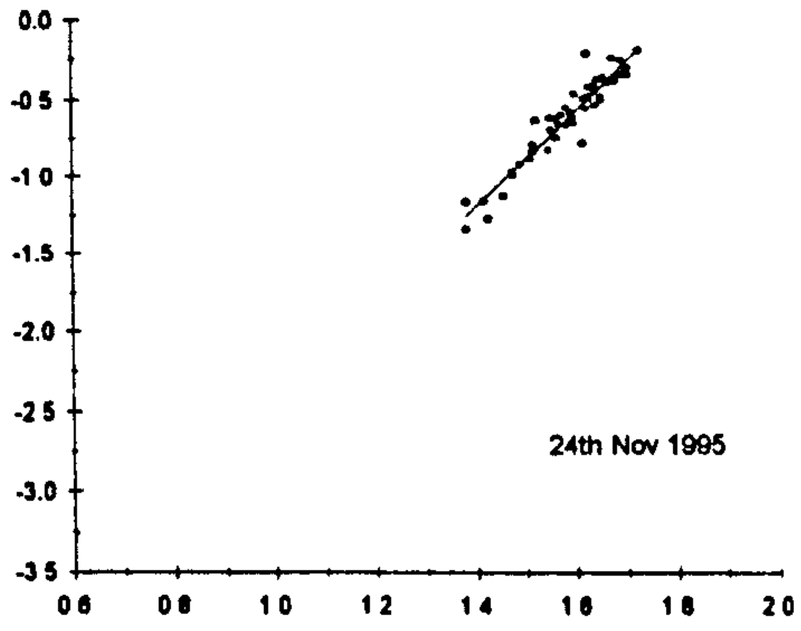

0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
18th Jun 1995


0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
20th Jan 1996


0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
23rd Sept 1995


0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
18th Feb 1996


dry tissue weight (g)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
26th Oct 1995


0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
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1.0
1.2
1.4
1.6
1.8
2.0
24th Nov 1995

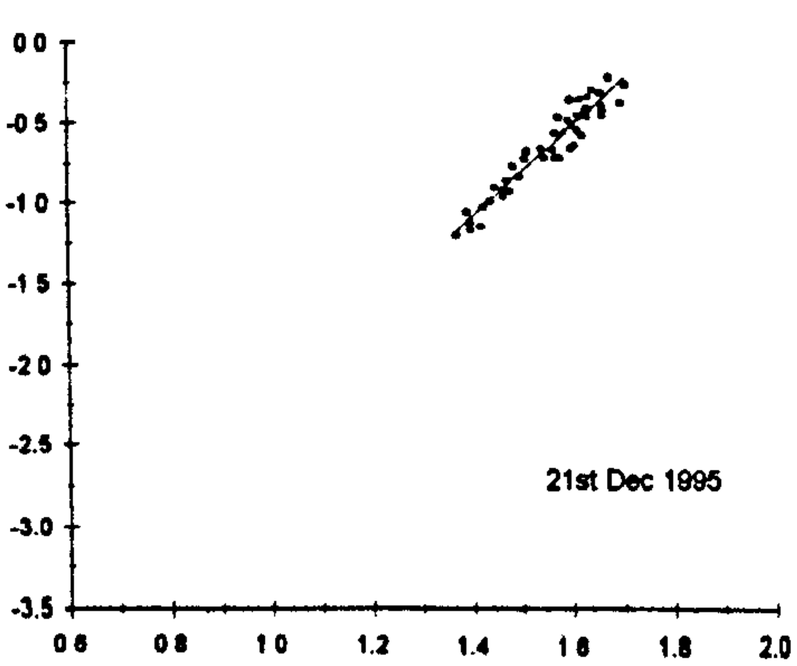

0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
21st Dec 1995
shell length (mm)

Table 3.5 Seasonal variation in condition index with shell length and log transformed dry tissue weights with shell length for monthly samples of *Mytilus edulis chilensis* from Darwin. Log transformed regression constants are derived from the equation $y = ax^b$ where y is dry tissue weight as the dependent variable and x is shell length as the independent variable.

Month	Condition Index vs shell length				Dry tissue weight vs shell length			
	n	a	b	F-value	n	a	b	F-value
Sept 1993	50	7.53	-0.086	22.92**	50	-4.94	2.577	1145.82**
Oct 1993	50	4.49	-0.030	5.22*	50	-5.55	2.846	1055.00**
Nov 1993	50	10.20	-0.104	13.01**	50	-5.31	2.838	582.39**
Dec 1993	50	1.43	0.070	7.89**	50	-6.44	3.471	376.56**
Jan 1994	50	4.52	-0.011	0.10 ns	50	-5.61	2.935	68.75**
Feb 1994	50	6.59	-0.040	4.93*	50	-5.38	2.852	901.85**
Mar 1994	48	2.59	0.029	0.45 ns	48	-6.11	3.236	40.73**
Apr 1994	50	5.80	-0.003	0.01 ns	50	-4.72	2.482	70.56**
May 1994	48	1.13	0.099	3.46 ns	48	-6.30	3.420	89.34**
Jun 1994	50	2.34	0.074	3.63 ns	50	-6.57	3.612	285.61**
Aug 1994	50	3.10	0.018	0.56 ns	50	-6.14	3.251	226.92**
Sept 1994	50	4.86	-0.005	0.15 ns	50	-5.39	2.847	844.14**
Oct 1994	50	6.50	-0.028	1.04 ns	50	-5.44	2.909	436.70**
Nov 1994	50	4.86	0.008	0.08 ns	50	-5.74	3.102	288.64**
Dec 1994	50	7.37	-0.022	0.13 ns	50	-4.92	2.641	38.23**
Jan 1995	50	4.27	0.050	1.52 ns	50	-5.71	3.121	236.20**
Feb 1995	50	6.69	0.005	0.04 ns	50	-5.44	2.985	408.99**
Mar 1995	50	7.23	-0.024	1.09 ns	50	-5.01	2.685	610.05**
Apr 1995	50	5.02	-0.004	0.03 ns	50	-5.32	2.826	294.37**
May 1995	50	8.43	-0.039	2.52 ns	50	-4.90	2.651	495.22**
Jun 1995	49	-0.16	0.162	4.64*	50	-5.68	3.093	96.52**
Aug 1995	50	3.22	0.055	9.87**	50	-5.52	2.977	326.92**
Sept 1995	50	5.92	-0.006	0.05 ns	50	-5.21	2.802	166.12**
Oct 1995	50	5.21	0.007	0.11 ns	50	-5.54	2.969	381.63**
Nov 1995	50	8.64	-0.022	1.35 ns	50	-5.21	2.875	1361.20**
Dec 1995	50	10.20	-0.083	17.10**	50	-4.74	2.542	583.18**
Jan 1996	50	7.95	-0.030	2.15 ns	50	-4.40	2.333	241.20**
Feb 1996	50	10.30	-0.065	8.74**	50	-4.52	2.447	648.36**

ns regression model does not fit data significantly; * significant fit at $p<0.05$; ** significant fit at $p<0.001$

Table 3.6 Seasonal variation in condition index with shell length and log transformed dry tissue weights with shell length for monthly samples of *Mytilus edulis chilensis* from Camilla Creek. Log transformed regression constants are derived from the equation $y = ax^b$ where y is dry tissue weight as the dependent variable and x is shell length as the independent variable.

Month	Condition index vs shell length				Dry tissue weight vs shell length			
	n	a	b	F-value	n	a	b	F-value
Sept 1993	47	10.10	-0.137	33.30**	48	-4.78	2.387	606.66**
Oct 1993	50	5.50	-0.042	8.89**	50	-5.70	2.840	1895.92**
Nov 1993	50	7.70	-0.074	53.22**	50	-5.35	2.689	1658.79**
Dec 1993	50	6.40	-0.059	45.14**	50	-5.55	2.773	1230.16**
Jan 1994	50	8.28	-0.099	47.90**	50	-5.12	2.519	1040.08**
Feb 1994	50	9.72	-0.148	43.55**	50	-4.68	2.183	481.96**
Mar 1994	50	6.74	-0.080	85.23**	50	-5.17	2.514	624.84**
Apr 1994	50	4.42	-0.040	5.60*	50	-4.65	2.223	30.71**
May 1994	50	4.72	-0.045	10.18**	50	-5.13	2.441	77.87**
Jun 1994	50	3.87	-0.015	0.47 ns	50	-6.46	3.359	156.26**
Aug 1994	50	5.61	-0.062	28.17**	50	-5.37	2.654	384.20**
Sept 1994	50	5.97	-0.058	10.85**	50	-5.48	2.698	209.94**
Oct 1994	50	5.71	-0.051	14.00**	50	-5.86	2.942	373.42**
Nov 1994	50	5.98	-0.064	10.47**	50	-5.33	2.633	179.49**
Dec 1994	49	3.75	-0.012	0.34 ns	49	-5.59	2.840	76.63**
Jan 1995	50	11.40	-0.147	24.93**	50	-4.82	2.450	124.88**
Feb 1995	50	10.40	-0.135	26.36**	50	-4.58	2.261	110.01**
Mar 1995	50	9.67	-0.122	32.60**	50	-5.04	2.550	217.32**
Apr 1995	50	4.70	-0.050	17.95**	50	-5.44	2.642	217.17**
May 1995	50	7.00	-0.072	8.11**	50	-5.36	2.723	194.00**
Jun 1995	50	4.72	-0.022	1.00 ns	50	-5.59	2.824	269.53**
Sept 1995	49	7.02	-0.063	4.79*	49	-5.48	2.750	117.60**
Oct 1995	49	6.21	-0.028	0.65 ns	49	-5.30	2.674	104.81**
Nov 1995	201	11.60	-0.109	51.94**	201	-5.01	2.649	2108.30**
Dec 1995	50	10.30	-0.067	4.54*	50	-5.32	2.840	353.51**
Jan 1996	50	10.30	-0.067	4.71*	50	-5.23	2.780	1450.11**
Feb 1996	50	16.20	-0.164	10.30**	50	-4.62	2.430	222.08**

ns regression model does not fit data significantly; * significant fit at $p<0.05$; ** significant fit at $p<0.001$

Table 3.7 Seasonal variation in condition index with length and log transformed dry tissue weights with length for monthly samples of *Mytilus edulis chilensis* from Goose Green. Log transformed regression constants are derived from the equation $y = ax^b$ where y is dry tissue weight as the dependent variable and x is shell length as the independent variable.

Month	Condition index vs shell length				Dry tissue weight vs shell length			
	n	a	b	F-value	n	a	b	F-value
Sept 1993	50	7.98	-0.076	31.88**	50	-4.72	2.480	591.16**
Oct 1993	50	7.26	-0.012	0.11 ns	50	-4.29	2.270	178.71**
Nov 1993	49	2.85	0.041	0.48 ns	49	-5.46	2.951	34.40**
Dec 1993	49	7.40	-0.052	0.67 ns	49	-4.33	2.266	13.93**
Jan 1994	49	5.65	-0.275	0.76 ns	49	-5.60	2.995	183.41**
Feb 1994	48	4.69	-0.018	0.18 ns	48	-4.67	2.421	24.20**
Mar 1994	50	1.90	0.061	3.17 ns	50	-6.04	3.294	100.75**
Apr 1994	50	5.73	-0.037	0.60 ns	50	-6.00	3.219	36.98**
May 1994	50	6.73	-0.047	1.15 ns	50	-4.81	2.554	43.74**
Jun 1994	49	7.05	-0.038	1.63 ns	49	-5.67	3.080	441.72**
Aug 1994	50	4.21	0.001	0.00 ns	50	-5.60	2.974	343.95**
Sept 1994	50	3.98	0.021	0.63 ns	50	-5.58	3.001	296.87**
Oct 1994	50	5.27	0.008	0.10 ns	50	-5.00	2.692	160.25**
Nov 1994	50	4.70	0.024	0.66 ns	50	-5.62	3.074	255.50**
Dec 1994	49	3.62	0.029	0.33 ns	49	-5.95	3.240	36.34**
Jan 1995	50	6.36	-0.026	1.07 ns	50	-5.15	2.762	217.91**
Feb 1995	50	7.83	-0.037	1.35 ns	50	-4.70	2.544	215.06**
Mar 1995	50	7.83	-0.042	2.63 ns	50	-5.31	2.896	373.02**
Apr 1995	50	3.57	0.024	1.61 ns	50	-4.92	2.599	233.92**
May 1995	47	3.51	0.044	1.55 ns	47	-5.96	3.308	66.01**
Jun 1995	50	4.97	-0.023	0.27 ns	50	-4.77	2.476	29.44**
Sept 1995	50	6.18	-0.022	0.42 ns	50	-4.61	2.464	53.81**
Oct 1995	50	5.42	0.009	0.12 ns	50	-5.29	2.890	298.67**
Nov 1995	50	9.11	-0.045	2.38 ns	50	-5.48	3.059	473.18**
Dec 1995	50	13.50	-0.133	16.04**	50	-5.05	2.832	586.28**
Jan 1996	50	12.00	-0.094	7.75**	50	-5.31	2.968	205.46**
Feb 1996	50	9.11	-0.028	1.16 ns	50	-5.05	2.836	524.57**

ns regression model does not fit data significantly; * significant fit at $p<0.05$; ** significant fit at $p<0.001$

where a significant linear relationship was observed (Tables 3.5, 3.6, 3.7). At Camilla Creek, the only site where sufficient samples were suitable for analysis using the general linear model, significant heterogeneity was detected between the fitted slopes of condition index on shell length data (Table 3.8A and B). Slope heterogeneity, which resulted in this line of analysis being abandoned, can be observed in Figure 3.6 where the condition indices of the March 1995 sample displayed a positive relationship with shell length followed by a negative relationship in samples collected in April and May. Covariate analysis carried out upon dry tissue weight on shell length data (Tables 3.9, 3.10, 3.11) similarly revealed significant heterogeneity between the regression slopes. An alternative method was therefore employed in order to identify any seasonal variation. Mean condition indices and dry tissue weights of a sub-sample of ten individuals ≈40 mm in shell length were calculated and plotted over time. Figure 3.10 provides evidence of a weak seasonal cycle in body condition which is made clearer when the times of peak reproductive condition (gonad indices) are superimposed. Peaks in body condition occurred during September - December 1993, December - February 1994/5, and November - February 1995/6, followed by a subsequent decrease (January - March) which coincided with the time of spawning. Although no further spawning occurred in any of the populations during the winter months, there was a marked increase in both condition index and dry tissue weight in populations from Darwin (Figure 3.10A) and Goose Green (Figure 3.10C) during April/May, followed by a sudden decrease between July and September. At Camilla Creek (Figure 3.10B) this fluctuation was only observed in the dry tissue weight data during 1995.

There was an overall increase in both condition index and dry tissue weight during the course of this study, regardless of season, at all three sites, with maxima being reached during the summer months in 1995/6. Curiously the winter of 1995 brought with it some of the lowest temperatures on record (Figure 2.4, Chapter 2) with mean minimum air temperatures remaining below zero from June through to August and snow occurring on more than 15 days each month over the same period (Figure 2.5, Chapter 2). Maximum values of condition index and dry flesh weight for a standard size mussel were reached in 1996, with a condition index of 10% occurring in mussels from Camilla Creek and a dry tissue weight of 0.3 g in mussels from Goose Green.

The relationship between condition index and dry tissue weight varied according to the study site. At Darwin and Goose Green very close agreement was observed between the condition index and the dry tissue weight, whereas marked differences occurred

Table 3.8A ANOVA table for the general linear model, with size as a single covariate between condition index and size on 23 selected monthly samples of *Mytilus edulis chilensis* from Camilla Creek (* significant at $p < 0.05$).

Source	DF	SS	MS	F	P
Size	1	5.923	5.922	304.89	<0.001*
Month	22	1.456	0.066	3.41	<0.001*
Month * Size	22	1.651	0.075	3.86	<0.001*
Error	1251	24.301	0.019		
Total	1296				

Table 3.8B The departure of single regression slopes from the average slope as determined by the general linear model presented in table 3.8A.

Term of regression	Coefficient	Standard deviation	t - value	P
Constant	1.74657	0.06617	26.39	<0.001*
Size	-0.71811	0.04113	-17.46	<0.001*
*Month * Size*				
September 1993	-0.1439	0.1051	-1.37	0.171
October 1993	0.40059	0.0903	4.44	<0.001*
November 1993	0.23522	0.08635	2.72	0.007*
December 1993	0.17653	0.09263	1.91	0.057
January 1994	-0.0596	0.09238	-0.65	0.519
February 1994	-0.428	0.1079	-3.97	<0.001*
March 1994	-0.0971	0.1127	-0.86	0.389
April 1994	-0.1456	0.3611	-0.40	0.687
June 1994	0.4358	0.2205	1.98	0.048*
August 1994	-0.0365	0.1942	-0.19	0.851
September 1994	0.0784	0.1623	0.48	0.629
October 1994	0.1284	0.1441	0.89	0.373
November 1994	-0.0818	0.2459	-0.33	0.739
January 1995	-0.451	0.2544	-1.77	0.077
February 1995	-0.6003	0.2589	-2.32	0.021*
March 1995	-0.3279	0.2131	-1.54	0.124
April 1995	-0.0004	0.1836	-0.00	0.998
May 1995	0.0748	0.1884	0.40	0.692
September 1995	-0.0662	0.2665	-0.25	0.804
November 1995	0.2979	0.0784	3.80	<0.001*
December 1995	0.3617	0.2054	1.76	0.079
January 1996	0.3347	0.2004	1.67	0.095
February 1996	-	-	-	-

Table 3.9A ANOVA table for the general linear model with size as a single covariate between dry tissue weight and size on 28 selected monthly samples of *Mytilus edulis chilensis* from Darwin (* significant at $p < 0.05$).

Source	DF	SS	MS	F	P
Size	27	1.1992	0.0444	4.32	<0.001*
Month	1	52.9235	52.9235	5150.69	<0.001*
Month * Size	27	0.9186	0.034	3.31	<0.001*
Error	1340	13.7685	0.0103		
Total	1395				

Table 3.9B The departure of single regression slopes from the average slope as determined by the general linear model presented in table 3.9A.

Term of regression	Coefficient	Standard deviation	t - value	P
Constant	-5.42909	0.06471	-83.89	<0.001*
Size	2.90802	0.04052	71.77	<0.001*
*Month * Size*				
September 1993	-0.331	0.09188	-3.60	<0.001*
October 1993	-0.0618	0.07332	-0.84	0.399
November 1993	-0.0695	0.1093	-0.64	0.525
December 1993	0.5634	0.1505	3.74	<0.001*
January 1994	0.0276	0.2554	0.11	0.914
February 1994	-0.05595	0.09036	-0.62	0.536
March 1994	0.3283	0.333	0.99	0.324
April 1994	-0.4256	0.2859	-1.49	0.137
May 1994	0.5121	0.3152	1.62	0.104
June 1994	0.704	0.167	4.22	<0.001*
August 1994	0.3431	0.1666	2.06	0.040*
September 1994	-0.0604	0.1383	-0.44	0.662
October 1994	0.0009	0.1419	0.01	0.995
November 1994	0.194	0.17	1.14	0.254
December 1994	-0.2669	0.3936	-0.68	0.498
January 1995	0.2129	0.1849	1.15	0.250
February 1995	0.077	0.2165	0.36	0.722
March 1995	-0.2231	0.1554	-1.44	0.151
April 1995	-0.0814	0.1778	-0.46	0.647
May 1995	-0.257	0.161	-1.60	0.111
June 1995	0.2783	0.3728	0.75	0.456
August 1995	0.0698	0.208	0.34	0.737
September 1995	-0.1059	0.2453	-0.43	0.666
October 1995	0.0611	0.1831	0.33	0.739
November 1995	-0.0325	0.158	-0.21	0.837
December 1995	-0.366	0.1882	-1.95	0.052
January 1996	-0.5746	0.1488	-3.86	<0.001*
February 1996	-	-	-	-

Table 3.10A ANOVA table for the general linear model with size as a single covariate between dry tissue weight and size on 27 selected monthly samples of *Mytilus edulis chilensis* from Camilla Creek (* significant at $p < 0.05$).

Source	DF	SS	MS	F	P
Size	26	1.5174	0.0584	3.75	<0.001*
Month	1	79.5378	79.5378	5108.41	<0.001*
Month * Size	26	1.1998	0.0461	2.96	<0.001*
Error	1442	22.4519	0.0156		
Total	1495				

Table 3.10B. The departure of single regression slopes from the average slope as determined by the general linear model presented in table 3.10A.

Term of regression	Coefficient	Standard deviation	t - value	P
Constant	-5.26366	0.06005	-87.65	<0.001*
Size	2.64002	0.03694	71.47	<0.001*
*Month * Size*				
September 1993	-0.25245	0.08467	-2.98	0.003*
October 1993	0.20039	0.08135	2.46	0.014*
November 1993	0.04967	0.07778	0.64	0.523
December 1993	0.13363	0.08345	1.60	0.110
January 1994	-0.12121	0.08232	-1.46	0.146
February 1994	-0.45702	0.09721	-4.70	<0.001*
March 1994	-0.1258	0.1016	-1.24	0.216
April 1994	-0.4163	0.3256	-1.28	0.201
May 1994	-0.1987	0.2183	-0.91	0.363
June 1994	0.7194	0.1988	3.62	<0.001*
August 1994	0.0144	0.1751	0.08	0.934
September 1994	0.0586	0.1463	0.40	0.689
October 1994	0.3029	0.1299	2.33	0.020*
November 1994	-0.0063	0.2217	-0.03	0.977
December 1994	0.2003	0.38	0.53	0.598
January 1995	-0.1896	0.2293	-0.83	0.408
February 1995	-0.379	0.2334	-1.62	0.105
March 1995	-0.0893	0.1921	-0.46	0.642
April 1995	0.0022	0.1655	0.01	0.989
May 1995	0.0835	0.1698	0.49	0.623
June 1995	0.1849	0.16	1.16	0.248
September 1995	0.11	0.2402	0.46	0.647
October 1995	0.0347	0.2156	0.16	0.872
November 1995	0.0091	0.07601	0.13	0.897
December 1995	0.2007	0.1852	1.08	0.279
January 1996	0.1408	0.1806	0.78	0.436
February 1996	-	-	-	-

Table 3.11A ANOVA table for the general linear model with size as a single covariate between dry tissue weight and size on 27 selected monthly samples of *Mytilus edulis chilensis* from Goose Green (* significant at $p < 0.05$).

Source	DF	SS	MS	F	P
Size	26	0.7188	0.0276	1.82	0.007*
Month	1	39.4888	39.4888	2604.49	<0.001*
Month * Size	26	0.7019	0.027	1.78	0.010*
Error	1286	19.4981	0.0152		
Total	1339				

Table 3.11B The departure of single regression slopes from the average slope as determined by the general linear model presented in table 3.11A.

Term of regression	Coefficient	Standard deviation	t - value	P
Constant	-5.22378	0.0901	-57.97	<0.001*
Size	2.82064	0.05527	51.03	<0.001*
*Month * Size*				
September 1993	-0.3399	0.09057	-3.75	<0.001*
October 1993	-0.5498	0.2557	-2.15	0.032*
November 1993	0.1305	0.3633	0.36	0.720
December 1993	-0.5547	0.4228	-1.31	0.190
January 1994	0.1752	0.1975	0.89	0.375
February 1994	-0.399	0.3915	-1.02	0.308
March 1994	0.4737	0 2872	1.65	0.099
April 1994	0.399	0.3774	1.06	0.291
May 1994	-0.2663	0.3708	-0.72	0.473
June 1994	0.2598	0.1607	1.62	0.106
August 1994	0.1542	0.2115	0.73	0.466
September 1994	0.1807	0.219	0.82	0.410
October 1994	-0.1282	0.2775	-0.46	0.644
November 1994	0.2542	0.2302	1.10	0.270
December 1994	0.4194	0.457	0.92	0.359
January 1995	-0.0584	0.2408	-0.24	0.808
February 1995	-0.2766	0.2567	-1.08	0.281
March 1995	0.0755	0.207	0.37	0.715
April 1995	-0.2214	0.233	-0.95	0.342
May 1995	0.4874	0.2769	1.76	0.079
June 1995	-0.3445	0.4014	-0.86	0.391
September 1995	-0.3563	0.3066	-1.16	0.245
October 1995	0.0700	0.2022	0.35	0.729
November 1995	0.2392	0.2021	1.18	0.237
December 1995	0.0122	0.1938	0.06	0.950
January 1996	0.1479	0.2078	0.71	0.477
February 1996	-	-	-	-

Figure 3.10 Seasonal changes in the average dry tissue weight (solid circles) and average condition index (solid squares) of ten mussels (~40 mm) from;

A. Darwin

B. Camilla Creek

C. Goose Green

Solid rectangles mark the timing of peak reproductive condition in the *Mytilus edulis chilensis* populations.

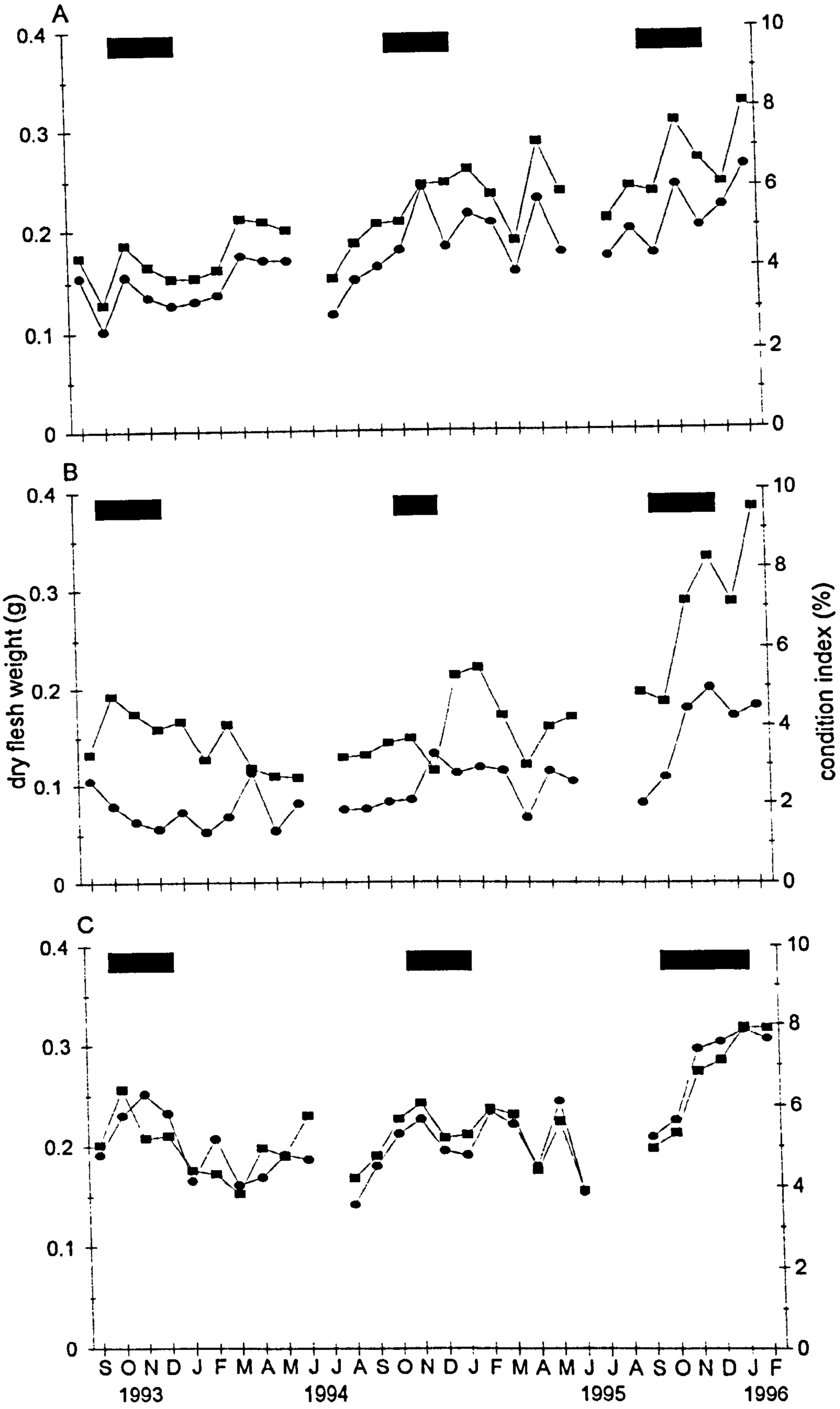
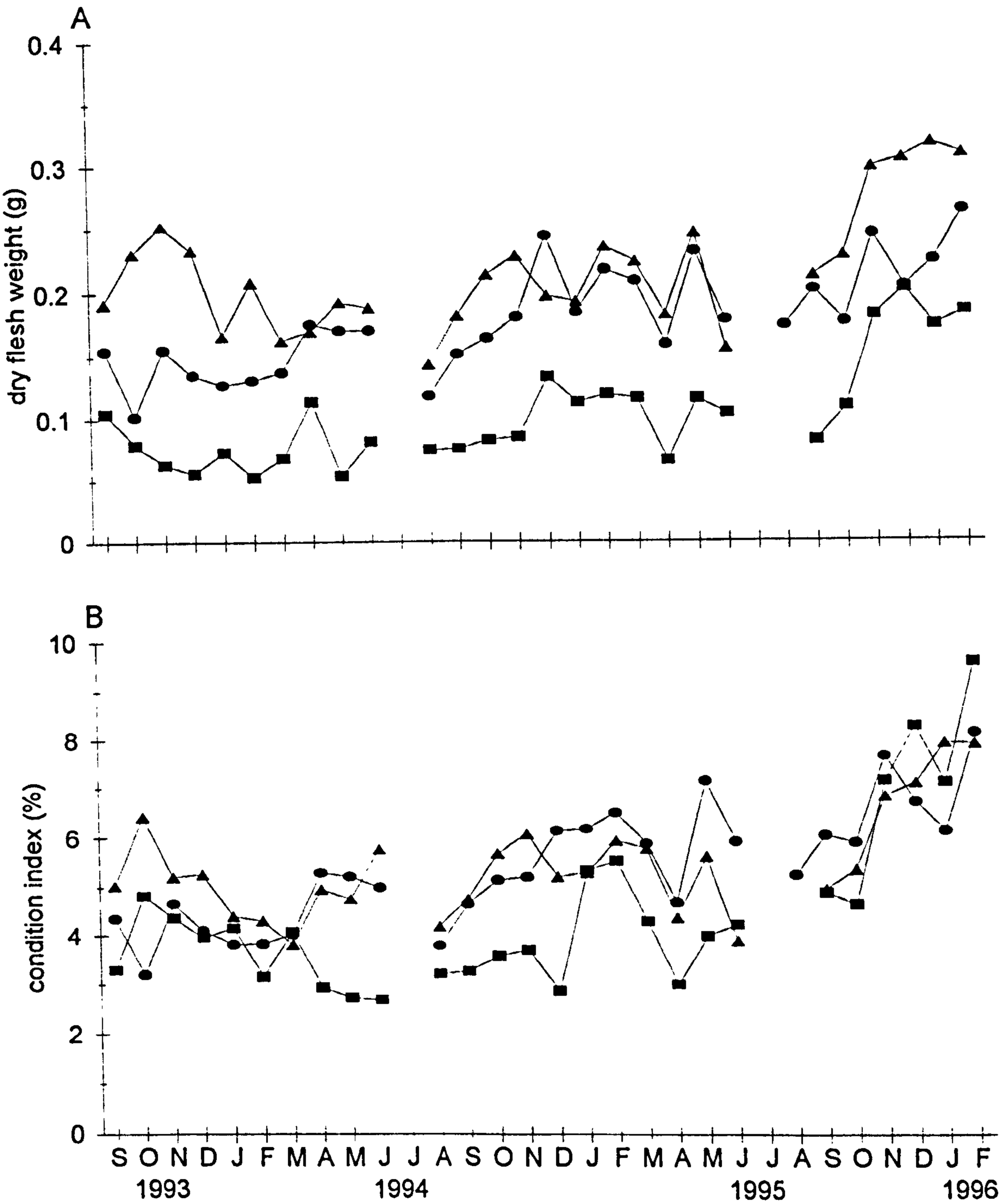

A
B
C
0.4
0.3
0.2
0.1
0
10
8
6
4
2
dry flesh weight (g)
condition index (%)
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996

Figure 3.11 Seasonal variation in the average body condition of ten mussels (~40 mm), *Mytilus edulis chilensis*, from Darwin (solid circles), Camilla Creek (solid squares) and Goose Green (solid triangles), using

A. dry tissue weight

B. condition index


A
0.4
0.3
0.2
0.1
0
dry flesh weight (g)
B
10
8
6
4
2
0
condition index (%)
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996

at Camilla Creek (Figure 3.10). Spearman rank order correlations examining the relationship between condition index, dry tissue weight, reproductive condition and temperature (Table 3.4) support in part some of the observations made here. A highly significant correlation between condition index and dry tissue weight was observed in mussels from Darwin and Goose Green, with significant but a considerably lower correlation at Camilla Creek. Correlation between both body and reproductive condition was only significant at Goose Green, whilst at Camilla Creek there was only evidence of a correlation between temperature and body condition.

Table 3.12 Mean, median, minimum and maximum condition indices and dry tissue weights of standard sized (40 mm) *Mytilus edulis chilensis* from Darwin, Camilla Creek and Goose Green.

Site	Condition Index (%)				Dry tissue weight (g)			
	mean	median	minimum	maximum	mean	median	minimum	maximum
Darwin	5.37	5.28	1.90	11.57	0.176	0.170	0.057	0.395
Camilla Creek	4.47	3.90	1.14	14.07	0.101	0.094	0.010	0.302
Goose Green	5.43	5.31	2.35	10.34	0.216	0.205	0.056	0.597

When overall dry tissue weight and condition indices for the three study sites were plotted together (Figure 3.11), there appeared to be consistent differences between sites. The condition of mussels from Camilla Creek appeared to be considerably lower than that of mussels from either Darwin or Goose Green, with mussels from Goose Green being in a higher condition overall. When compared statistically, significant differences were observed in both dry tissue weight ($H = 322.28$, d.f. = 2, $p < 0.01$) and condition index ($H = 56.29$, d.f. = 2, $p < 0.01$). Dunn's test for multiple comparisons revealed that dry tissue weights of mussels at the three sites were all significantly different from each other (Table 3.12) with Goose Green > Darwin > Camilla Creek. Condition indices of mussels from the populations at Darwin and Goose Green, on the other hand, exhibited no significant differences, although these two populations had significantly higher condition indices than those found at Camilla Creek.

3.3.4. Settlement

The meiofauna sample splitter used during this study was found to divide the samples

in a completely random fashion, with no significant variation between the chambers after splitting 10 replicate samples (Table 3.13). Also no significant differences could be discerned between replicate samples (Table 3.13). Therefore three of the eight sample chambers were randomly selected to assess the numbers of *M.e.chilensis* spat settled on the artificial substrate units (ASU).

Table 3.13 Two-way ANOVA table for the distribution of *Mytilus edulis* spat in 10 replicate samples split into 8 chambers of a meiofauna sample splitter.

Source	DF	SS	MS	F - value
Replica	9	26.3	2.9	0.25^{ns}
Chamber	7	83.2	11.9	1.01^{ns}
Error	63	746.3	11.8	
Total	79	855.8		

^{ns} not significant, $p > 0.05$

The numbers (mean ± one standard error) of *M.e.chilensis* spat (per square metre) settling on to ASU between November 1994 and February 1996 at each study site are presented in Figure 3.12. A single main spat settlement occurred during the austral summer and early autumn months (December - March/May), after which considerably lower settlement occurred throughout the winter at both Darwin and Goose Green. At Camilla Creek settlement was negligible with only a single mussel spat being found on the ASU in the February 1995 and February 1996 samples.

Settlement densities varied considerably according to site and year. At Darwin a fairly intense settlement period occurred from January to March 1995 where densities as high as around 5000 spat.m^{-2} were observed. At Goose Green settlement densities were not as high, around 500 spat.m^{-2}, but settlement remained at a relatively high level over a longer time period, December through to May. Settlement the following year appeared to be either considerably lower or possibly later with numbers increasing slightly from December 1995 to February 1996.

The relatively high peak in settlement at Darwin, the lower but more extended peak at Goose Green and the very low settlement at Camilla Creek observed in Figure 3.12

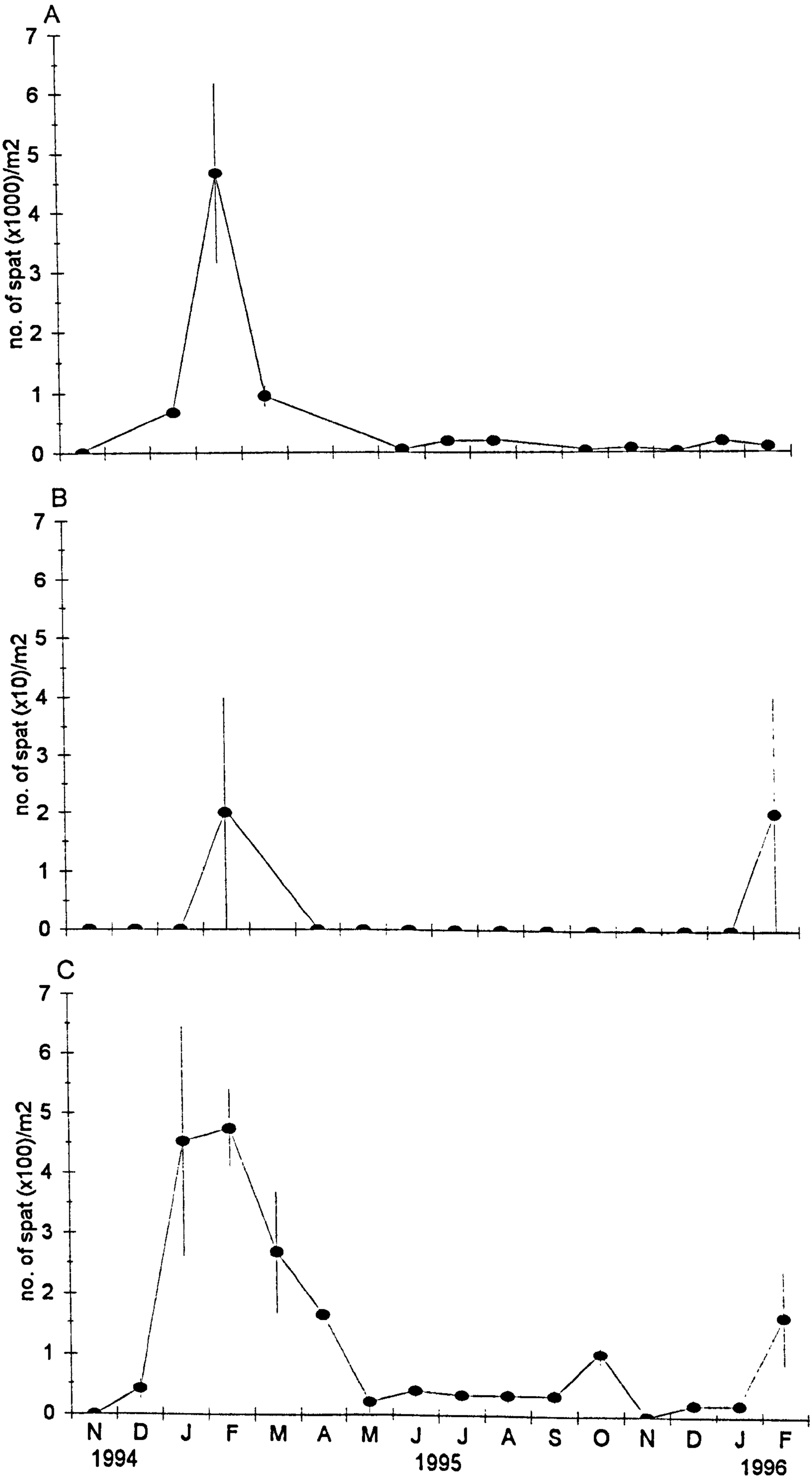
Figure 3.12 Mean (± 1 standard error) numbers of *Mytilus edulis chilensis* spat settling onto artificial substrate units deployed at

A. Darwin

B. Camilla Creek

C. Goose Green

Note: the scale of the y-axes vary according to the study site.


A
no. of spat (x1000)/m2
7
6
5
4
3
2
1
0
B
no. of spat (x10)/m2
7
6
5
4
3
2
1
0
C
no. of spat (x100)/m2
7
6
5
4
3
2
1
0
N D J F M A M J J A S O N D J F
1994
1995
1996

indicated there were considerable differences between the overall settlement densities at the three sites. When examined statistically, significant differences were identified ($H = 44.36$, d.f. = 2, $p < 0.01$). Multiple comparisons revealed that settlement densities at both Darwin and Goose Green were significantly higher than at Camilla Creek ($D = 6.01$ and $D = 4.23$, d.f. =2, $p < 0.05$, respectively), whilst no overall differences between Darwin and Goose Green could be discerned ($D = 2.16$, d.f. = 2, $p > 0.05$).

Length frequency histograms, in which it was possible to identify the settlement cohorts of *M.e.chilensis* spat for the period November 1994 through to February 1996, are presented in Figures 3.13, 3.14, 3.15. At Darwin a small cohort (density of < 50 spat.m^{-2}) of mussels approximately 7 mm in shell length was present in the November 1994 sample which can be followed through subsequent samples collected in January and March 1995. The arrival of this cohort coincided with the time when intense settlement of considerably smaller mussels was observed on the ASU deployed adjacent to the mussel bed. In August 1995 a second mode of mussels approximately 12 mm in shell length appeared, but within three months could barely be identified in the population. December 1995 marked the onset of a period of heavy settlement which continued into February 1996. Densities of between 300 and 500 mussel spat.m^{-2}, at around 7 mm in shell length were observed joining the adult population. This very clear settlement period was mirrored by settlement densities onto the ASU of 100 - 200 spat.m^{-2} (Appendix 3) during January and February 1996.

At Camilla Creek there was very little evidence of settlement onto the natural population during the study period. The large unimodal population did not appear to vary in either size or shape throughout the year (Figure 3.14). However, mussels < 10 mm in shell length were observed joining the adult population in November - January 1994/5 and January-February 1996 coinciding with the main spawning periods. Slightly larger individuals (12 - 15 mm) were observed in October - December 1994, prior to the main spawning period. *Mytilus edulis chilensis* spat (approximately 20 m^{-2}) settling onto the ASU coincided with settlement times of these small (< 10 mm) mussels joining the adult population.

Cohorts of mussels settling into the Goose Green population were small. In November 1994 a broad cohort of 7 - 25 mm mussels (approximately 20 m^{-2}) was observed, which by March 1995 appeared to have separated into two clear modes. The timing of the arrival of these mussels onto the adult bed broadly coincided with that observed

Figure 3.13 Length frequency histrograms for monthly/bi-monthly population samples of *Mytilus edulis chilensis* from Darwin. Arrows denote newly settled juvenile mussels.

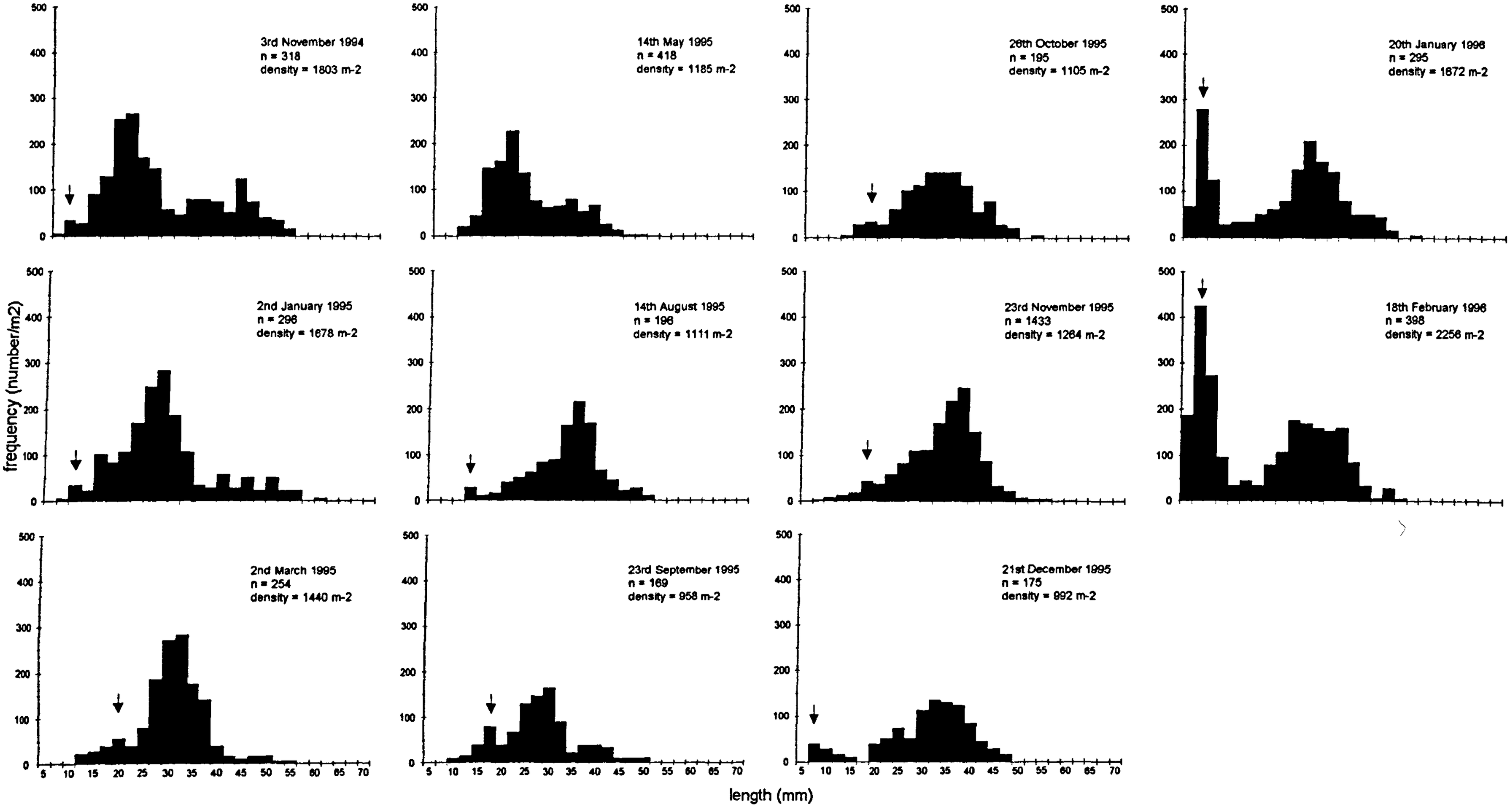

3rd November 1994
n = 318
density = 1803 m-2
14th May 1995
n = 418
density = 1185 m-2
26th October 1995
n = 195
density = 1105 m-2
20th January 1996
n = 295
density = 1672 m-2
2nd January 1995
n = 296
density = 1678 m-2
14th August 1995
n = 196
density = 1111 m-2
23rd November 1995
n = 1433
density = 1264 m-2
18th February 1996
n = 398
density = 2256 m-2
2nd March 1995
n = 254
density = 1440 m-2
23rd September 1995
n = 169
density = 958 m-2
21st December 1995
n = 175
density = 992 m-2
frequency (number/m2)
length (mm)

Figure 3.14 Length frequency histrograms for monthly/bi-monthly population samples of *Mytilus edulis chilensis* from Camilla Creek. Arrows denote newly settled juvenile mussels.

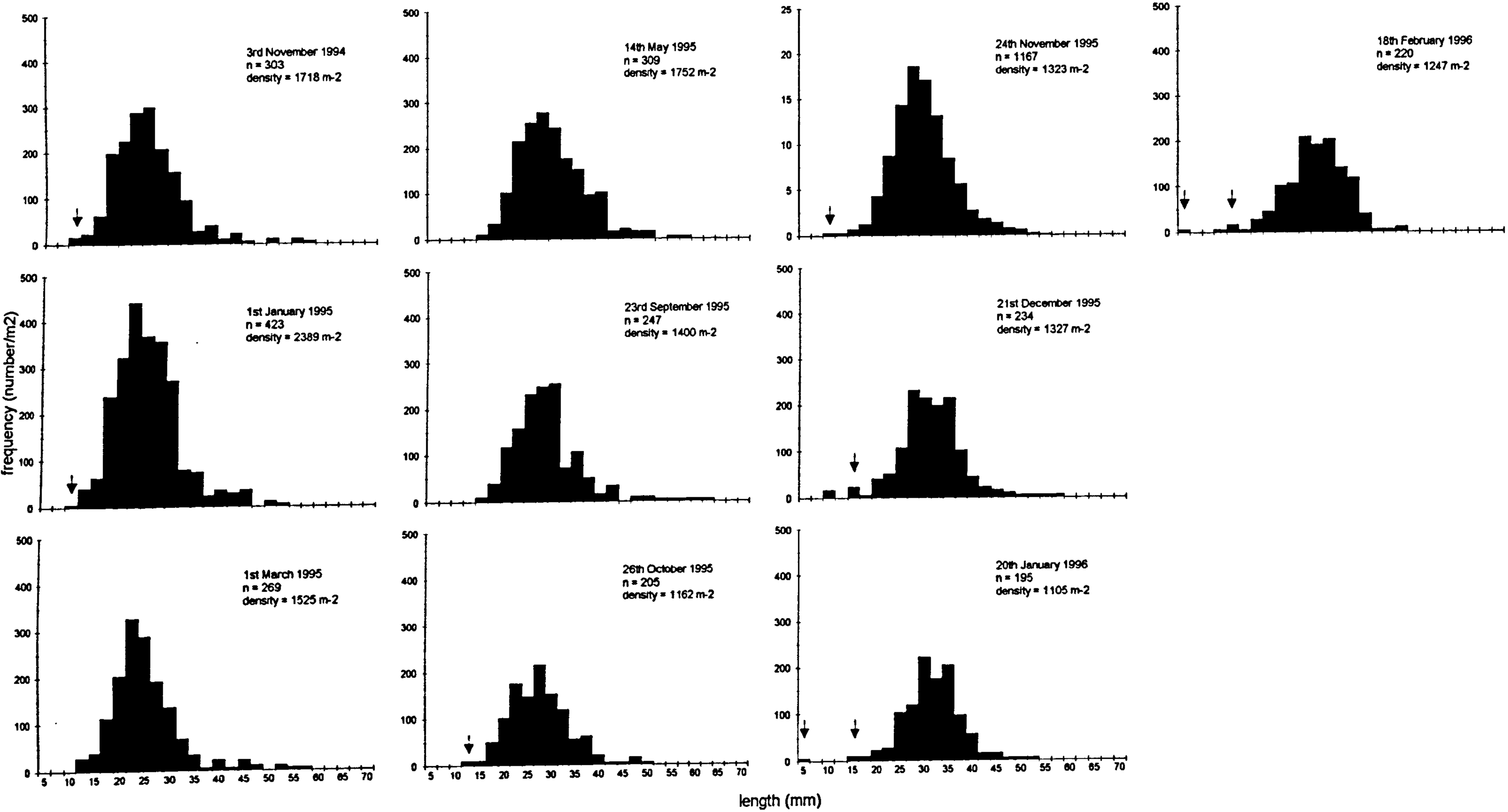
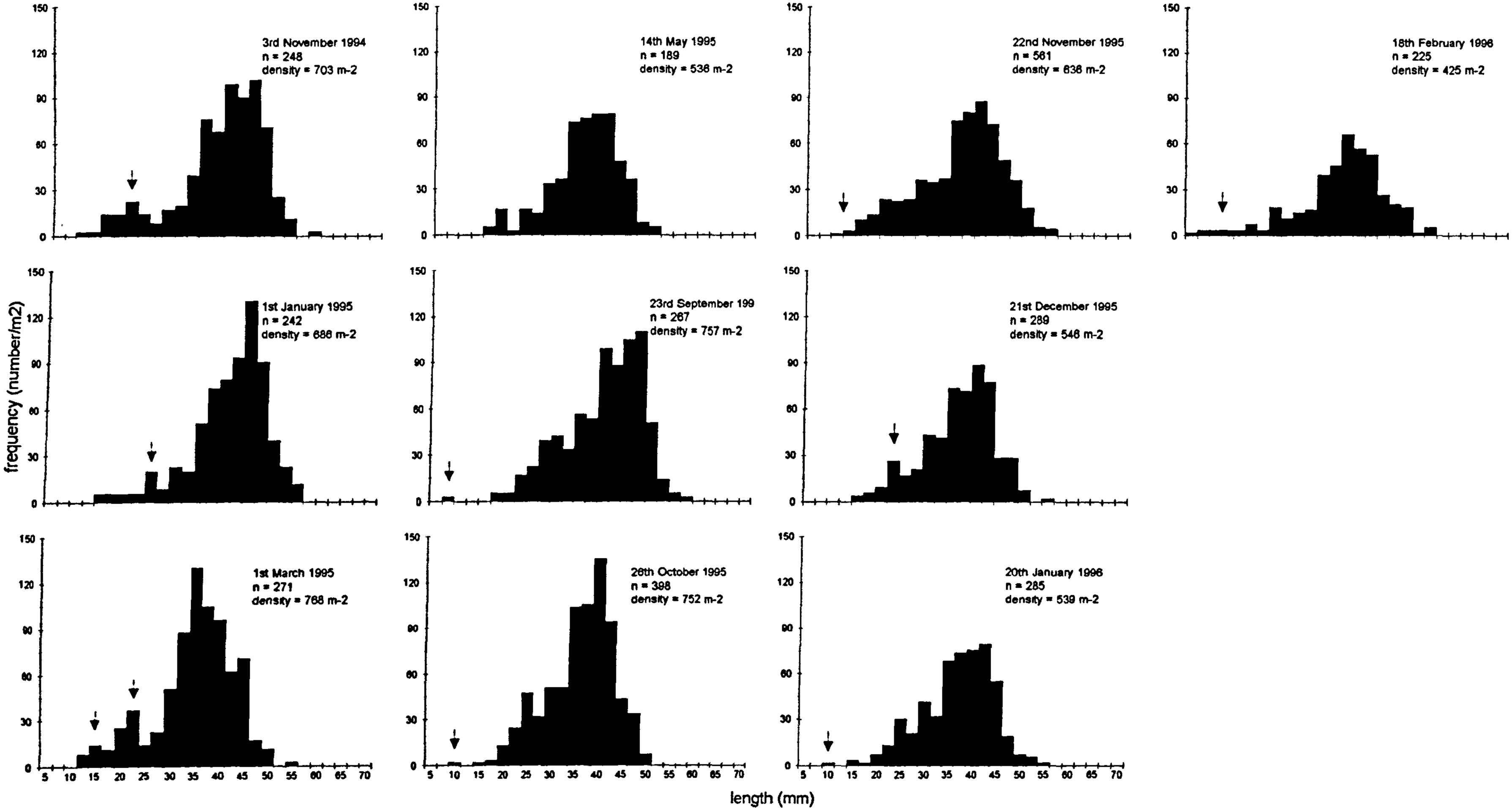

3rd November 1994
n = 303
density = 1718 m-2
14th May 1995
n = 309
density = 1752 m-2
24th November 1995
n = 1167
density = 1323 m-2
18th February 1996
n = 220
density = 1247 m-2
1st January 1995
n = 423
density = 2389 m-2
23rd September 1995
n = 247
density = 1400 m-2
21st December 1995
n = 234
density = 1327 m-2
1st March 1995
n = 269
density = 1525 m-2
26th October 1995
n = 205
density = 1162 m-2
20th January 1996
n = 195
density = 1105 m-2
frequency (number/m2)
length (mm)

Figure 3.15 Length frequency histrograms for monthly/bi-monthly population samples of *Mytilus edulis chilensis* from Goose Green. Arrows denote newly settled juvenile mussels.


3rd November 1994
n = 248
density = 703 m-2
14th May 1995
n = 189
density = 536 m-2
22nd November 1995
n = 561
density = 836 m-2
18th February 1996
n = 225
density = 425 m-2
1st January 1995
n = 242
density = 686 m-2
23rd September 199
n = 267
density = 757 m-2
21st December 1995
n = 289
density = 548 m-2
1st March 1995
n = 271
density = 768 m-2
26th October 1995
n = 398
density = 752 m-2
20th January 1996
n = 285
density = 539 m-2
frequency (number/m2)
length (mm)

onto the ASU. In September 1995 new settlement was observed, with low numbers (< 10 spat.m^{-2}) of mussels < 15 mm in shell length. In January/February 1996 further settlement occurred, with mussels 5 - 15 mm in shell length joining the adult population. The arrival of the summer cohort similarly coincided with settlement onto the ASU (10 - 160 spat.m^{-2}).

With the exception of a spring settlement of relatively large *M.e.chilensis* into the adult populations, there is a broad overall agreement between the timing of settlement onto ASU and that into the adult populations. Settlement densities, however, were somewhat different, with settlement into the adult population at considerably lower levels than that observed onto adjacent ASU. Only in early 1996 was the trend reversed, when settlement into the adult population at Darwin was unusually high, with settlement densities reaching 300 - 500 spat.m^{-2}.

3.3.5. Fecundity and Reproductive output

Figure 3.16A illustrates the relationship between fecundity (maximum number of eggs produced by individual mussels) and mussel size (shell length). Overall, fecundity increases with mussel size, although the degree to which size determines fecundity varies according to the study site. At Camilla Creek fecundity is consistently low and appears to be independent of mussel size, whereas at Darwin and Goose Green fecundity increases with size, although there is considerable variation, particularly in mussels from Darwin. In order to examine the data further, fecundities and mussel length data were log transformed and regressed using least squares linear regression. Regression was suitable only for mussels from Darwin and Goose Green; at Camilla Creek there was no linear relationship between fecundity and size. Log transformed regressions for Darwin and Goose Green are presented in Figure 3.16B.

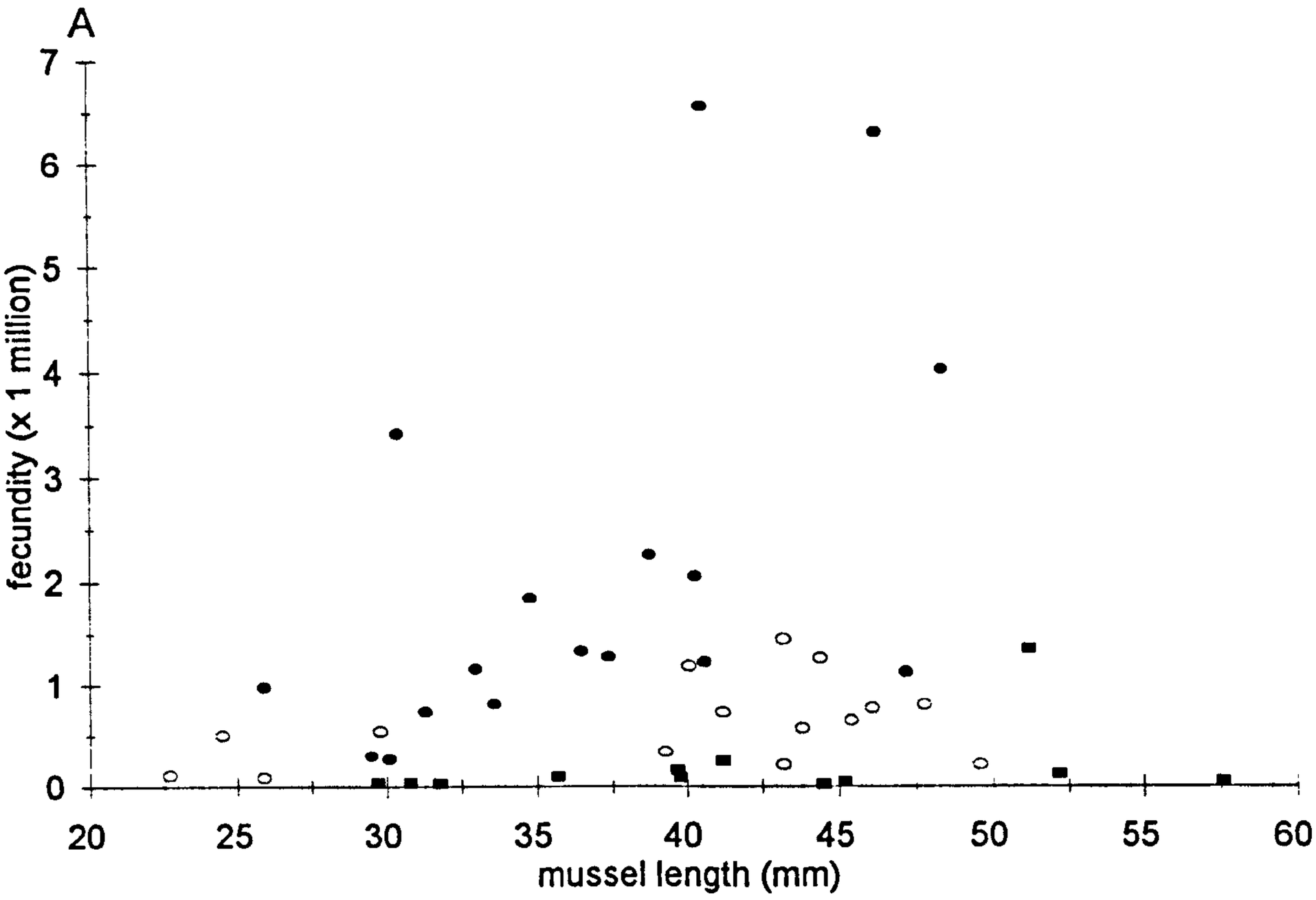
Variation in fecundity between the two sites was identified by applying the general linear model with a single covariate. The regression slopes were found to be homogeneous ($F = 0.65$, $p > 0.05$, d.f. = 1) but the elevations were significantly different ($F = 16.65$, $p < 0.01$, d.f. = 1). The fecundity of a standard-size mussel (40 mm) was therefore calculated using the regression constants in Table 3.14. The fecundity of a standard sized mussel from Camilla Creek was calculated using the raw data and is also presented in Table 3.14. The maximum number of eggs available for spawning at Camilla Creek was very low, an order of magnitude less than at Darwin

Figure 3.16

A. Maximum fecundity estimates of *Mytilus edulis chilensis* with size at Darwin (solid circles), Camilla Creek (solid squares) and Goose Green (open circles)

B. Log transformed fecundity estimates with fitted regression lines at Darwin (solid circles and solid line) and Goose Green (open circles and broken line).

A


fecundity (x 1 million)
0
1
2
3
4
5
6
7
20
25
30
35
40
45
50
55
60
mussel length (mm)

B

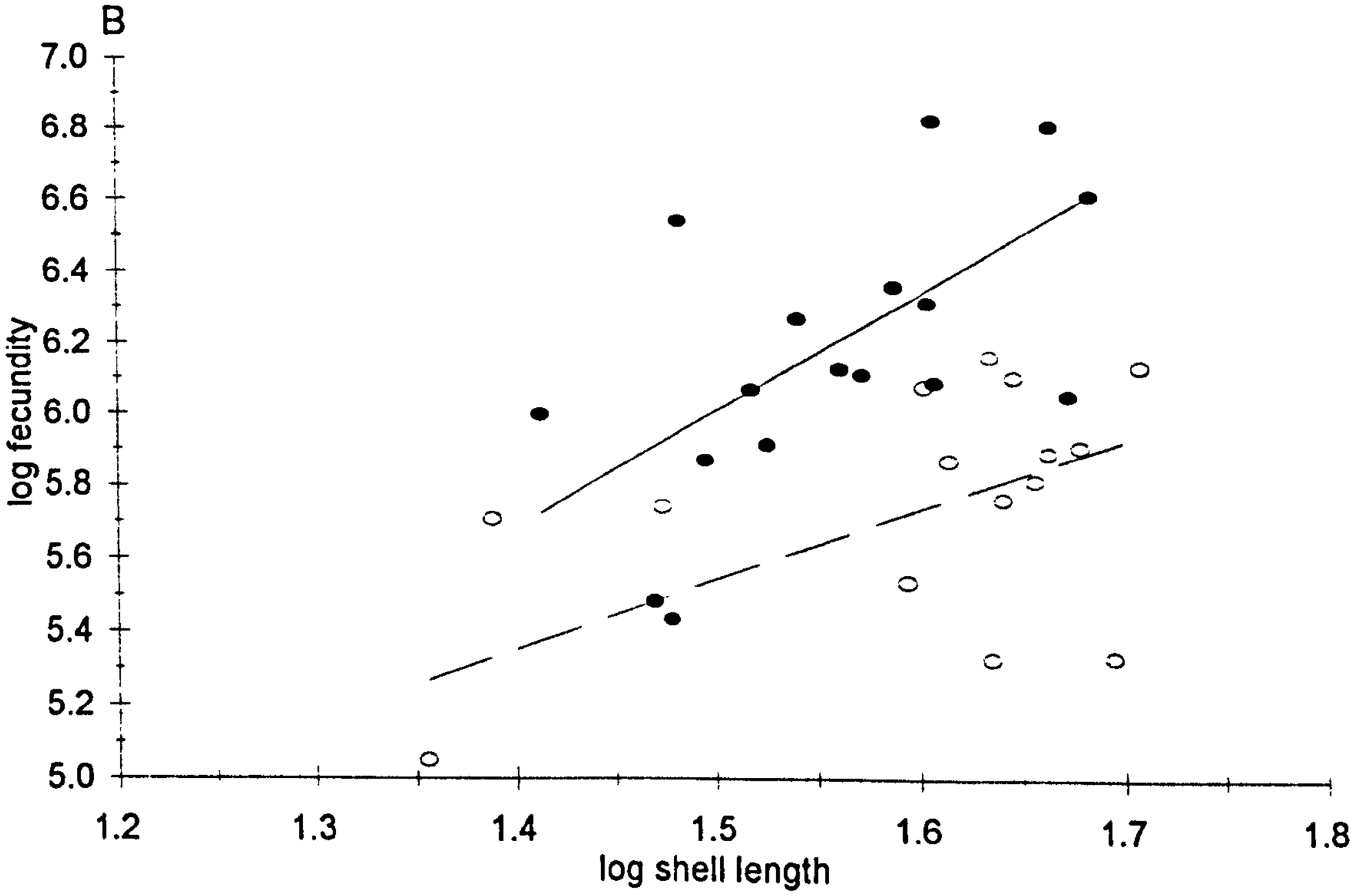
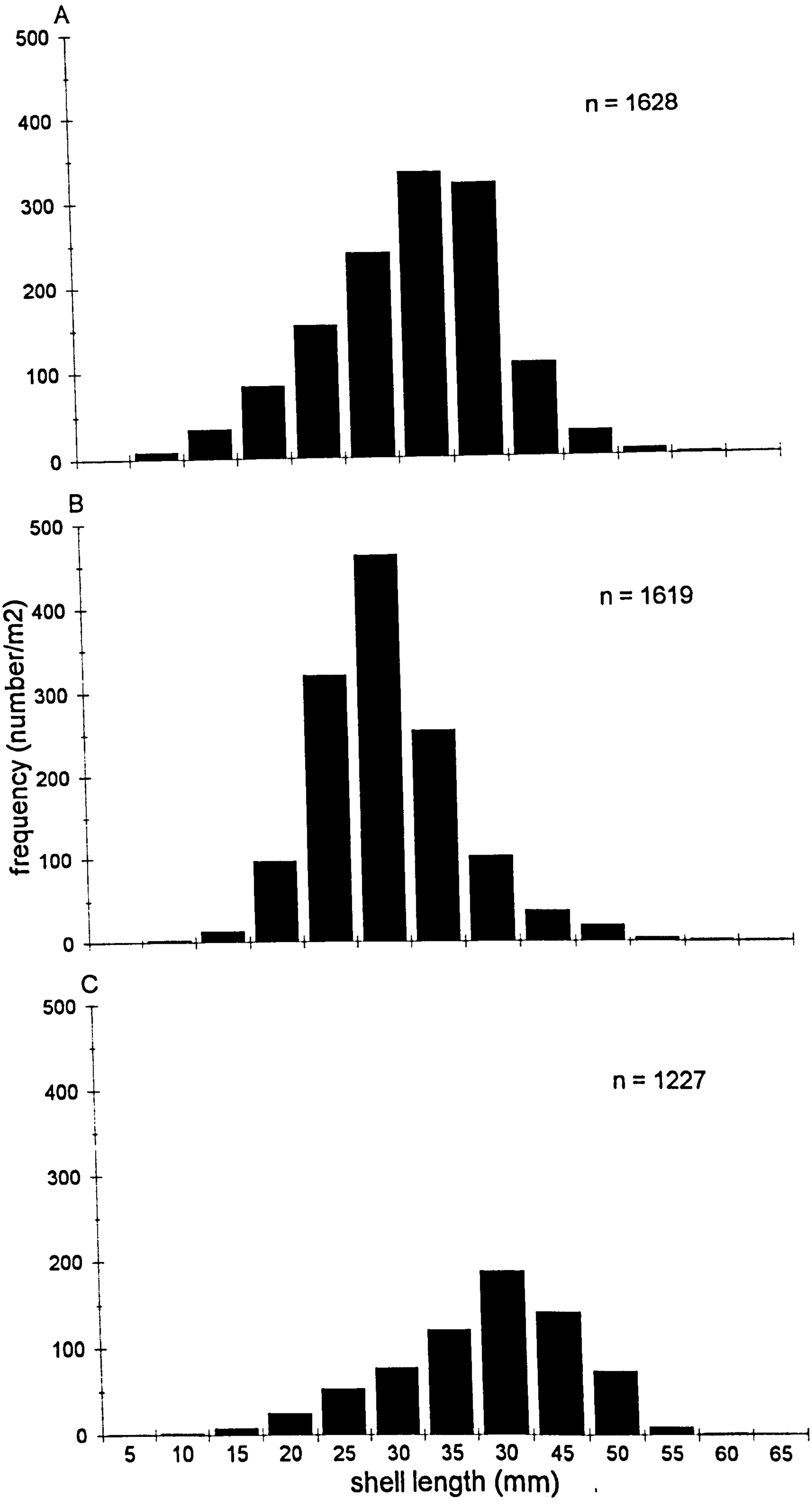

log fecundity
5.0
5.2
5.4
5.6
5.8
6.0
6.2
6.4
6.6
6.8
7.0
1.2
1.3
1.4
1.5
1.6
1.7
1.8
log shell length

Figure 3.17 Population length frequency distributions of *Mytilus edulis chilensis* from

A. Darwin

B. Camilla Creek

C. Goose Green


A
500
400
300
200
100
0
n = 1628
B
500
400
300
200
100
0
frequency (number/m2)
n = 1619
C
500
400
300
200
100
0
5
10
15
20
25
30
35
30
45
50
55
60
65
n = 1227
shell length (mm)

and Goose Green, where between one and two million eggs are produced annually by a standard 40 mm mussel.

Table 3.14 Regression constants for log transformed fecundity and shell length data, with the fecundity of a standard sized (40 mm) mussel from populations of *Mytilus edulis chilensis* at Darwin, Camilla Creek and Goose Green.

Site	a	b	F -value	Fecundity of a 40 mm mussel
Darwin	1.435	3.034	8.70*	1.924×10^6
Camilla Creek	3.616	0.736	0.35^{ns}	0.108×10^6
Goose Green	2.966	2.029	8.29*	1.667×10^6

a = intercept; b = slope, in the regression equation log y = log x . b + a

* significant at $p < 0.05$, ^{ns} not significant, $p > 0.05$

The length frequency distributions of mussels from the three study sites (Figure 3.17) together with the size related fecundity estimates, from both regression analysis and raw data, were used to calculate the population reproductive output. Table 3.15 contains the reproductive output of the population determined using both visual and stereological methods to estimate spawning percentages, as well as the reproductive output when determined directly from the raw data and calculated from regression equations. Using stereological estimates, the reproductive output of the population at Camilla Creek was poor, with an output of approximately half that found in the population from Goose Green and a quarter of that provided by the population at Darwin. There was very good agreement between estimates of the reproductive output when determined by regression analysis and from the raw data. However, visual estimates of the spawning percentages consistently under-estimated the true population potential, as determined stereologically.

The overall median weight of 10^6 eggs was determined as 123 mg, as no significant differences in the size of the eggs could be discerned between study sites when these were compared using the non-parametric Mood Median test ($\chi^2 = 1.74$, d.f. = 2, $p > 0.05$). Therefore the total weight of eggs spawned at each site was estimated, and found to be 219 $g.m^{-2}$, 54 $g.m^{-2}$, 114 $g.m^{-2}$ at Darwin, Camilla Creek and Goose Green, respectively.

Table 3.15 Population reproductive output (number of eggs.m^{-2}) for *Mytilus edulis chilensis* from Darwin, Camilla Creek and Goose Green.

Site	Reproductive output (number of eggs.m^{-2})		
	Stereological estimate		Visual estimate
	actual data	regression	actual data
Darwin	1.771×10^9	1.783×10^9	1.189×10^9
Camilla Creek	0.439×10^9	-	0.113×10^9
Goose Green	0.930×10^9	1.101×10^9	0.739×10^9

The overall proportions that eggs contribute to the total mussel tissue are presented in Table 3.16. Visual and stereological estimates were in close agreement, with tissue of mussels from Darwin and Goose Green comprising between 50 and 60% of eggs; at Camilla Creek just 8 - 10% of mussel tissue consisted of eggs. No obvious consistent relationship between the proportion of tissue weight which is comprised of gametes and mussel size was detected, probably due to the small sample sizes and the relatively small size range of mussels which were successfully induced to spawn.

Table 3.16 Estimates of the proportion of mussel tissue which comprises eggs in *Mytilus edulis chilensis* from Darwin, Camilla Creek and Goose Green.

Site	Stereological estimate		Visual estimate	
	n	%	n	%
Darwin	10	47	23	54
Camilla Creek	10	8	25	10
Goose Green	15	61	25	58

n = number of mussels examined

3.4. Discussion

The stages of gametogenesis observed in *Mytilus edulis chilensis* from the Falkland Islands were similar to those described for *Mytilus edulis* elsewhere (Seed, 1969a; Wilson & Seed, 1974). *Mytilus edulis chilensis* is gonochoristic with no incidences of

hermaphroditism detected during this study. The populations contained almost equal numbers of males and females, which could not, however, be distinguished by external characters. Only at Camilla Creek did the ratio of males and females depart from unity, here a ratio of 1.48:1 in favour of males was observed. Previous literature suggests that *Mytilus* has separate sexes with approximately equal numbers of males and females (Seed, 1976; Sunila, 1981; Kautsky, 1982a; Brousseau, 1983; Sprung, 1983). Unequal numbers of juvenile male and female mussels, selective mortality or sampling bias could be possible explanations for the apparent unequal sex ratio at Camilla Creek.

Reproductive condition of mussels is known to be affected by both age and size (Bayne & Worrall, 1980; Kautsky, 1982a; Sprung, 1983; Thompson, 1984; Rodhouse *et al.*, 1986). Young mussels grow rapidly and convert little or no energy into reproduction reaching sexual maturity within one year, although the size at which this happens depends largely on local growth rates (Rodhouse *et al.*, 1986). With increasing size there is a gradual transition from somatic growth to reproduction (Thompson, 1979; Kautsky, 1982a). Investigation into the reproductive condition of different sized *M.e.chilensis* from the three study populations revealed that both medium (35 - 45 mm) and large (45 - 55 mm) mussels from Camilla Creek had significantly lower gamete volume fractions than small (25 - 35 mm) individuals. A possible reason for the low reproductive condition in medium and large mussels could be the presence of the valviferan isopod *Edotia doellojuradoi* within the mussel mantle cavity (see Chapter 5). Although infection rates appeared to decrease slightly in larger mussels, the abundance (number per mussel) generally increased with host size (Gray *et al.*, 1997). In an ensuing chapter I have shown that the isopod has a detrimental effect upon the host's reproductive condition. Thus although not conclusive, the presence of infesting isopods could in part be responsible for the low reproductive condition observed in larger mussels. Mussels between 25 and 35 mm (small) in shell length from Camilla Creek were around 2 years old (Chapter 4) and would generally have been expected to have reached sexual maturity. Small mussels from Goose Green exhibited a lower reproductive condition than their larger conspecifics, suggesting that although sexual maturity has been attained in the small individuals, these do not appear to allocate the same amount of energy to reproduction as large individuals. *Mytilus edulis chilensis* from Darwin, on the other hand, exhibited a reproductive condition which did not vary with size, suggesting sexual maturity had been attained and similar quantities of energy amongst all size

ranges had been allocated to reproduction. Routine monthly samples collected during this study consisted of 35 - 45 mm individuals, all of which would have reached sexual maturity.

Seasonal variations in the gonad index and volume fraction of various tissue components indicate that gametogenesis was initiated during the austral spring (August - September) with peak reproductive condition being attained during the early summer months (November - December). A single major spawning period occurred during summer (December - March), after which mussels entered a reproductively quiescent phase. Occasional cases of early stage redevelopment were observed, however, the full extent of this second gametogenic cycle was not realized until the following spring when rapid maturation of the gonads occurred. The timing and duration of the gametogenic cycle was similar at all three sites, and was not dissimilar to that observed in *Mytilus* populations elsewhere (Seed, 1976; Seed & Suchanek, 1992). Food supply and seawater temperature are probably the two principal environmental factors controlling the timing and duration of gametogenesis. In the present study the timing of gametogenesis coincided with increasing seawater temperatures in the spring. Both the gonad index and gamete volume fraction increased with increasing temperature and achieved their maximal values as summer sea water temperatures approached 12°C. This strongly suggests that temperature is an important controlling factor. However, Kautsky (1982a) found that mussels from the Baltic, when placed in favourable feeding conditions, ripened in January when temperatures were close to zero and when the gonad index of the natural population was still close to its lowest value. Lubet and Aloui (1987) have suggested that a 'temperature window' probably exists, outside which gametogenesis is negligible, but inside which the reproductive strategy depends principally on food availability.

The duration of the spawning period is a significant feature of the annual cycle. In some *Mytilus* populations a single short spawning period lasting only a few weeks occurs (Chipperfield, 1953; Kautsky 1982a; Newell *et al.*, 1982). In other populations protracted reproductive periods resulting from repeated spawnings may occur (Wilson & Hodgkin, 1967; Wilson & Seed, 1974; Seed & Brown, 1977; Lowe *et al.*, 1982; Brousseau, 1983; Dix & Ferguson, 1984; Fell & Balsamo, 1985; McKenzie, 1986; Emmett *et al.*, 1987; King *et al.*, 1989; Kimball & McElroy, 1993) and appear to be characteristic of many cultivated mussels growing under particularly favourable nutrient conditions (Lutz, 1980; Rodhouse *et al.*, 1984a; Zhang, 1984; Wilson, 1987;

Wallace, 1990). Kennedy (1977) and Dix and Ferguson (1984) both observed a major spawning period in late winter/early spring and subsequent minor spawnings during summer and autumn in *Mytilus edulis aoteanus* from New Zealand and *Mytilus edulis planulatus* from Tasmania, respectively. However, *M.edulis planulatus* from mainland Australia is predominantly a winter spawner (Wilson & Hodgkin, 1967), though the gonad does continue to redevelop throughout the spring and summer months. In the Northern Hemisphere, populations of *M.edulis* from warmer more southerly waters generally spawn earlier than those from cooler northern waters (Seed & Suchanek, 1992 and references therein) and in Britain *M.edulis* on the west coast spawns earlier than on the colder North Sea coast at the same latitude (Seed, 1975). The relatively short summer spawning period of *M.e.chilensis* and lack of subsequent spawnings suggests that food supply may be a limiting factor at other times of the year.

Male *M.e.chilensis* at Darwin and Camilla Creek appeared more advanced in gametogenesis than female mussels of a similar size. Seed (1969a) and Kautsky (1982a) observed a similar uncoupling in *M.edulis* populations from the east coast of Britain and the Baltic and suggested that this may be an artefact of the arbitrary gonad index scheme used to assess the reproductive condition. However, in the present study this uncoupling also occurred when reproductive condition was assessed using the more quantitative stereological volume fraction scheme. Newell *et al.* (1982) also using the stereological volume fraction method, found that during gonad maturation male *M.edulis* from the east coast of the United States had higher gamete volume fractions than females. It is not improbable, however, that the production of sperm occurs at a faster rate than that of ova with their relatively large reserves of yolk (Seed, 1976).

The consistently low reproductive condition of the *M.e.chilensis* population from Camilla Creek may be explained by a number of factors, perhaps the most important of which is sampling bias. The fact that reproductive condition is lower in larger mussels (see page 98) and that the size range of the Camilla Creek mussels examined routinely fell into this category may account for the overall lower condition. However, the relatively high levels of silt observed at Camilla Creek (Chapter 2) compared to the other two sites may also be a contributory factor, particularly since the overall body condition (condition index and dry tissue weight) is significantly lower at Camilla Creek when compared to the body condition of mussels from Darwin and Goose Green. Although the exact quantities of silt at Camilla Creek are not known,

Beatty and Aldrich (1989) have reported an increase in overall body condition in relatively poor condition *M.edulis* from Lough Foyle (N.Ireland) when mussels were elevated 60 cm above the seabed. They suggested that the mussels were either exposed to increased proportions of phytoplankton and/or reductions in the proportion of silt.

The degree of heterogeneity in the slopes of regression plots for both condition index and dry tissue weight on shell length data for *M.e.chilensis* exhibited no evidence of a seasonal pattern. Khamdan (1994) was able to identify the time at which young, recruiting pearl oysters, *Pinctada radiata*, from Bahrain (Arabian Gulf), reached sexual maturity and started contributing to the overall reproductive condition of the population, by observing the rotation of regression lines (and therefore the allometric relationship, see Chapter 4) of dry tissue weight on shell length. Sexual maturity was approached when the allometric relationship shifted from being predominantly negative (adult population) to being almost isometric when the generally isometric young oysters spawned. Brotohadikusumo (1994) suggested that the rotation of regression lines, of condition index on shell length, whereby the relationship changed from one of a positive nature to one that was negative in the Indonesian blood clam, *Anadara antiquata*, could be related to spawning and redevelopment and/or the arrival of juvenile clams into the adult population. Sampling, in which an inadequate size range of *M.e.chilensis* was examined, was probably the main factor contributing to the apparently random heterogeneity of the regression slopes in this investigation.

The dry tissue weight of a standard sized individual has been used successfully by some workers (eg Kautsky, 1982a; Rodhouse *et al.*, 1984a) to observe the gametogenic and nutrient cycles of mussel populations. Generally variations in the dry tissue weights of a standard sized mussel are reflected in histological changes of the gonad, although occasional anomalies have occurred. Rodhouse *et al.* (1984a) observed a large decrease in ash free dry weight (AFDW) of wild intertidal *M.edulis* populations from Killary Harbour, Ireland, during September immediately following a sharp increase in July/August. However, no spawning had occurred. Hilbish (1986) suggested that the anomaly present in the AFDW data of Rodhouse *et al.* (1984a) was a result of uncoupled tissue and shell growth, following observations that provided evidence that shell and tissue growth do not occur simultaneously. Kautsky (1982b) and Mallet and Carver (1993) have both observed a similar uncoupling of tissue and shell growth in populations of *M.edulis* from the Baltic and Nova Scotia, respectively.

Both condition indices (which include shell and tissue growth) and dry tissue weights of standard sized *M.e.chilensis* were observed at the three study sites in the Falkland Islands. The consistently high correlation between condition index and dry tissue weight indicates that the uncoupling of tissue and shell growth is negligible.

Rodhouse *et al.* (1984a), Dix and Ferguson (1984) and Kimball and McElroy (1993) all observed strong seasonal patterns in the condition of *M.edulis* and related these to the gametogenic and nutrient storage cycles. In all cases variations in the condition and gametogenic events were thought to be attributable to temporal or quantitative food supply. It can be suggested, therefore, that both temperature and food supply are important factors influencing tissue growth and the gametogenic cycle. Increasing temperatures in the Falkland spring coincide with the initiation of the gametogenic cycle and mean maximum temperatures broadly coincide with the peak in gonad maturity. Following spawning and the reduction in body condition, there appears to be a build up of nutrients whereby the condition of mussels increases, however, no further spawning is observed after March. These stored nutrient reserves are subsequently depleted, possibly as a result of increased metabolic demands, during the winter months when mussels often experience sub-zero temperatures for several months and when they may also have little or no access to available food (Chapter 2). Only with the onset of spring do the reproductive and body condition of mussels both increase. Although temperature is thought to be a principal factor in controlling the broader aspects of the annual reproductive cycle (Seed & Suchanek, 1992), any factor which affects the availability of food, or the ability of mussels to assimilate food, will alter the nutrient storage cycle, and thus the timing of gametogenic events (Newell *et al.*, 1982).

The overall increase in both condition index and dry tissue weight of *M.e.chilensis* from all three study sites over time is particularly interesting. The increase is more pronounced following the winter of 1995 during which temperatures remained below zero from June through to August and snow occurred on 15 days each month, often resulting in the study sites remaining under ice for several weeks (Chapter 2). Mussels appear to be allocating considerably more energy to tissue growth with time, which may be a direct consequence of the severe winter conditions. Routine water sampling would be required in order to determine whether food supply was playing an important role in controlling the overall reproductive cycle, and whether there was any correlation between food quality and quantity and the short or long term fluctuations in the

seasonal cycle of condition and reproduction.

Overall, dry tissue weights and condition indices are rather low, 0.10 - 0.22 g and 4.5 - 5.4 %, respectively, in comparison to commercially grown mussel populations. Lutz (1980) found condition indices ranged between 30 and 50 % in 50 mm *M.edulis* grown on commercial rafts in Maine, USA; Dare (1976) observed dry tissue weights of 0.3 - 0.5 g for 40 mm *M.edulis* grown on commercial beds (5% aerial exposure) at Morecambe Bay, England; whilst 47 % aerially exposed *M.edulis* from the Conwy estuary, North Wales, had condition indices of 40 - 50 % (Baird, 1966) and Aldrich and Crowley (1986) observed condition indices of 40 and 27 % in 40 mm *M.edulis* commercially grown on rafts and intertidally in Ireland. These tissue weights and condition indices suggest that the intertidal populations of *M.e.chilensis* studied in the Falkland Islands generally have a poor condition in comparison to mussels from other temperate waters and would not be particularly suitable for commercial exploitation.

Marked seasonal patterns in abundance of both early and late plantigrades have been observed in many *M.edulis* populations (Bayne, 1964; King *et al.*, 1989; King *et al.*, 1990), although sporadic and often unpredictable pulses of recruitment of late plantigrades into the adult population have also been well documented (eg Seed, 1969a; Dare, 1976). Following Bayne (1964) early *M.edulis* plantigrades were observed settling onto filamentous substrata in the Menai Strait (N.Wales), approximately four weeks after the mussel populations spawned (April - June). Four to eight weeks later (June - July) the number of early plantigrades decreased, as a result of growth and/or departure from these filamentous substrata. A period of more than 30 days elapsed between the first definite spatfall of larvae in early June and the appearance of young mussels on the adult beds in late July. Direct settlement of *M.edulis* onto the adult beds has also been observed in several studies (eg McGrath *et al.*, 1988; King *et al.*, 1990) whilst Bøhle (1971) and Kautsky (1982a) have speculated that direct settlement occurs in the Baltic, following the lack of mussels >400 µm in plankton samples.

In the present study no mussels < 2 mm were found on the adult bed suggesting that direct settlement at these sites does not occur. Primary settlement of early plantigrades onto the filamentous artificial substrata occurred coincidentally with the spawning period (December - March), although low numbers continued to settle throughout much of the year, despite the lack of any subsequent spawning. Relatively

large, (5 - 15 mm), mussels which were observed arriving onto established adult beds during spring and early summer months (September - December) prior to the main spawning period (December - March), could be either secondary settling juveniles from the previous year which have over-wintered in some place other than the adult bed, where they may have experienced some degree of post-settlement mortality, or possibly immigrant mussels from populations which have spawned considerably earlier than those documented in the present study. Mussels with a shell length of around 5 mm settling onto the adult beds during the summer months, almost coincidentally with spawning and the arrival of early plantigrades onto the artificial substrate units, are probably secondary settlers, late plantigrades resulting from spawning in the same year. Bayne (1964) found that secondary settlement onto the adult population of *M.edulis* in the Menai Strait, North Wales, could occur just 4 weeks after spawning. Unfortunately the absence of regular and frequent samples over this main settlement period in the Falkland Islands leaves the precise timing of the arrival and departure of primary and secondary settling mussels unclear.

The unusually high settlement densities observed on the adult beds at Darwin during 1995/6 may have been a direct result of the previous severe winter. Large patches of the mussel bed were removed during winter storms and the scouring action of persistent coastal ice left large areas of the shore available for colonisation. The mussels also exhibited an increased body and reproductive condition during the summer of 1995/6, compared to 1994/5, indicating that an increase in the energy allocated to tissue growth/reproduction may have resulted in the production of higher numbers of settling larvae. However, settlement densities onto the ASU were lower than in the previous year suggesting that numbers of larvae produced were not necessarily higher. The winter of 1994 was not as severe as that of 1995, and densities of mussels settling onto the adult beds at Darwin were considerably lower than in 1995, despite the relatively high settlement onto the artificial substrata. These settlement discrepancies may be due to several factors; 1) the mussel larvae of the 1994 cohort may have experienced considerably higher mortality rates following primary settlement, than those in 1995; 2) the space available for colonisation in 1994/5 could have been considerably less than in 1995/6 due to the milder conditions in the previous winter; and 3) sampling of the artificial substrata may not have been frequent enough. The four week periods between deployment and collection of the ASU's may have been too long, as the early plantigrades may have settled and subsequently left the primary settlement sites, particularly in 1995/6.

Although settlement was observed at all three study sites, numbers of plantigrades and juvenile mussels settling onto both artificial substrate units and the adult bed at Camilla Creek were relatively low. This relatively sheltered estuary experiences rather high levels of silt deposition. The effects of silt upon the settlement of *M.edulis* have been well documented (Bayne, 1964; Dare, 1976; Young, 1983; McGrorty *et al.*, 1990; McGrorty & Goss-Custard, 1991; 1993). Generally, increased silt levels have a detrimental effect upon the settlement of mussel spat. Bayne (1964) observed that when larvae were inhaled by adult mussels in clear filtered water, they were often exhaled alive, but with only a slight amount of silt in the water, inhaled larvae became entangled in mucus and pseudofaecal material and died. Dare (1976) noted that in Morecambe Bay, settlement succeeded only on ground devoid of mud and loose accumulation of shells. Young (1983) investigated the effect of sediment type upon byssal attachment in *M.edulis* and found that mussels did not attach byssus pads to mud or silt particles less than 0.85 mm in diameter. Later, Igic (1988) found that heavy silting was totally limiting to the settlement of *Mytilus galloprovincialis*. McGrorty *et al.* (1990) found that *M.edulis* larvae in estuaries with soft sediments settled predominantly on existing beds amongst the byssus threads of adults. McGrorty and Goss-Custard (1991, 1993) further showed that *M.edulis* spat had a strong aversion to soft substrata and that the softness of the substratum had a highly significant, negative effect upon the density of mussel settlement. The byssus threads of adult mussels were often covered in mud and thus inaccessible to settling larvae, and those that did settle were subsequently smothered by further deposits of mud.

Populations of *M.e.chilensis* generally increased in fecundity with increasing size, although mussels from Camilla Creek exhibited relatively low fecundity regardless of size. Young (small) mussels are known to grow rapidly and convert little or no energy into reproduction. With increasing size, there is a transition from somatic growth to reproduction so that in the largest mussels most production is channelled into gamete synthesis, resulting in relatively high fecundities in these larger (older) individuals (Thompson, 1979; Bayne & Worrall, 1980; Kautsky, 1982a; Sprung, 1983; Rodhouse *et al.*, 1986). Thompson (1979) and Kautsky (1982a) found that reproductive tissues could account for as much as 50 % of the tissue weight in some large individuals. The average proportions of the total tissue weight that the reproductive tissues comprised in *M.e.chilensis* were 47, 8 and 61 % at Darwin, Camilla Creek and Goose Green respectively.

The relatively low fecundity of mussels from the Camilla Creek population was further realized when the numbers of eggs spawned by standard sized mussels were calculated. A 40 mm female from Camilla Creek produced only 0.11 x 10^6 eggs compared to conspecifics at Darwin and Goose Green which produced 1.92 x 10^6 and 1.67 x 10^6 eggs respectively. These latter values are broadly similar to those documented for *M.edulis* elsewhere. Thompson (1979) found that a 42 mm mussel from Nova Scotia produced 2 x 10^6 eggs, whilst Bayne *et al.* (1983) observed fecundities of between 0.57 x 10^6 and 1.91 x 10^6 in *M.edulis* from the English and Welsh coasts. Larger individuals (70 mm), however, can produce 7 - 8 x 10^6 eggs (Seed & Suchanek, 1992).

The reproductive output of mussel populations is largely dependent upon their size (age) structure. The population of *M.e.chilensis* from Camilla Creek is comprised mainly of mussels between 15 and 35 mm in shell length, and has a correspondingly low reproductive output when compared to the populations at Darwin and Goose Green, where the majority of mussels are between 25 - 45 mm and 35 - 50 mm in shell length, respectively. However, it should be remembered that small mussels from Camilla Creek have a significantly higher reproductive condition when compared to medium and large individuals, hence the low reproductive output of the entire population may be a result of factors other than population structure. It is known that reproductive output is influenced by environmental variables such as temperature, food supply and aerial exposure. Bayne *et al.* (1983) reported ten-fold differences between the minimum and maximum values for egg production, reproductive effort and reproductive value in *M.edulis* from six contrasting sites on English and Welsh coasts. Thompson (1979) observed inter-annual changes in fecundity in *M.edulis* from Nova Scotia and Newfoundland and suggested that the mussels adjust the proportion of energy allocated to reproduction according to the available food ration. Bayne and Worrall (1980) selected two contrasting sites near Plymouth (England) in which mussels exhibited different growth rates and reproductive outputs. One population received a rich food supply which resulted in greater overall production, mussels spawned twice a year and allocated up to 60 % of their total production to reproduction, whilst the other population exhibited low growth rates, spawned only once a year and allocated only 26 % of total production to reproduction due to a considerably reduced food supply. The three Falkland populations investigated during the present study experienced very similar air and seawater temperatures and aerial exposure times (Chapter 2). Only the quality/quantity of food, the high levels of silt

deposition and/or the presence of the isopod, *Edotia doellojuardoi*, within the mussel mantle cavity may have accounted for the differences in reproductive output of the mussels from Camilla Creek and those from the other two sites. *E.doellojuradoi* does have some detrimental effect upon the gamete volume fraction of host mussels (Chapter 6), though it is doubtful that this alone could explain the overall reduced reproductive condition of this Camilla Creek population.

3.4.1. Conclusions

The absence of hermaphrodites and the approximately equal numbers of male and female *Mytilus edulis chilensis* is consistent with that observed in populations of *M.edulis* elsewhere.

The onset of gametogenesis coincided with increasing seawater temperatures during spring (August - September) and peak reproductive condition was attained during the early summer months (November - December). The occurrence of a single major spawning period during summer (December - March) and the lack of redevelopment until the following spring, is consistent with that documented in other populations of *M.edulis* in which food is a major limiting factor. The broad agreement between the initiation and completion of gametogenesis and the coinciding rise and fall of seawater temperatures implies some broad level of control.

The gametogenic cycle as well as nutrient storage and depletion were identified when the seasonal cycles of condition were examined. These variations appear to be directly related to food supply. The overall increase in body condition within all mussel populations suggests that over time progressively more energy is being allocated to tissue growth.

Although there were peaks in the settlement of *M.e.chilensis* both onto artificial substrate units and into the established population following spawning of the established adults, the low and somewhat sporadic settlement into the established populations at other times of the year indicates that settlement cannot always be predicted from the knowledge of the reproductive cycle alone and that some settlement and juvenile growth must occur on substrata away from the established populations.

Reproductive output of *M.e.chilensis* populations provide a significant input into the pelagic coastal system, broadly similar to populations of *M.edulis* elsewhere. The consistently low reproductive condition, body condition and reproductive output as well as the virtual absence of juvenile mussels at Camilla Creek appears to be related to food supply and/or silt levels.

Chapter 4

Growth - absolute and allometric

4.1. Introduction

The growth of *Mytilus edulis* has been extensively studied, not only because of its commercial importance but also because its age and the history of its growth, including a record of the environmental conditions under which shell deposition took place, are permanently recorded in the shell structure (see Seed, 1976; Lutz & Rhoads, 1980; Seed & Richardson, 1990; Seed & Suchanek, 1992 for reviews). Growth in bivalves is conveniently expressed as a linear measurement of the shell. However, on some occasions, an individual may be increasing in biomass whilst its growth in terms of other linear dimensions, for example length, may vary seasonally with age and in response to changes in environmental conditions. Moreover, negative growth of tissue has been observed in *M.edulis* populations, despite the continued deposition of shell material (Seed, 1980; Kautsky, 1982b).

Growth is generally determined in one of two ways (Seed, 1980). Either the size of the whole organism is related to age (= absolute growth), or the rate of growth of one parameter is related to that of another (= allometric growth). Several methods have been used successfully to estimate the absolute growth of *M.edulis* these include: 1) the modal progression in length frequency distributions (Böetius, 1962; Baird, 1966; Bayne & Worrall, 1980; Loo & Rosenberg, 1983; Rodhouse *et al.*, 1984b; Page & Hubbard, 1987); 2) surface growth rings (Seed, 1969b; Thiesen, 1973; Wallace, 1980; Suchanek, 1981; Kautsky, 1982b; Davenport *et al.*, 1984; Hilbish, 1986); 3) growth patterns within the shell (Lutz, 1976; Richardson, 1989; Richardson *et al.*, 1990a,b); 4) measurement of marked/caged animals (Seed, 1969b; Harger, 1970; Dare & Edwards, 1976; Kautsky, 1982b; Page & Hubbard, 1987); 5) laser and photographic techniques (Strömgren, 1975; Davenport & Glasspool, 1987); and 6) physiological estimates (Bayne *et al.*, 1976, 1979; Widdows *et al.*, 1981, 1984; Navarro & Winter, 1982; Thompson, 1984; Widdows & Donkin, 1992; Loo, 1992).

The analysis of length frequency distributions, either by probability plot methods (Harding, 1949; Cassie, 1954), or by the separation of normal distributions into their

component size (age) classes (Bhattacharya, 1967), enable the age and growth of populations to be determined as well as providing a valuable insight into the effects of environmental conditions upon the population structure (Cerrato, 1980). However, prolonged recruitment periods and variable growth rates, which often typify many populations of *M.edulis*, generally result in the merging of individual age classes (Seed, 1969b; Kautsky, 1982b).

The absolute growth of individual animals may be described in terms of the average size attained at specific ages. Growth in *M.edulis,* however, is known to vary considerably. Individual mussels of initially similar size and/or age, grown under apparently identical conditions can show large variations in their growth rates, which can be partially related to their genotype (Innes & Hayley, 1977; Freeman & Dickie, 1979). Since observations of growth using size alone leads to often highly variable estimates of age, it is necessary to support this morphometric data by determining the age of individual specimens within the population. The analysis of growth patterns within the shell structure has proved to be a valuable method for determining the age and growth rate of many bivalves (Lutz, 1976; Richardson *et al.*, 1979; 1995; Lutz & Rhoads, 1980; Richardson, 1987; 1989; Richardson & Walker, 1991).

The shell of *M.edulis* consists of three layers, a thin outer periostracum consisting of the quinone tanned protein, conchiolin, a middle prismatic layer, and an inner nacreous layer. The nacreous layer is comprised of layers of aragonitic tablets; these are separated by interlamellar organic matrix and are deposited by the general outer surface of the mantle, thus effectively thickening and strengthening the shell. The middle prismatic layer consists of simple prisms of calcite which are separated from each other by a wall of conchiolin and are deposited by the mantle epithelium around the growing margin of the shell (Seed & Richardson, 1990).

Sections through the nacreous and prismatic layers reveal a series of distinct bands. In the nacreous shell these appear as dark lines which alternate with lighter, more widely spaced regions (Lutz, 1976), whilst in the prismatic layer they consist of dark microscopic growth bands separated by wider more transparent growth increments (Richardson, 1989; Richardson, *et al.*, 1990b). The nacreous lines are formed annually during the winter months (Lutz, 1976) whilst the prismatic growth bands are deposited during periods of tidal emersion such that under a normal semi-diurnal tidal regime two bands are produced each day (Richardson, 1989). These prismatic tidal

microgrowth bands are used to determine short-term (eg tidal) and long-term (eg annual) rates of growth. Growth increments within the prismatic layer vary along the shell in response to seasonal environmental changes. During the summer, when shell growth is rapid, wide increments are produced, whilst in the winter shell deposition slows and increments become progressively narrower, resulting in an easily measured record of annual growth (Richardson, 1989; Richardson *et al.*, 1990b). This invaluable record of growth history recorded within the shell structure of *M.edulis* has been used extensively to assess the age and growth rates of individual mussels (Lutz, 1976; Richardson, 1989; Richardson *et al.*, 1990b; 1995).

Surface growth checks within the periostracum, have also been successfully used in studies of mussel growth (Seed, 1969b; Kautsky, 1982b; Davenport *et al.*, 1984). However, the presence of disturbance checks and the loss in detail arising from shell abrasion, shell boring algae and the convergence of bands at the shell margin in older individuals, together with factors influencing the clarity of the bands, such as the position of the animal on the shore in relation to tidal immersion, and the latitudinal origin of the organism, may complicate the interpretation of growth patterns. The technique of observing microgrowth patterns within the prismatic shell layer, however, is generally successful in distinguishing disturbance bands from those which are annual in origin, and is particularly useful for resolving growth over short time scales (Richardson, 1989); and assessing the effects of environmental stressors on shell growth (Thompson & Richardson, 1993). Furthermore, the growth history, including a detailed record of environmental conditions such as temperature, human disturbance, predator attacks and detrimental algal blooms, to which the animal was subjected during its life, is faithfully recorded within the shell structure.

By virtue of its resource-limiting environmental conditions, a habitat imposes a maximum size beyond which further growth proceeds slowly, if at all. Fast-growing individuals approach this asymptotic limit relatively quickly, whilst in areas of slow growth this limit may only be approached by much older individuals. Animals which have reached the maximum size imposed by a given environmental regime and which are subsequently transplanted to more favourable conditions, respond by a further period of enhanced growth until the new asymptote of the environmental regime is reached (Seed, 1973; 1980; Kautsky *et al.*, 1990; Stirling & Okumus, 1994; Sukhotin & Maximovich, 1994).

Quantitative expressions of growth including von Bertalanffy and Gompertz type equations have been used successfully to describe and compare the growth of several *Mytilus* populations (Theisen, 1973; Bayne & Worrall, 1980; Rodhouse *et al.*, 1984a; Thompson, 1984). In the von Bertalanffy equation :

$$L_t = L_\infty [1 - e^{-k(t-t_0)}] \qquad \text{Equation 4.1}$$

where L_t represents the shell length of an animal at time t; L_∞ is the maximum asymptotic shell length; k is the growth constant reflecting the rate at which maximum size is approached and t_0 is a constant representing theoretical time when $L_t = 0$. The Gompertz equation is similar but utilises the logarithm of length :

$$\log_{10} L_t = \log_{10} L_\infty [1 - e^{-k'(t - t_i)}] \qquad \text{Equation 4.2}$$

where k′ is the rate constant, and t_i a constant representing time when $L_t = L$.

Theisen (1973) used both equations for describing the growth of *M.edulis* and concluded that the von Bertalanffy equation, which provided a more realistic estimate of asymptotic length, gave a good fit for individuals above one third of their maximum size, whilst the sigmoidal Gompertz equation was better suited to smaller mussels. Bayne and Worrall (1980) successfully used the Gompertz equation to assess the growth of *M.edulis* populations in south-west England. Both of these equations assume that growth is determinate and that some maximum attainable size exists for any given population. Yet growth may not always be determinate, at least over the realized life span and may not therefore, cease at any fixed adult size (Seed, 1980; Gardner & Thomas, 1987). For this reason polynomial equations have sometimes been used to describe shell growth in preference to the more commonly used growth equations (eg Davenport *et al.*, 1984).

Extensive investigations into the effects of various factors on the growth of *M.edulis* have been carried out (see Seed & Richardson, 1990; Seed & Suchanek, 1992 for reviews), although the causative factors responsible for controlling the growth of field

populations are often very difficult to determine due to the complex interactions of several often inextricably linked variables. The relationship between growth and temperature is clearly demonstrated (Ursin, 1963; Thiesen, 1973) when shell length is plotted against age in day degrees (a product of the average daily temperature and the number of days); however these growth rates are not always consistent, suggesting that factors other than temperature are probably involved (Wilson, 1977; Thompson, 1984). In western Sweden, Loo and Rosenberg (1983) found that low temperatures (< 5°C) did not seem to limit mussel growth whenever they coincided with the phytoplankton bloom. More recently, Loo (1992) found mussels actively ingesting seston and growing at -1°C. In northern Norway, Wallace (1980) observed that mussels growing close to experimental fish cages were able to maintain summer growth rates through the long Arctic winter by utilizing particles of fish food. Food supply, which is closely linked to tidal level, is probably the single most important factor determining growth rate, since if food is unavailable sustained growth cannot occur. Growth rate in *M.edulis* is inversely correlated with tidal level, reflecting the progressively reduced feeding times with increasing aerial exposure (Baird, 1966; Seed, 1969b). Although several studies (Kautsky, 1982b; Loo & Rosenberg, 1983; Rodhouse *et al.*, 1984a) have reported a general decrease in growth rate with water depth, Page and Hubbard (1987) found that growth at 9 m was faster than at 2 m and 18 m. These differences were evidently not associated with water temperature, which declined with depth, but rather with variations in food concentration.

Although the increase in size of most organisms, including mussels, is commonly expressed as linear growth, mussels do exhibit progressive changes in their relative proportions with increasing body size, resulting from differential growth vectors operating at different points around the mantle margin. The relationship between any two size variables (x and y) can be expressed by the allometric equation :

$$y = ax^b \qquad \text{Equation 4.3}$$

where a and b are constants. The exponent or growth coefficient b represents the relative growth rate of the two variables, while a is the value of y when x is unity. In its linearised form this becomes :

$$\log y = \log a + b \log x \qquad \text{Equation 4.4}$$

The slope (b) and intercept (a) of such transformed data are estimated by regression analysis (Brown *et al.*, 1976; Aldrich & Crowley, 1986; Kautsky *et al.*, 1990; Stirling & Okumus, 1994). Such allometric studies enable simple and useful comparisons to be made of the variability in shell proportions amongst different mussel populations, and thus provide valuable insights into the relationship between shell shape and environmental change in both space and time, as well as determining the degree of ontogenetic control which may be exhibited during shell growth (Seed, 1980).

In this chapter the periodicity of the prismatic microgrowth bands is determined, and the age and growth rate of *Mytilus edulis chilensis* from three study sites investigated using surface growth rings, microgrowth patterns present within the shell structure and the modal progression of length-frequency distributions. Variations in the age, growth rate, shell shape and allometric relationships between various body dimensions of individuals from different tidal levels are investigated, and the growth of marked and transplanted individuals assessed.

4.2. Material and methods

4.2.1. Absolute growth

4.2.1.1. Shell growth - surface checks and internal growth patterns

4.2.1.1.1. Sample collection, marking experiments and shell sections

In order to investigate the growth history of individual mussels as well as populations of *Mytilus edulis chilensis* in the Falkland Islands, samples of 'large' (largest individuals in the population) mussels were collected from the low, mid and upper zones of the mussel beds at Darwin, Camilla Creek and Goose Green during the summer months (October - January) in 1994/5. Only shells with an intact periostracum were selected for analysis. Shells with severe abrasion and extensive infestations of a shell-boring alga were often difficult to interpret largely as a result of the obliteration

of the external and occasionally the internal growth bands. Where possible the age of individual mussels was determined by counting the number of surface growth rings on approximately 15 individuals and measuring to 0.1 mm using dial vernier calipers the distance between the umbo and each ring. These surface growth rings were assumed to be annual in origin, being a direct result of periods of suspended shell growth during the cool austral winter months.

In order to estimate short term growth of the natural mussel population, mussels from the mid region of the mussel zone (position noted on a map) at the three study sites were file marked *in situ* by making a small cleft in the posterior growth margin of the shell. These ground-marked mussels were collected at two month intervals over a period of two years from the time of marking, and the growth increments measured. Additionally, sub-tidal mesh cages (20 x 20 x 20 cm^3) containing 100 file-marked mussels, collected from the upper region of the mussel zone at Darwin, were deployed in September/October 1993, from the bridge at Darwin (see Plate 2.1, Chapter 2) and within the wreck of the 'Vicar of Brae' moored alongside the farm jetty at Goose Green, and in September 1994, at FIPASS, the flotation jetty at the east end of Stanley harbour. Mussels were collected from each cage at intervals of 1, 2 and 6 months, over the 18 month period following deployment. Short term growth was assessed by measuring the distance (to 0.1 mm) from the file mark to the posterior growth margin using dial vernier calipers.

To investigate the periodicity of microgrowth bands present in the prismatic layer of the shell, small mussels (≈20 mm shell length) were collected from the low part of the shore at Camilla Creek on a spring tide, marked by a 24 hour emersion period and returned to the shore in mesh cages situated in the low, mid and upper zones of the natural mussel bed. Ten mussels were subsequently collected at 2, 4 and 8 week intervals prior to examination following shell sectioning.

Shell sections of *M.e.chilensis* from the natural population (low, mid and upper zones) as well as all marked experimental individuals were prepared so that the internal microgrowth patterns of individual mussels could be studied. Shells were washed using mild detergent and warm water, and the inner shell surface labelled using a pencil; one valve was then selected for embedding. Shell valves were embedded in Metaset resin (Buehler UK Ltd) in order to provide support during the sectioning process. All embedded shells were sectioned through the umbone, along the anterior -

posterior axis, using a diamond saw. The cut surface of one section from each mussel was then ground smooth on increasingly finer grades of wet and dry paper attached to a rotating table. Ground sections were finally polished using household Brasso, washed in mild liquid detergent, taking care not to scratch the ground surface, and etched for 30 minutes in 1 % Decal. Excess Decal was immediately rinsed off the newly etched shell sections in running tap water, prior to being left to dry in the air. Once dry, acetate peel replicas were prepared. Near molten replication material (Agar Scientific Ltd, UK), previously immersed in ethyl acetate solvent, was applied to the etched shell section surface. Dry acetate peels were removed, trimmed and mounted onto glass microscope slides and subsequently viewed using a light microscope.

4.2.1.1.2. Estimation of growth from shell sections

Examination of acetate peel replicas of polished and etched shell sections reveal a series of distinct growth bands within the middle and inner shell layer (see Plate 4.1). Where possible the number of lines present within the inner shell layer, (umbone and nacreous) were estimated for individuals taken from the low, mid and upper zones of each population, and their origin determined following comparison with the number of winter growth checks present within the prismatic shell layer of the same mussels.

The periodicity of microgrowth bands present within the middle prismatic layer (Plate 4.1) was determined from the file-marked mussels caged at Camilla Creek by counting the number of bands present between the point of marking and the growing edge and comparing the number of observed bands with the predicted number of tidal emersions that the mussels would have experienced over the equivalent period of time. Chi-squared analysis was used to test for any significant differences between the observed and expected number of microgrowth bands. In order to estimate short-term growth rates and investigate any relationship between band number and incremental growth, the growth increment from the point of marking to the growing edge was measured to the nearest 1 µm using a calibrated eye-piece graticule.

Once the periodicity of the growth bands was established using the file-marked experimental animals, the annual growth and longevity of individual *M.e.chilensis* from different shore levels and sites could then be determined by observing changes in the microgrowth band patterns present in the middle prismatic layer. Each annual check within each shell was identified by the narrowing winter growth bands and marked

directly onto the acetate peel replica. The age of each mussel was noted and the distance between each successive check and the umbo measured to the nearest 0.1 mm using vernier calipers. Von Bertalanffy growth constants were determined from measurements of size at age using the software package Fishparm, and fitted growth curves were subsequently generated and plotted for all mussel populations.

4.2.1.2. Length frequency analysis

In an attempt to investigate the age and growth of *M.e.chilensis* using the method of length frequency analysis, random monthly, bimonthly and quarterly samples were collected from the middle of the mussel zone at each study site (see Chapter 2 for site descriptions). Population growth was estimated using the method of Bhattacharya which resolves the length frequency data into individual size (age) classes.

The length data of each sample were grouped into 2.5 mm intervals and the percentage frequency of each size class calculated and plotted for each sample at Darwin, Camilla Creek and Goose Green. Estimations of the modal sizes in the size frequency distributions were carried out using the Modal Progression Analysis (MPA) module in the ELEFAN version 1.1 software package (Electronic Length Frequency; Gayanilo *et al.*, 1988), which is a slight modification of the original Bhattacharya (1967) method. Size (age) class distributions and associated statistical parameters (χ^2 and separation index) were identified and attributed to different cohorts of the population.

4.2.2. Allometric growth

Samples of ≈ 50 *M.e.chilensis* over the entire size range of the population were collected from the low, mid and upper regions of the mussel bed at each study site. Shell length, height, width, weight and dry tissue weight were determined for each individual mussel. Figure 4.1 illustrates the shell dimensions which were measured to 0.1 mm using dial vernier calipers. Routine monthly samples of 50 mussels from the mid region of the mussel zone at each site provided further information on the relationship between the parameters of shell length, shell weight and dry tissue weight. The shell and tissue of individual mussels were placed into pre-weighed aluminium boats, dried in an oven at 60°C for 24 hours and 3 days respectively, and

subsequently re-weighed to the nearest 0.1 mg. All data were log transformed and the allometric relationships between pairs of variables determined. Significant departure from isometric growth was assessed by a t-test :

$$t_{(d.f.)} = \frac{b - \beta}{S.E.\ of\ b} \qquad \text{Equation 4.5}$$

where b is the exponent in the equation $y = ax^b$, and is generally known as the growth coefficient. β is the expected value of b if growth is isometric. When the dimensions of x and y are the same, β = 1 corresponds to isometry; when they differ, for example when y is a weight (L^3) and x is linear (L), then β = 3 corresponds to isometry. S.E. is the standard error and d.f. the degrees of freedom (n-1).

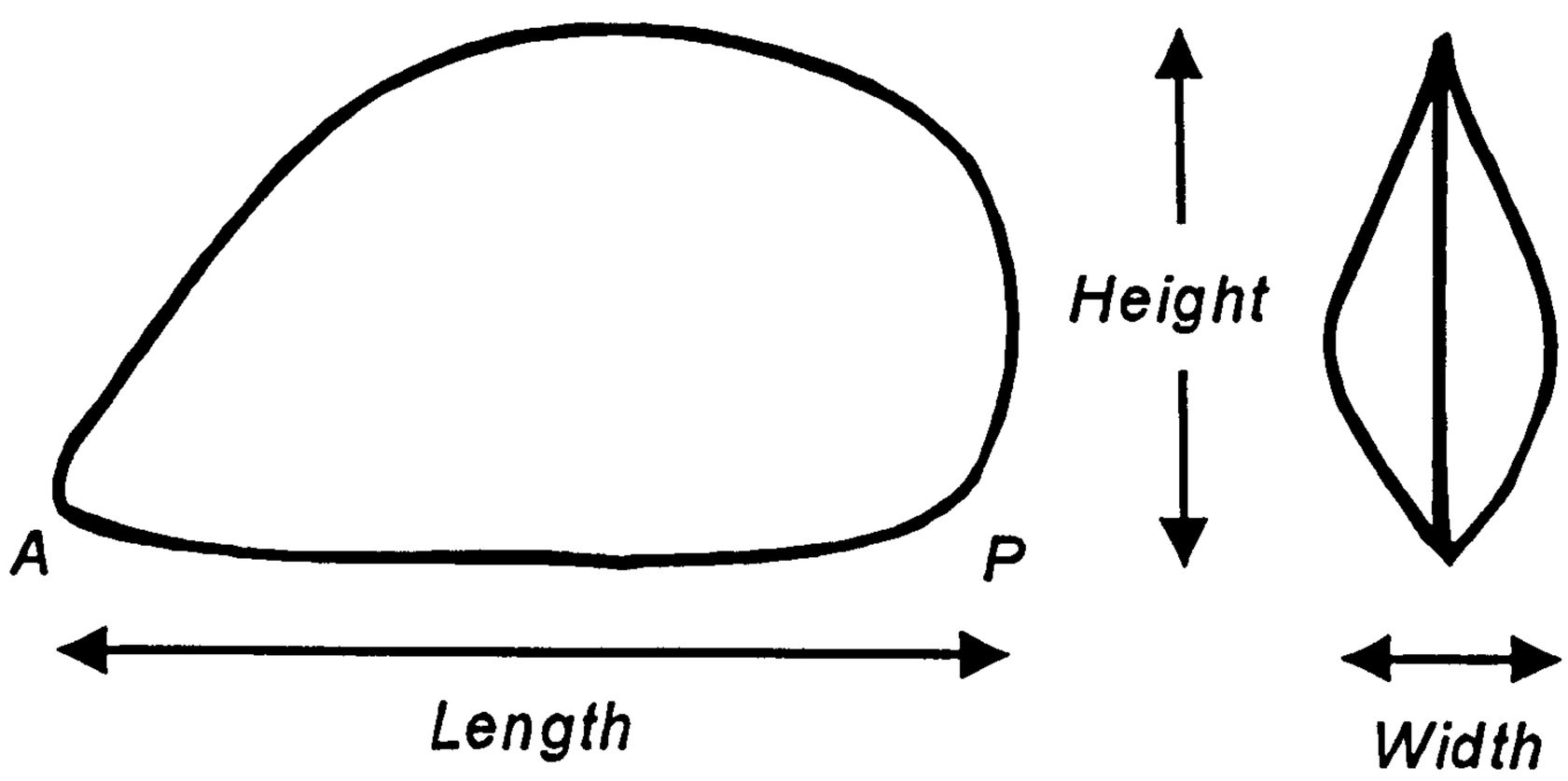


Figure 4.1 Schematic diagram of a mussel shell viewed laterally and anterio-posteriorally to illustrate the dimensions used during this study. A, anterior, P, posterior.

Differences in the allometric relationships of particular size variables (x and y) between study sites and shore levels were identified by applying the general linear model with a single covariate (GLM, Minitab). Wherever possible the parameter of shell length, which is generally the most accurate and easiest to obtain, was used as the independent variable.

4.3 Results

4.3.1. Absolute growth

4.3.1.1. Shell growth - surface checks and internal growth patterns

4.3.1.1.1. Description and interpretation of microgrowth band patterns

When viewed under the light microscope, acetate peel replicas of polished and etched *Mytilus edulis chilensis* shells revealed a similar shell structure to that previously described in *Mytilus edulis* (Lutz, 1976; Richardson *et al.*, 1990b; Seed & Richardson, 1990; Seed and Suchanek, 1992). Three shell layers were identified; 1) a thin outer periostracum, 2) a middle prismatic layer, and 3) an inner nacreous layer. A series of distinct bands were observed both in the nacreous and prismatic layers (Plate 4.1). The periodicity of the dark microscopic bands which separate wide transparent growth increments present in the prismatic shell layer was first determined.

The tides in the Falkland Islands have a mixed semi-diurnal pattern (Plate 4.2C). During the neap and early period of the spring lunar cycle there is a marked difference in tidal height between the morning and evening low tide, whereas little or no differences in tidal heights are observed during the early part of the neap period. Animals living in the lower part of the shore would therefore experience only one emersion a day during certain times of the lunar cycle when the largest differences in tidal height occurred. In this study mussels were situated in cages at 0.64, 0.82 and 0.95 m above chart datum (see Chapter 2) corresponding to the low, mid and high zones of the mussel bed, respectively. Mussels at 0.64 m and to a lesser extent those at 0.82 m, would have experienced diurnal emersion during the course of the experimental period (Plate 4.2C). The number of microgrowth bands deposited and the incremental growth attained by these experimentally marked mussels are presented in Table 4.1. Very little agreement between the number of microgrowth bands expected and those observed deposited in the shells was found in mussels from the mid and upper zones. Only in mussels from the low shore cage was there any agreement between the number of expected emersion bands and observed bands, and this only occurred in mussels collected after experimental periods of 14 and 28 days. Chi-squared analysis revealed that in all cases, except for individuals from the low region of the zone with an experimental period of 14 days, the number

Plate 4.1

A. Schematic illustration of a shell section of *Mytilus edulis chilensis*;

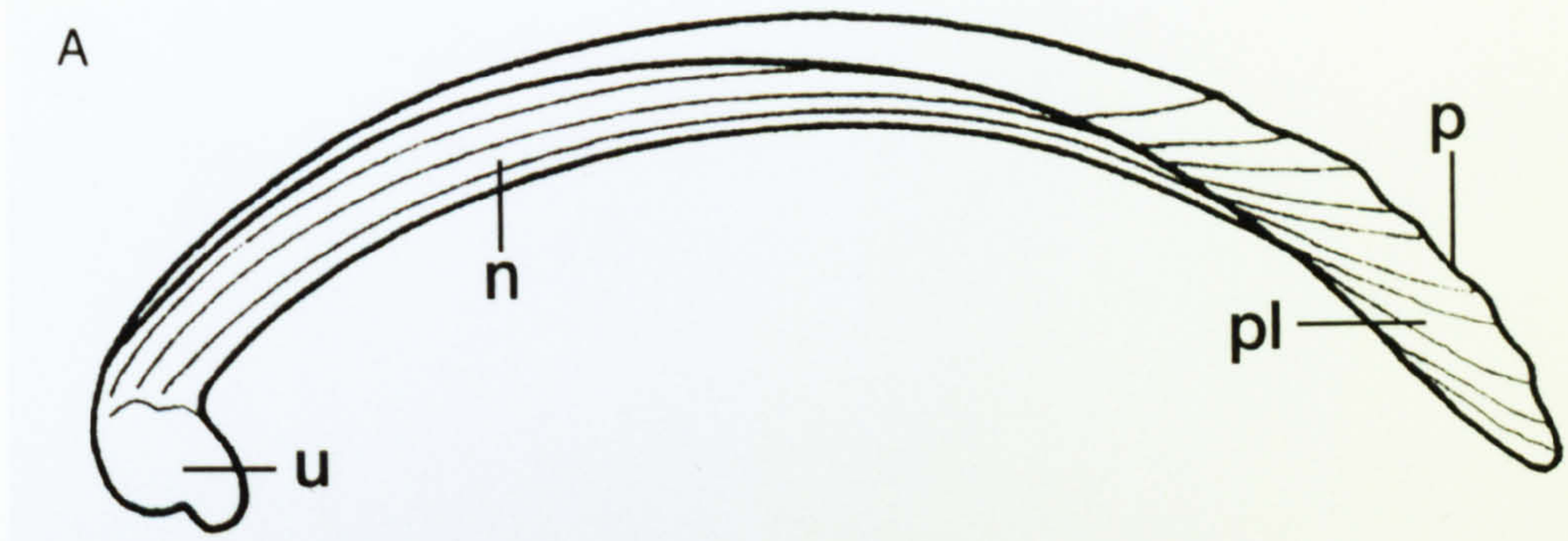
B. Photomicrograph illustrating the position of a winter growth check within the prismatic shell layer of a mussel from the low shore population at Darwin;

C. Photomicrograph illustrating the position of a disturbance check within the prismatic shell layer of a mussel from the low shore population at Darwin;

D. Photomicrograph illustrating the presence of growth lines within the umbone region of a mussel from the low shore at Goose Green;

E. Photomicrograph illustrating the presence of annual lines within the nacreous shell layer of a mussel from the low shore at Goose Green;

p, periostracum, u, umbone; pl, prismatic shell; n, nacreous shell; arrows denote growth checks and lines. Scale bar = 500 µm (B, C); 100 µm (D, E).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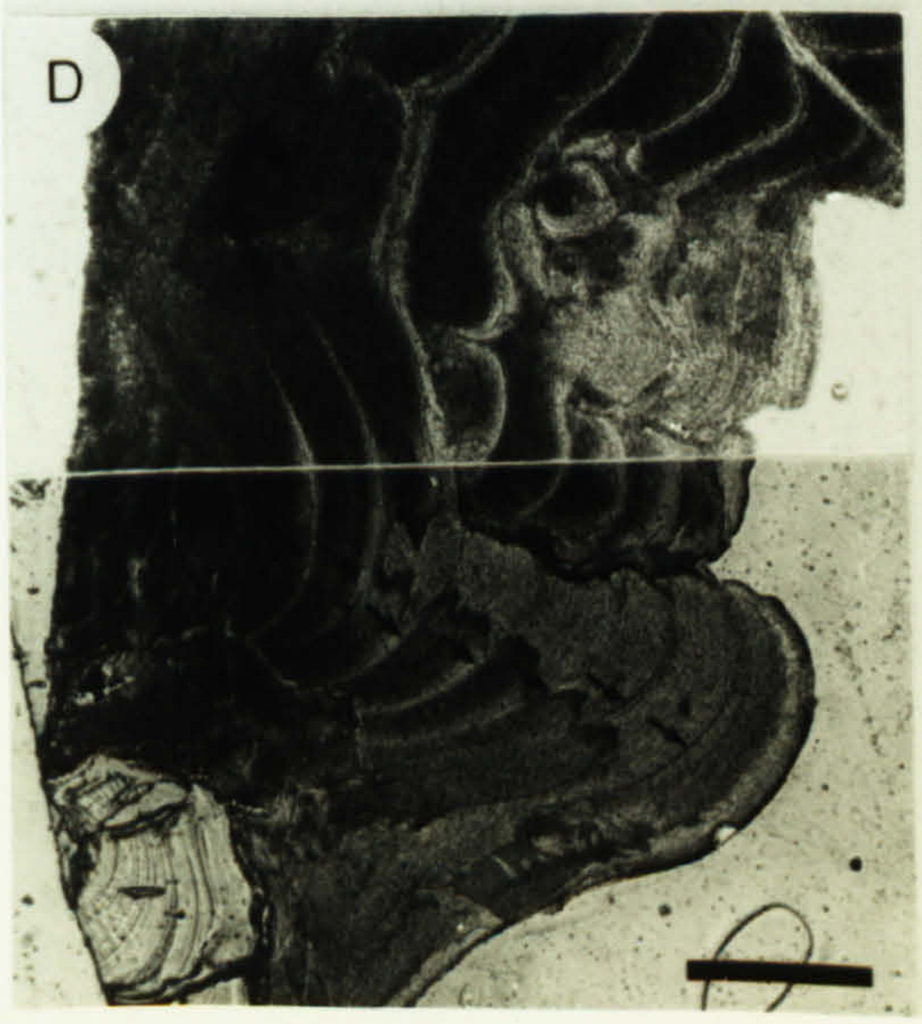
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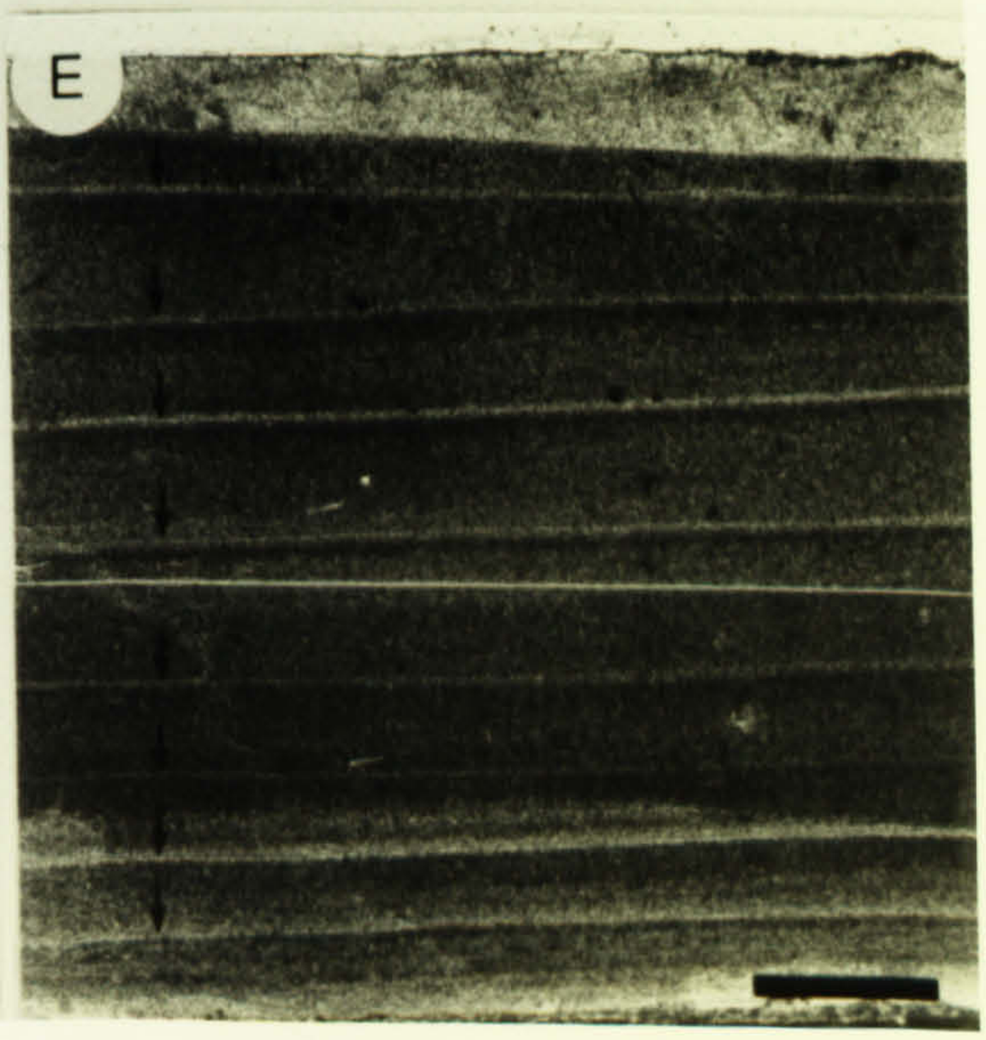
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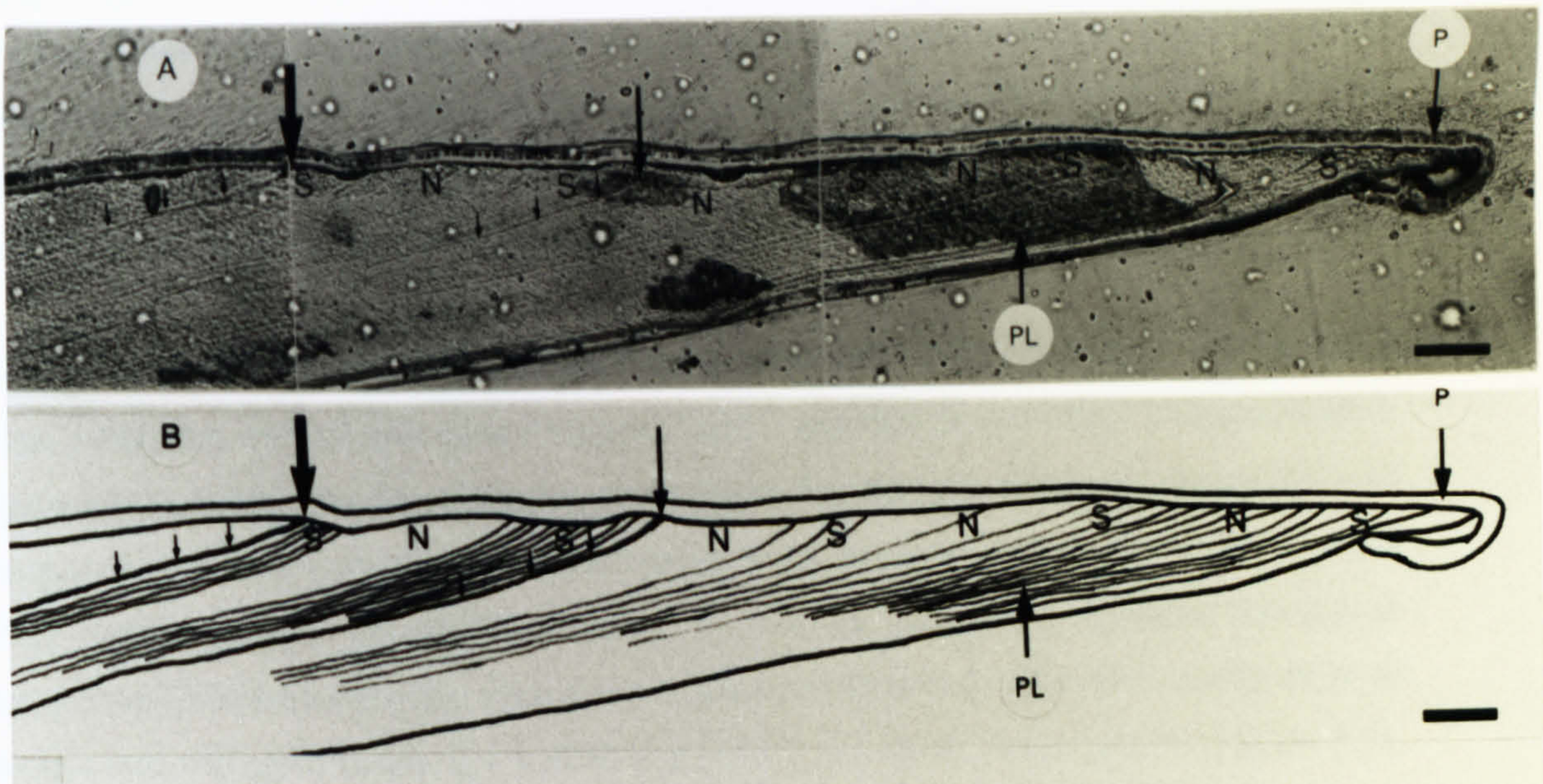
Plate 4.2

A. Photomicrograph illustrating the microgrowth band pattern within the prismatic shell layer of a *Mytilus edulis chilensis*, which was file-marked and emersed for 24 hours (large arrow) and grown at 0.82 m above chart datum at Camilla Creek for 56 days. P, periostracum, PL, prismatic layer, S, spring period; N, neap period; the large arrow indicates the 24 hour emersion band, whereas the smaller arrows indicate the position of this band within the shell section; the medium-sized arrow indicates a prominent band deposited at low tide during a period of anomalously high air temperature, whilst small arrows highlight this band within the shell section. Scale bar = 100 µm.

B. Schematic diagram of the photomicrograph in A above to highlight the 24 hour emersion band, the proximity of the growth banding during spring and neap tides and the clearly defined band deposited during emersion when an anomalously high air temperature occurred.

C. Predicted tidal cycle during the experimental period; solid line marks position the of the mid shore experimental cage, c.d. = + 0.82 m.

D. Continuous seawater and air temperature records logged by TinyTalk temperature logger (Orion Ltd). Asterisk highlights the anomalously high temperature (20 th January 1996) which occurred when the mussel was emersed for several hours during a spring low water. On other occasions the mussels were not emersed when there were unusually elevated air temperatures.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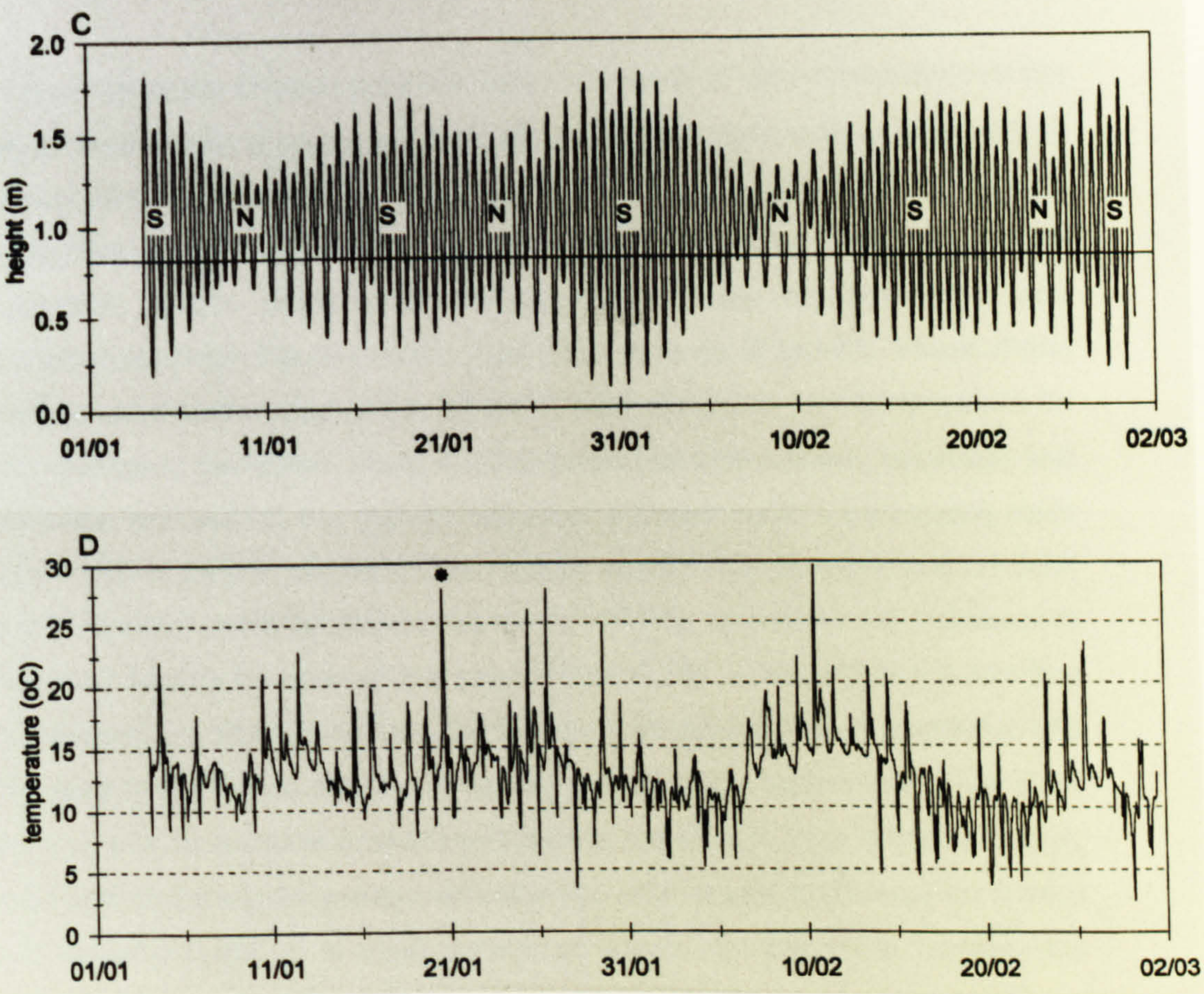
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of bands observed was significantly different from the numbers of tidally produced bands expected (Table 4.1). Thus, although there appears to be slight agreement in the low shore animals, overall deposition of individual bands does not appear to be regulated by the tidal cycle. Curiously, the number of bands deposited in 14 day experimental individuals from the low shore, was in agreement with the number of experimental days as well as the expected number of tidal emersions.

Mussels from the high shore cage grew much slower (≈ 0.011 mm.day $^{-1}$) than those from either the mid or low shore cages (0.015 and 0.018 mm.day $^{-1}$, respectively). Figure 4.2 illustrates the low correlation between the number of bands deposited and the growth increment deposited over a period of 56 days for individual mussels at the three shore levels, where the number of bands deposited remains the same regardless of increment size. The low correlation observed, was particularly clear at the low and mid zone levels, r_s = 0.496 and 0.500, $p > 0.05$, respectively (Figure 4.2), indicating that band deposition is not a direct consequence of the rate of growth.

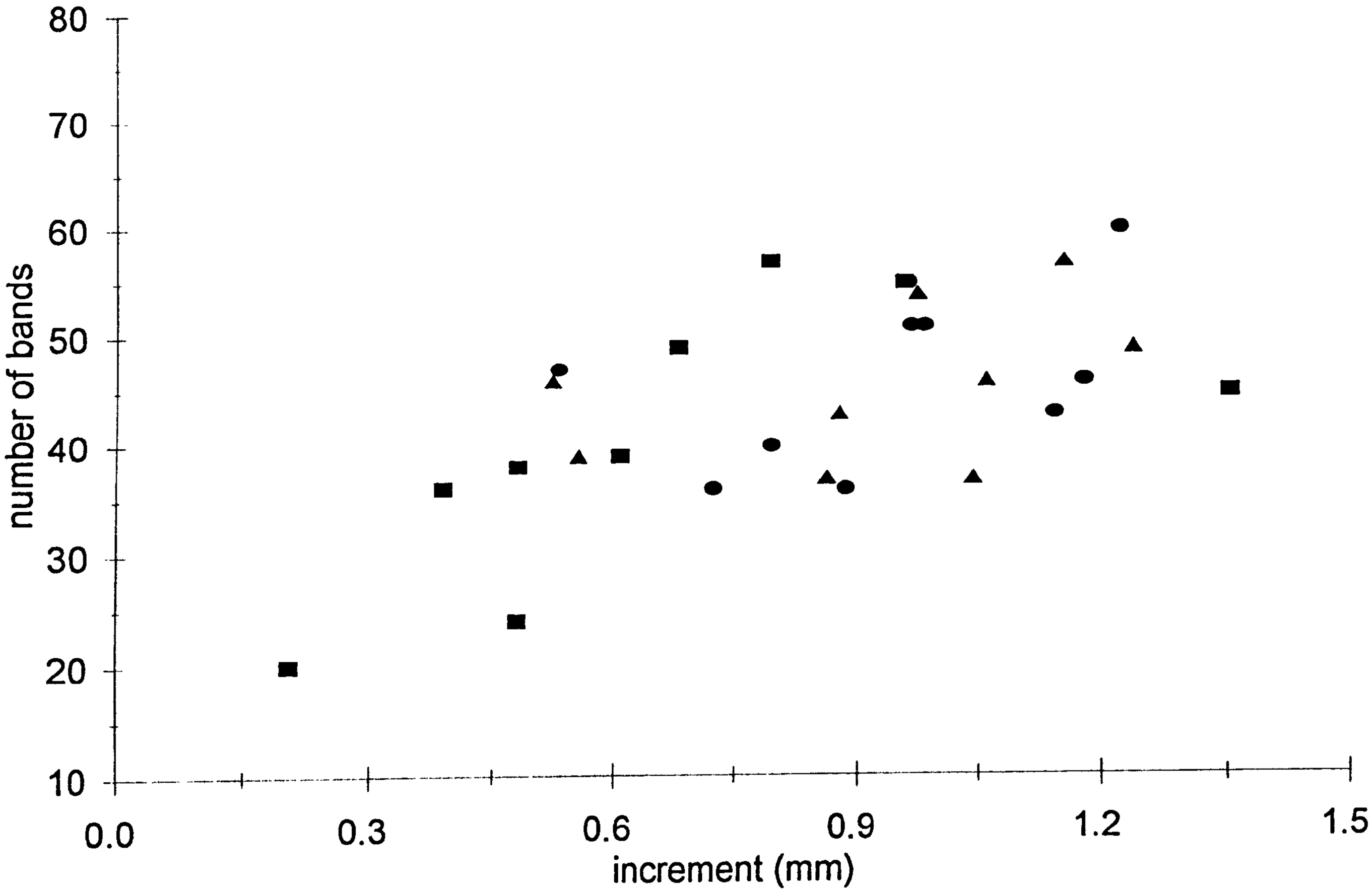
Generally the preservation of band patterns within the shell of *M.e.chilensis* from the mid and upper shore levels is poor, and their deposition appears to bear little relation to tidal or daily factors. However, within any group of shells, occasional individuals which exhibited fast growth rates tended to have more clearly defined and demarcated banding patterns. In this study most shells collected from the low shore, and occasional individuals from the mid shore, had clear records of growth. These shells were therefore used to investigate the effect of tidal emersion and temperature on growth rates and band formation. Plate 4.2 illustrates the tidal pattern, seawater and air temperatures, and part of the growth record of a mussel with a particularly clear pattern of growth, from the middle of the mussel zone. The file mark and 24 hour emersion period which were administered on the 4/1/95, as well as the subsequent tidal pattern can clearly be seen in the shell (Plate 4.2A). A tracing of the banding patterns to highlight important features within the shell section is also illustrated (Plate 4.2B). The shell was marked during the spring period of the lunar cycle. Four clear spring bands can be observed immediately following marking (5 th and 6 th January), after which a period of neap tides occurred when the animal was immersed for 5 days resulting in the deposition of a weak pattern of bands. On the 12 th January the mussel was emersed at low tide and for the following 6 days (11 bands can be clearly observed) a clear banding pattern was produced. At the end of these spring low tides the mussel was subjected to a period of neap tides where it remained continually

Table 4.1 The number of microgrowth bands deposited and the incremental growth in the shells of *Mytilus edulis chilensis*, following marking by 24 hour emersion and transplantation to low, mid and high shore cages at Camilla Creek, for periods of 14, 28 and 56 days.

Level	Experimental period (1995)	Duration of experiment (days)	No. of expected emersions	No. of shells examined	No. of bands observed (mean ±1 S.D.)	Growth increment (mm)	Growth rate (mm.day-1)	χ^2 value for expected versus observed band number
High	4th Jan - 18th Jan	14	27	7	12.58 ± 2.47	0.19 ±0.06	0.013	53.22*
	4th Jan - 1st Feb	28	49	7	21.85 ± 6.06	0.29 ± 0.11	0.010	109.76*
	4th Jan - 1st Mar	56	104	9	41.88 ± 11.25	0.66 ± 0.34	0.011	363.24*
Mid	4th Jan - 18th Jan	14	20	9	13.22 ± 1.71	0.27 ± 0.10	0.019	21.85*
	4th Jan - 1st Feb	28	43	8	21.62 ± 3.11	0.36 ± 0.12	0.012	86.58*
	4th Jan - 1st Mar	56	95	10	46.50 ± 7.96	0.94 ± 0.22	0.016	253.61*
Low	4th Jan - 18th Jan	14	12	6	9.16 ± 3.76	0.26 ± 0.10	0.018	9.92^{ns}
	4th Jan - 1st Feb	28	29	9	24.33 ± 10.22	0.63 ± 0.42	0.022	35.59*
	4th Jan - 1st Mar	56	67	9	45.33 ± 7.15	0.92 ± 0.25	0.016	69.18*

* significant at $p < 0.05$
^{ns} not significant

Figure 4.2 The relationship between the number of microgrowth bands and the incremental growth in *Mytilus edulis chilensis* which had been marked and transplanted to low (closed triangles), mid (closed circles) and high (closed squares) shore levels at Camilla Creek during a 56 day experimental period.


80
70
60
50
40
30
20
10
number of bands
0.0
0.3
0.6
0.9
1.2
1.5
increment (mm)

immersed only to be emersed twice a day for very short periods of time, resulting in the formation of very faint growth bands in an otherwise clear area of the shell. This spring/neap lunar pattern was obvious in all the shells examined from the three tidal levels.

During the course of the experimental period five anomalously high air temperatures in excess of 25°C were recorded (Plate 4.2D). Only on one occasion did the high temperatures (≈ 28°C) coincide, during a period of spring tides, when the mussels were emersed. A prominent microgrowth band was deposited (20 th January 1995) as a result of the unusually high air temperature and relatively longer emersion time that the mussel experienced (compare Plate 4.2A and B with Plate 4.2D). The other four unusually high temperatures occurred during periods of neap tides when the mussels were only emersed for relatively short time periods. Neap tide emersion during these periods of high air temperatures was insufficient to result in the formation of a well defined band. Examination of all the experimental shells revealed that the prominent band deposited on the 20 th January 1995 could be observed in 50 %, 70 % and 10 % of mussels from high, mid and low shore levels, respectively. Mussels from the low shore would have been emersed for the shortest period of time and would therefore not have been expected to have been affected to the same degree as mussels from the upper shore levels.

The mixed semi-diurnal nature of the tides in the Falkland Islands results in the deposition of a distinct pattern of closely deposited diurnal growth bands which can be observed in Plate 4.2A and B towards the growing shell margin. Clear widely spaced and less distinct bands are characteristic of a period of mixed semi-diurnal tides when the mussels only experience diurnal emersion.

4.3.1.1.2. Seasonal growth and longevity

The appearance of the outer surface of a selection of mussel shells collected from the three study sites are shown in Plates 4.3 and 4.4. In some shells surface growth rings were relatively easy to identify. For example in Plate 4.3A a mussel from the low shore population at Goose Green has very clearly defined surface rings over most of the shell surface. However, in other shells these rings are more difficult to distinguish, often rendering the analysis of surface rings difficult or impossible. During this study several factors were found to affect the appearance of the surface rings; 1) *abrasion*

Plate 4.3 Photographs of the shell surface of *Mytilus edulis chilensis;*

A. with clear surface rings, from the low shore population at Goose Green;

B. with severe abrasion, from the mid shore population at Goose Green;

C. with blisters on the shell surface due to the infestation by shell boring algae.

Scale bar = 10 mm.


A
B
C

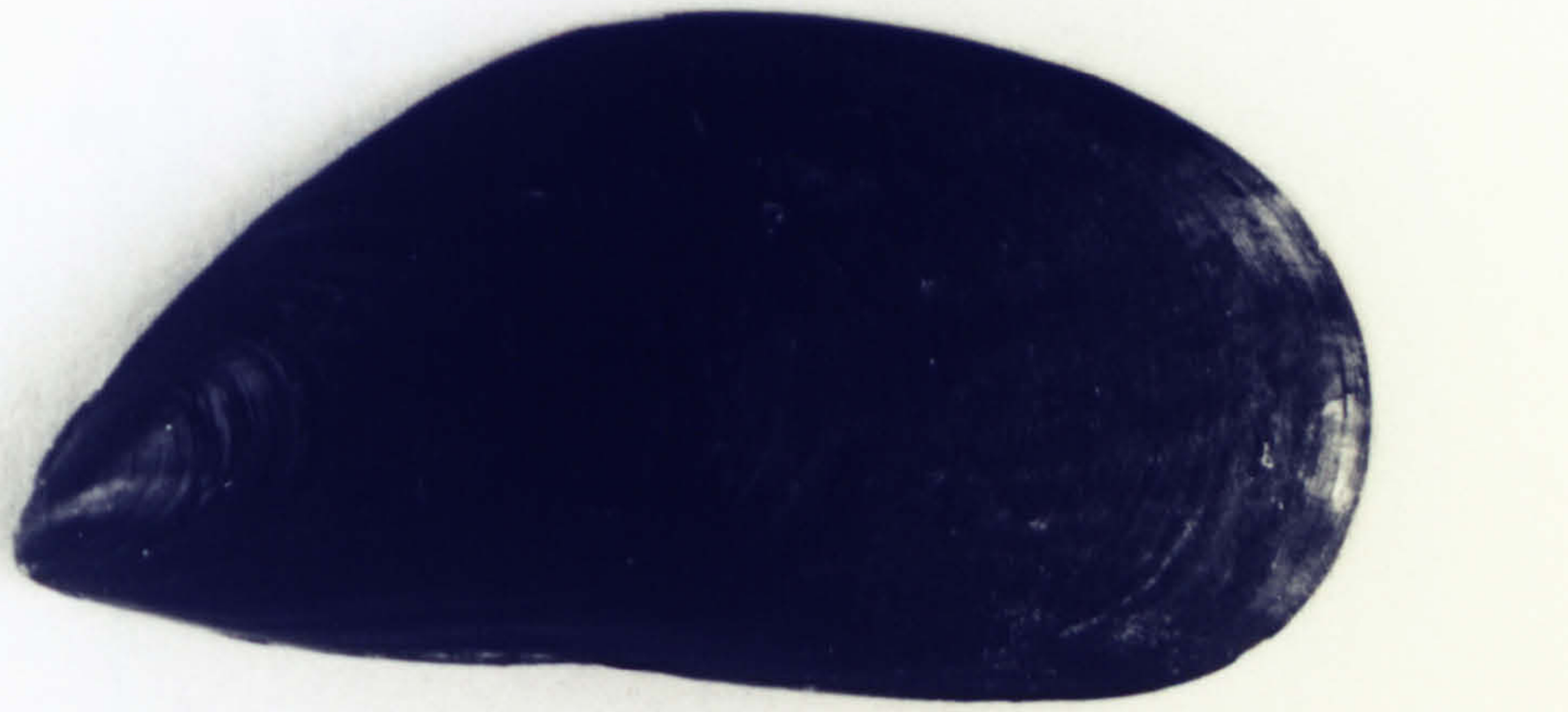
Plate 4.4 Photographs of the shell surface of *Mytlius edulis chilensis;*

A. with an intact periostracum, but no clear surface rings, from the low shore population at Darwin;

B. with a poor record of surface rings in a relatively old, slow growing individual from the high shore population at Goose Green.

Scale bar = 10 mm

A



B

Table 4.2 Shell length (mm) at age (years) and maximum age of *Mytilus edulis chilensis* derived from surface growth rings.

Site	Zone	Shell length (mean ± s.e.) at age						Maximum age (years)
		1	2	4	6	8	10	
Darwin	high	14.02 ± 0.82	17.72 ± 1.05	25.34 ± 1.18	30.01 ± 1.61	33.35 ± 2.28	37.90 ±0.00	10
	mid	14.95 ± 1.33	18.40 ± 1.78	25.56 ± 2.27	29.83 ± 2.07	34.72 ± 2.55	30.30 ± 0.00	13
	low	24.40 ± 3.29	30.52 ± 2.27	41.58 ± 3.19	49.34 ± 3.78	53.48 ± 5.11	57.27 ± 4.78	13
Camilla Creek *	mid	25.35 ± 1.68	31.87 ± 2.10	40.10 ± 2.25	46.28 ± 1.94	52.72 ± 2.07	56.70 ± 2.28	15
Goose Green	high	10.38 ± 0.78	15.54 ± 0.78	24.08 ± 1.05	30.99 ± 1.28	35.79 ± 1.51	38.28 ± 1.64	14
	mid	14.32 ± 1.00	18.18 ± 1.06	24.82 ± 1.30	31.92 ± 1.31	37.62 ± 1.20	42.48 ± 0.68	13
	low	22.09 ± 2.20	30.43 ± 2.26	43.66 ± 2.03	56.53 ± 2.33	66.94 ± 1.67	73.53 ± 2.01	13

* shells only available from the mid zone of the mussel bed

of the external shell surface. The periostracum was frequently completely removed, particularly in the umbone region, and in severe cases part of the middle prismatic layer had also been worn away. Plate 4.3B illustrates such abrasion in a shell collected from the mid shore at Darwin, although shell abrasion was also observed in mussels from the middle of the mussel zone at Goose Green; 2) *blisters* on the shell surface caused by shell boring algae. These sometimes prolific areas of infestation often result in the surface rings being completely obscured (Plate 4.3C). Mussels from the mid and high regions of the mussel zones at all three study sites in particular suffered from high infestations of shell boring algae; 3) *rapid shell growth*. Mussels from the low shore at Darwin (Plate 4.4A), in which the periostracum is totally intact, do not have any obvious surface rings which can be readily identified; 4) *low resolution* and *crowding of rings*. Surface rings in shells collected from old, slow growing populations particularly in the high zone of the mussel bed, are also sometimes difficult to identify (Plate 4.4B).

Table 4.2 contains size at age data for *M.e.chilensis* from the three study sites obtained from surface growth rings present in the periostracum. All of the mussels examined appeared to be relatively long lived with the oldest individuals, 15 years old, observed in the Camilla Creek populations from the middle of the mussel zone. Mussels from high shore at Darwin appear to live for a slightly shorter time than their mid and low shore counterparts. At Goose Green the oldest mussels were found in the upper region of the mussel zone.

Growth rates vary with both shore level and study site. Generally mussels from the low shore exhibit considerably faster growth rates than those from either the mid or high shore levels. The size of the mussel when the first winter ring is deposited varies according to tidal elevation. Mussels from the upper part of the shore grow to about 10 and 14 mm in shell length in their first year, at Goose Green and Darwin respectively, whilst those from the mid part of the zone reach a size of 14, 15 and 25 mm at Goose Green, Darwin and Camilla Creek respectively, in their first year. Low shore mussels from all the sites attain a size of 22 - 24 mm in the first year. At 4 years, mussels from the low shore at Darwin and Goose Green were ≈40 mm in shell length, compared to shell lengths of ≈25 mm in mussels from both the mid and high regions. This difference in size with shore level is retained whilst the animals increase in age, although low shore mussels from Goose Green attain a considerably larger shell length than those from Darwin (74 mm and 57 mm at 10 years, respectively) and

mussels from the high zone are usually slightly smaller than those from the mid zone. Due to insufficient data for the mussel population at Camilla Creek it is not possible to consider the effects of tidal elevation on growth rate; however, it can be noted that mussels from the mid shore level (≈0.82 m above c.d.) reach a similar size to those from the low shore levels at Darwin and Goose Green (≈0.38 m and 0.23 m above c.d.).

Bands present in the nacreous layer (including the umbone region) could be observed in polished and etched shell sections. Alternating light lines and dark wide areas of incremental growth were clearly seen in the nacreous layer of 96% of the shells examined (Plate 4.1). Growth lines present in this nacreous shell layer were counted, where possible, for all mussels examined, and compared to the ages of individual mussels estimated using other methods of age determination, such as surface ring analysis and prismatic layer band analysis. Occasionally individuals were discounted from the analysis due to unclear band patterns which were the result of either poor resolution of the banding, or the presence of organic material on the shell section surface, which obscured the bands beneath. Growth bands present in the umbone were more difficult to identify due to excessive shell abrasion in this the oldest part of the shell, resulting in part of the growth history being lost. Plate 4.1 illustrates the appearance of the growth bands in the umbone and nacreous shell layer in a particularly clear specimen. The number of lines observed in the umbone region and the nacreous layer are in general agreement.

Seasonal variations in the width of the microgrowth patterns present in the middle prismatic layer were also examined in order to assess the age and to determine the growth rate of individual mussels. Acetate peel replicas of polished and etched shell sections revealed a series of alternating dark growth lines and wide clear increments within the prismatic shell layer. The relative width of the growth increments varies along the shell according to seasonal environmental changes. During the summer, when shell growth is fast, wide increments are deposited, but with the onset of cooler autumn months shell deposition gradually slows down and the increments become progressively narrower during the winter. This seasonal narrowing of the microgrowth bands is clearly illustrated in Plate 4.1B, a photomicrograph of a mussel from the lower reaches of the mussel bed at Goose Green. Disturbance checks, which result in sudden interruptions to the normal pattern of banding, and which are not seasonal in origin (Plate 4.1C), were identified and separated from the seasonal growth checks, which are characterised by a gradual narrowing of the increments.

Table 4.3 contains size at age data for *M.e.chilensis* from the three sites and from all positions in the mussel zone determined from the seasonal narrowing of the tidal bands in the prismatic layer. The maximum age of mussels at the study sites did not reflect any pattern relating to the tidal elevation of the mussels. At Darwin the oldest mussels ranged between 6 and 8 years. At Camilla Creek the oldest mussels (10 years of age) were collected from the middle of the mussel bed. Mussels from the low shore level lived for up to 7 years, whilst those from the upper region were relatively short lived, with a maximum age of 5 years being observed. At Goose Green the oldest mussels (11 years old) were from the low part of the shore, whilst those from the high shore reached up to 9 years of age and those from the mid shore 8 years. The maximum estimated ages provide some indication of the differences which result from using the different methods of age determination. At all sites and tidal levels the maximum age was higher when surface growth rings were used to age the mussels. At Darwin, mussels from the mid shore were as much as 6 years older when aged using the surface growth ring method, compared to the more accurate method using internal microgrowth patterns. This lack of agreement between the two methods suggests that the mussels are subjected to a considerable number of disturbances during their lives, all or most of which are recorded in the shell structure. Many surface rings are mistakenly identified as annual growth checks, but they can be identified and discounted when prismatic band patterns are examined.

The growth rates of *M.e.chilensis*, determined using the narrowing of the microgrowth bands, vary considerably according to site and shore level. The size at which the first growth check is laid down provides no evidence of any trend with shore level at the three sites. By the first year mussels from the low shore at Darwin and Goose Green, reach 15 and 12 mm in shell length, respectively, whilst mussels from the mid and high shore deposit the first growth check at 13 and 6 mm and 13.5 and 8 mm, respectively. By the time the mussels reach 4 years old there is a clear difference in the growth rates with decreasing tidal elevation. Mussels from the upper limit of the mussel bed exhibit the lowest rates of growth, at Darwin and Goose Green, with sizes of ≈ 28 mm in shell length being attained after 4 years. In the middle and lower regions of the mussel zone individuals reach 35 and 47 mm after 4 years, respectively. It is only when mussels reach 8 years that the growth rates of low shore Darwin and Goose Green mussels begin to show a difference, with those from Goose Green being up to 10 mm longer than those from Darwin. Mussels from Camilla Creek, attain relatively large sizes at both low and high shore levels when compared

Table 4.3 Shell length (mm) at age (years) and maximum age of *Mytilus edulis chilensis* derived from winter growth checks in the prismatic layer

Site	Zone	Shell length (mean ± s.e.) at age						Maximum age (years)
		1	2	4	6	8	10	
Darwin	high	13.41 ± 1.50	22.69 ± 2.35	28.88 ± 0.66	37.20 ± 0.00			6
	mid	12.78 ± 1.73	23.85 ± 1.68	35.42 ± 1.26	38.48 ± 1.22			7
	low	14.69 ± 1.59	31.12 ± 1.21	48.77 ± 2.47	60.48 ± 4.00	60.55 ± 4.00		8
Camilla Creek	high	18.89 ± 2.36	35.36 ± 1.57	50.09 ± 1.44				5
	mid	14.89 ± 1.76	30.69 ± 1.69	48.62 ± 1.98	58.87 ± 1.69	57.88 ± 1.22	65.90 ± 0.00	10
	low	18.15 ± 1.43	31.98 ± 1.99	48.05 ± 1.42	55.39 ± 1.73			7
Goose Green	high	8.30 ± 0.90	15.64 ± 0.52	27.91 ± 0.88	36.20 ± 0.95	38.43 ± 1.42		9
	mid	6.05 ± 0.47	19.61 ± 1.33	35.45 ± 1.43	40.87 ± 0.71	41.55 ± 0.46		8
	low	12.13 ± 1.06	26.32 ± 1.77	46.68 ± 1.25	63.34 ± 0.82	71.20 ± 1.09	77.43 ± 0.60	11

to those from the mid shore. Sizes attained at the time when the first growth check is deposited in mussels from Camilla Creek are similar regardless of shore level (≈18 mm in the low and high zone and 15 mm in the mid). By the time mussels reach 4 years of age they are ≈49 mm, exhibiting no differences in size with respect to tidal elevation. Only mussels from the lower part of the shore at Darwin and Goose Green reach ≈49 mm in shell length at 4 years old. Therefore it might be suggested that overall, mussels from Camilla Creek have faster growth rates than those from either Darwin or Goose Green, reaching 49 mm in shell length after four years, irrespective of their position on the shore.

Overall, growth rates calculated from the pattern of microgrowth bands were considerably higher than those estimated by surface ring analysis. For example mussels from the mid region of the mussel zone at Darwin were estimated to take only 4 years to reach a size of ≈35 mm using the microgrowth band method, whereas the same size mussel took 8 years to reach this size when the age and growth rate were estimated using surface growth rings.

Figure 4.3 illustrates the relationship between the estimated ages of the mussels using; A) surface rings and prismatic winter growth checks, and B) nacreous lines and prismatic winter growth checks. Spearman rank order correlation carried out on the estimated ages of all the mussels examined revealed a strong correlation between nacreous lines and prismatic winter growth checks (r_s = 0.927), and a weaker, although just significant, correlation between surface rings and prismatic winter growth checks (r_s = 0.399). Nacreous lines, as well as umbone lines, are therefore annual in origin and can also be used to assess the age and growth rate of individual mussels.

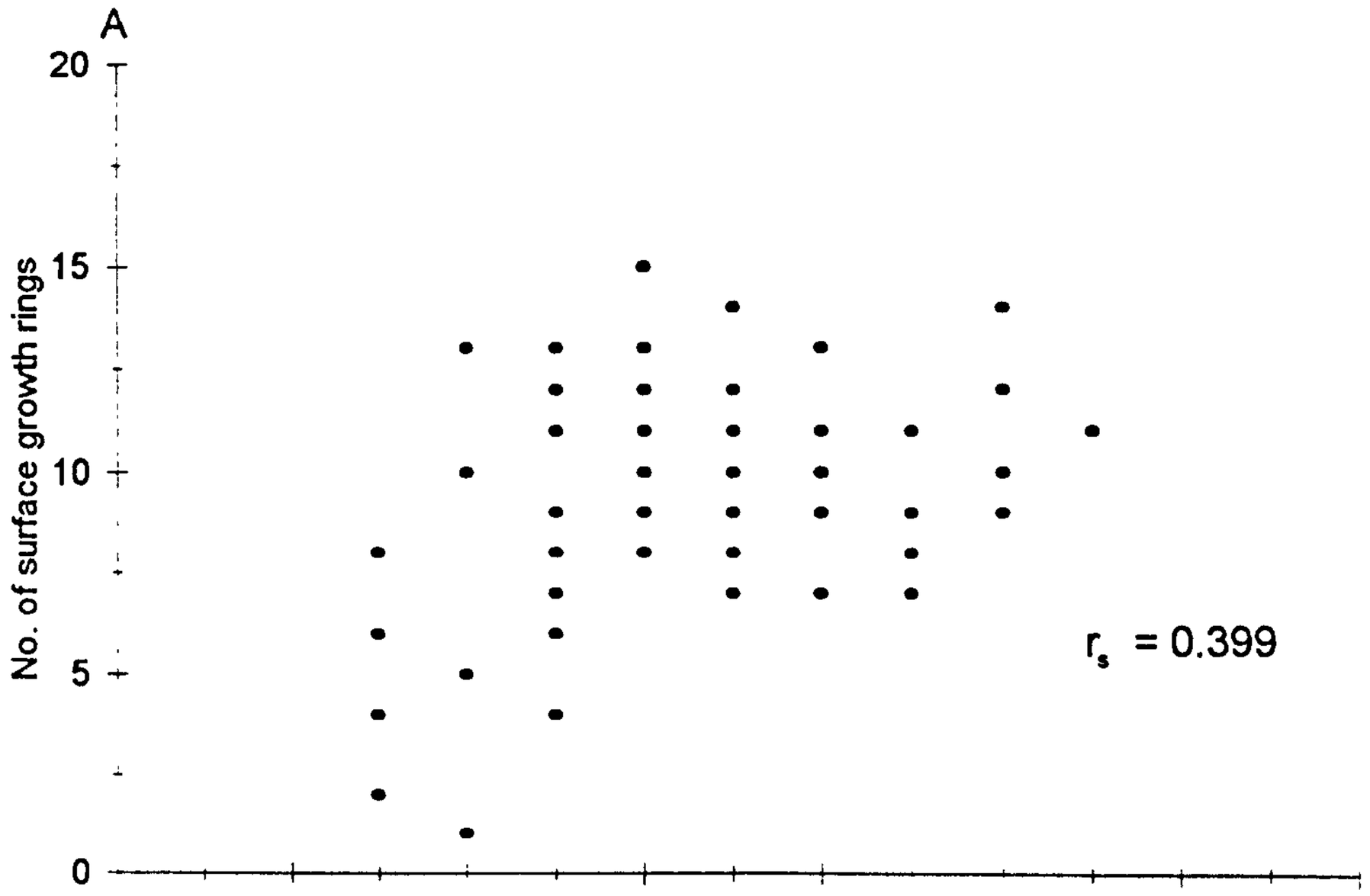
The growth constants derived using Fishparm, where the seasonal growth checks within the prismatic shell layer have been fitted to the von Bertalanffy growth model, are presented in Table 4.4. The predicted maximum attainable size (from the von Bertalanffy growth equation), L_∞, is generally highest in the low shore populations and lowest in mussels from the upper part of the shore. At Camilla Creek the difference in L_∞ at different shore levels is marginal. K, the rate at which the asymptotic size is approached is highest in mid shore mussels from both Darwin and Goose Green, with values being slightly lower in high shore mussels and lowest in those from the low shore. Low shore mussels from Darwin and Goose Green appear to be relatively large and fast growing, although those from Goose Green have a relatively low k value, and do not approach their asymptotic size until they are relatively old, whilst those from the

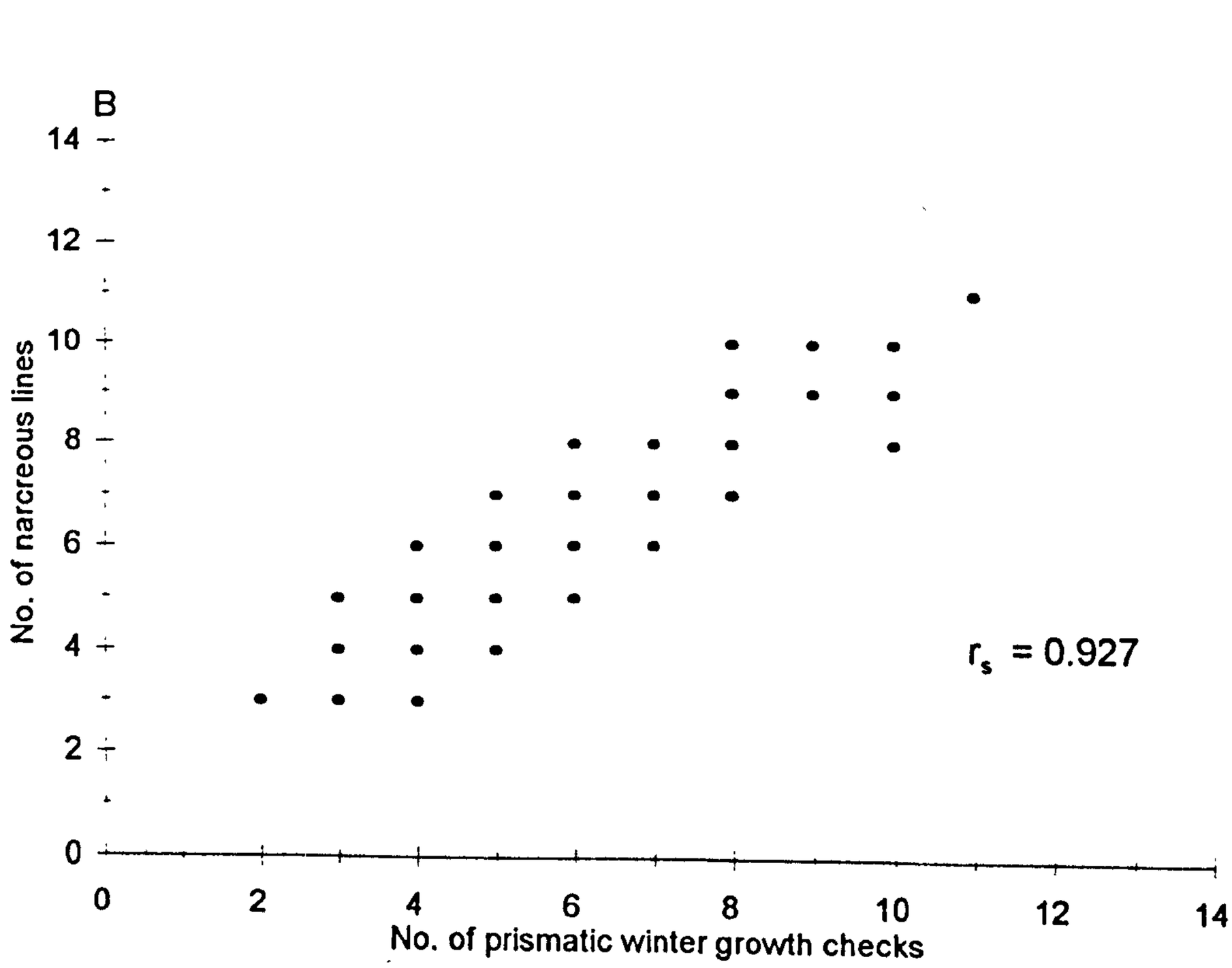
Figure 4.3 The relationship between

A. surface growth rings and prismatic layer winter growth checks and

B. nacreous lines and prismatic layer winter growth checks.

The surface growth rings, nacreous lines and prismatic layer winter growth checks were all used to estimate the age of *Mytilus edulis chilensis*. r_s, Spearman Rank Order correlation coefficient.


A
20
15
10
5
0
No. of surface growth rings
r_s = 0.399


B
14
12
10
8
6
4
2
0
No. of narcreous lines
r_s = 0.927
0
2
4
6
8
10
12
14
No. of prismatic winter growth checks

mid shore grow rapidly and reach their asymptotic size much earlier. High shore mussels from Goose Green are slow growing with relatively low k values, resulting in small, old mussels. At Darwin, high shore mussels are slightly slower growing than those from the middle of the mussel zone, and reach a marginally smaller size than their mid zone conspecifics. Mussels from Camilla Creek exhibit negligible differences between their k values; those from the upper region of the mussel bed approach the asymptotic size at a marginally faster rate than do mussels from either mid or low regions.

Table 4.3 Growth constants derived from the von Bertalanffy growth model using prismatic winter growth checks in *Mytilus edulis chilensis* from the three study sites in the Falkland Islands

Site	Zone	n	L_∞ (mm)	k
Darwin	high	32	36.3	0.491
	mid	63	40.5	0.542
	low	61	66.1	0.396
Camilla Creek	high	50	59.0	0.521
	mid	89	63.5	0.401
	low	68	60.9	0.391
Goose Green	high	65	47.6	0.235
	mid	80	45.3	0.702
	low	110	91.4	0.198

Von Bertalanffy growth curves are presented in Figure 4.4A, B, C. Low shore mussels from both Darwin and Goose Green exhibit faster growth rates and are larger than both mid and high shore mussels, whilst mid shore mussels are marginally larger and faster growing, than their high shore counterparts. At Camilla Creek there is very little difference between both growth rate and size of mussels regardless of position on the shore.

Figure 4.4D compares the growth curves estimated using the von Bertalanffy growth model using the prismatic layer winter growth checks for the low shore populations from Darwin, Camilla Creek and Goose Green. Also included in the figure are the von Bertalanffy growth curves estimated using surface growth rings for low shore mussels

Figure 4.4 Von Bertalanffy growth curves for *Mytilus edulis chilensis* populations predicted from prismatic winter growth checks for high (open squares), mid (open triangles) and low (open circles) shore levels at

A. Darwin,

B. Camilla Creek,

C. Goose Green.

D. A comparison of the growth curves predicted from prismatic winter growth checks in low shore populations from Darwin (closed circles), Camilla Creek (closed squares) and Goose Green (closed triangles); growth curves predicted from surface growth rings, for the low shore Darwin population (open circles) and the subtidal population examined by Davenport *et al.* (1984), (closed bows).

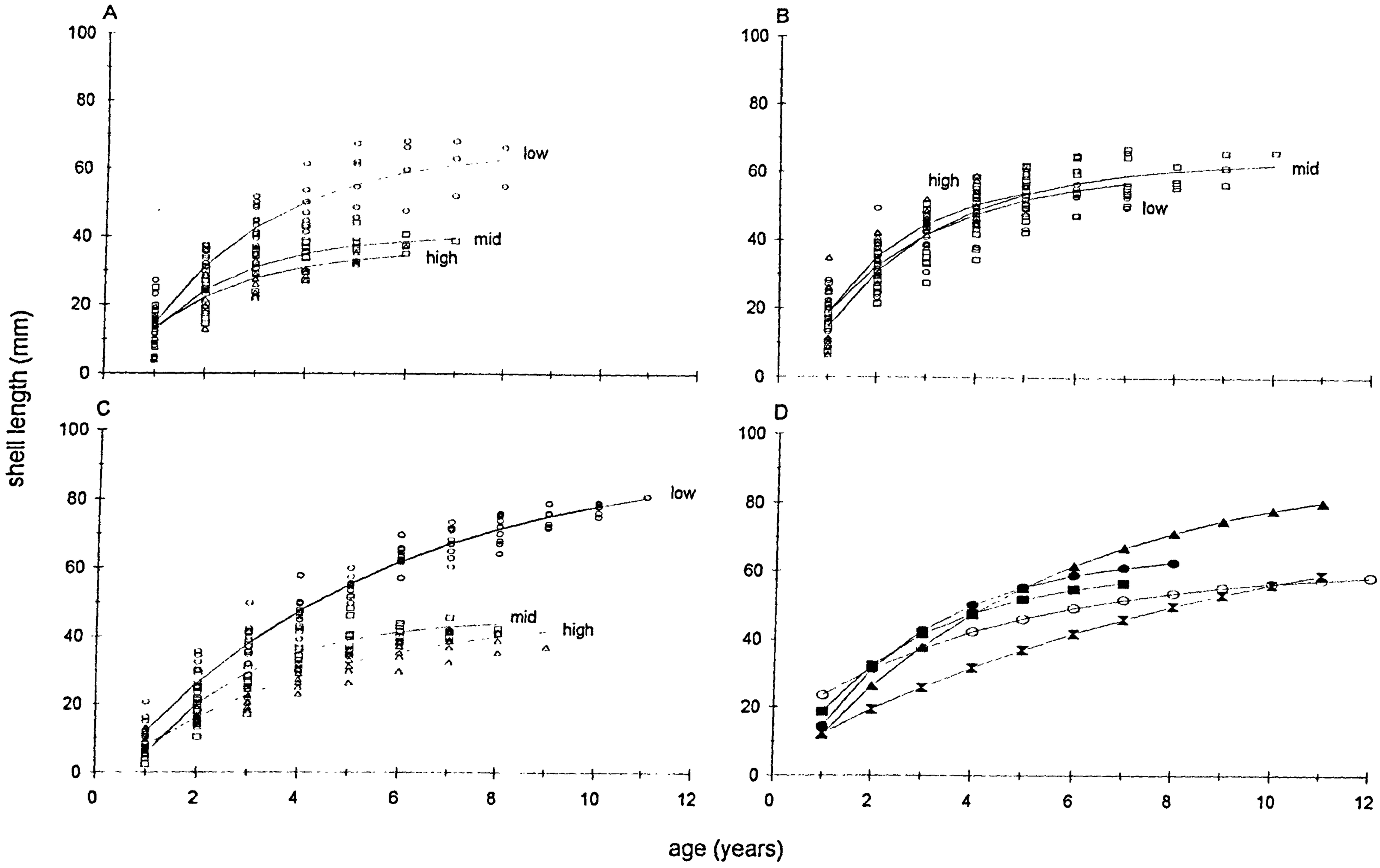
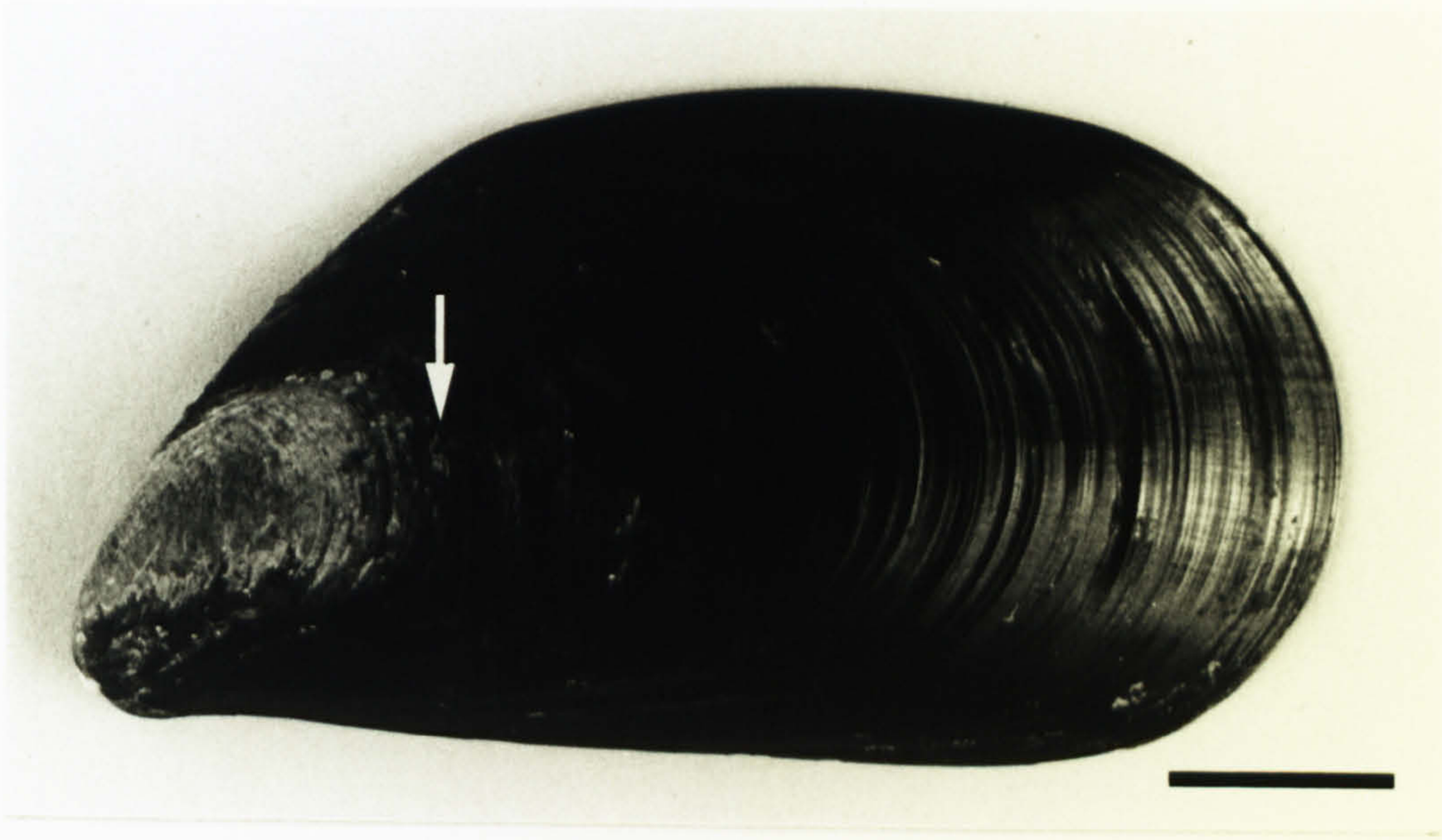

A
B
C
D
low
mid
high
shell length (mm)
age (years)
0
20
40
60
80
100
2
4
6
8
10
12

Plate 4.5

A. Photograph of the shell surface of a high shore *Mytilus edulis chilensis* from Darwin, which was grown for one year in a subtidal cage suspended from the bridge at Darwin. The arrow denotes the time of marking and transplantation. Scale bar = 10 mm.

B. Growth of the natural population of mussels from the high shore at Darwin (closed circles), together with the mean (± 1 standard error) length of mussels subsequently transplanted to subtidal cages at Darwin (open circles), FIPASS in Stanley (open triangles) and the 'Vicar of Brae' at Goose Green (open squares).

A



B

from Darwin and the polynomial estimate ($y = 4.728 + 7.995x - 0.332x^2 + 0.005x^3$, where y = length, mm and x = number of growth bands) from surface ring data for *M.e.chilensis* from Stanley, presented by Davenport *et al.* (1984). The estimated growth curves for the low shore population at Darwin differ considerably according to the method of analysis employed. Estimates from an analysis of the surface rings suggest that the mussels are slow growing and are relatively longer lived, despite depositing the first ring at a slightly larger size than is found when the microgrowth bands are observed. The growth curve of Davenport *et al.* (1984) suggests that mussels are slow growing, more so than the slow growing mussels observed in this study from Darwin. However, the asymptotic length for the mussels examined by Davenport *et al.* (1984) is considerably higher than that predicted for the low shore Darwin population.

Marking experiments carried out to estimate the short term growth of individual *M.e.chilensis* from the natural shore populations, during which mussels were file-marked *in situ* at the growing shell margin were fairly unsuccessful. Even though most mussels that were marked could be identified and collected several months later, the majority of these animals had failed to grow. In most cases the growing edge of the shell had thickened on the inner shell surfaces, but no linear growth could be detected. File-marked and transplanted mussels, on the other hand, showed a clear mark and exhibited considerable linear shell growth. The shell surface of a mussel which was file-marked and transplanted to a subtidal cage deployed at the bridge at Darwin is illustrated in Plate 4.5A. The notch resulting from the file-mark is clearly visible, and the rapid increase in growth following transplantation can be clearly observed. The mussel in Plate 4.5A was in the subtidal cage for 1 year during which time it increased in size from 21.2 mm (at which size it would have been ≈3 years old) to 58.1 mm. Plate 4.5B illustrates the growth rates of the natural population of high shore mussels from Darwin as well as mussels transplanted to the bridge at Darwin, the 'Vicar of Brae' at Goose Green and the flotation jetty, FIPASS, in Stanley harbour. Mussels (initial size ≈28 mm) transplanted to the cages at Darwin and Stanley increased in size dramatically after just 12 months, reaching ≈54 mm and ≈47 mm respectively. Mussels transplanted to the subtidal cage at Goose Green did not grow very well, in fact they grew even less than the natural high shore population at Darwin which were tidally emersed. After 12 months all mussels from the subtidal cage at Goose Green had died, indicating that poor conditions existed at the site which were not suitable for growth in this particular environment.

4.3.1.2. Length frequency distributions

Length frequency histograms of *M.e.chilensis* from the three study populations are shown in Figures 4.5. 4.6 and 4.7. Obvious modes already present within each population as well as newly recruited individuals, were identified and followed where possible over time. Due to the lack of consecutive samples during the first year of sampling (1993 - 1994), the identification of successive cohorts was not possible, therefore this year is not considered further.

Marked recruitment periods into the established population are rather difficult to identify, although mussels ≥ 2 mm in shell length were observed at all three sites during the summer months (December - February) of 1995/6. In the previous summer recruitment was much lower, and recruiting mussels were somewhat larger (2 - 15 mm). A few mussels were also observed recruiting at other times of the year, for example, at Darwin a cohort of relatively large (10 - 15 mm) individuals arrived in August and September 1995, and at Goose Green low numbers of small (5 - 10 mm) mussels started to arrive during September 1995; these were subsequently joined by slightly larger individuals (15 - 20 mm) in November, increasing the overall size of the mode.

Following the separation of the polymodal distributions into individual modes using the method of Bhattacharya, cohorts of relatively small mussels already present, as well as new recruits arriving into the population, could generally be traced throughout the study period. At Darwin the cohort (≈ 18 mm) present in September 1994 and the recruiting cohort in November 1994 could be traced through to October and December 1995, respectively (Figure 4.5, 4.8A). The two relatively large cohorts recruiting in August and December 1995 could be followed until the last sample collected in February 1996. Figure 4.8A shows a plot of the average mussel lengths of recruiting and established modes, with time.

Cohorts within the Camilla Creek population were more difficult to follow (Figure 4.6, 4.8B). The prominent cohort present in the September 1994 sample was successfully followed until February 1996, although new recruits joining the adult population in September 1994 could not be followed after January 1995. In November 1995 a few recruits (10 - 15 mm) appeared within the population, but only in December could this cohort be recognised by the method of Bhattacharya. Further recruitments of yet

Figure 4.5 Length frequency distributions of *Mytilus edulis chilensis* from Darwin. Numbers denote estimated cohorts fitted using the method of Bhattacharya.

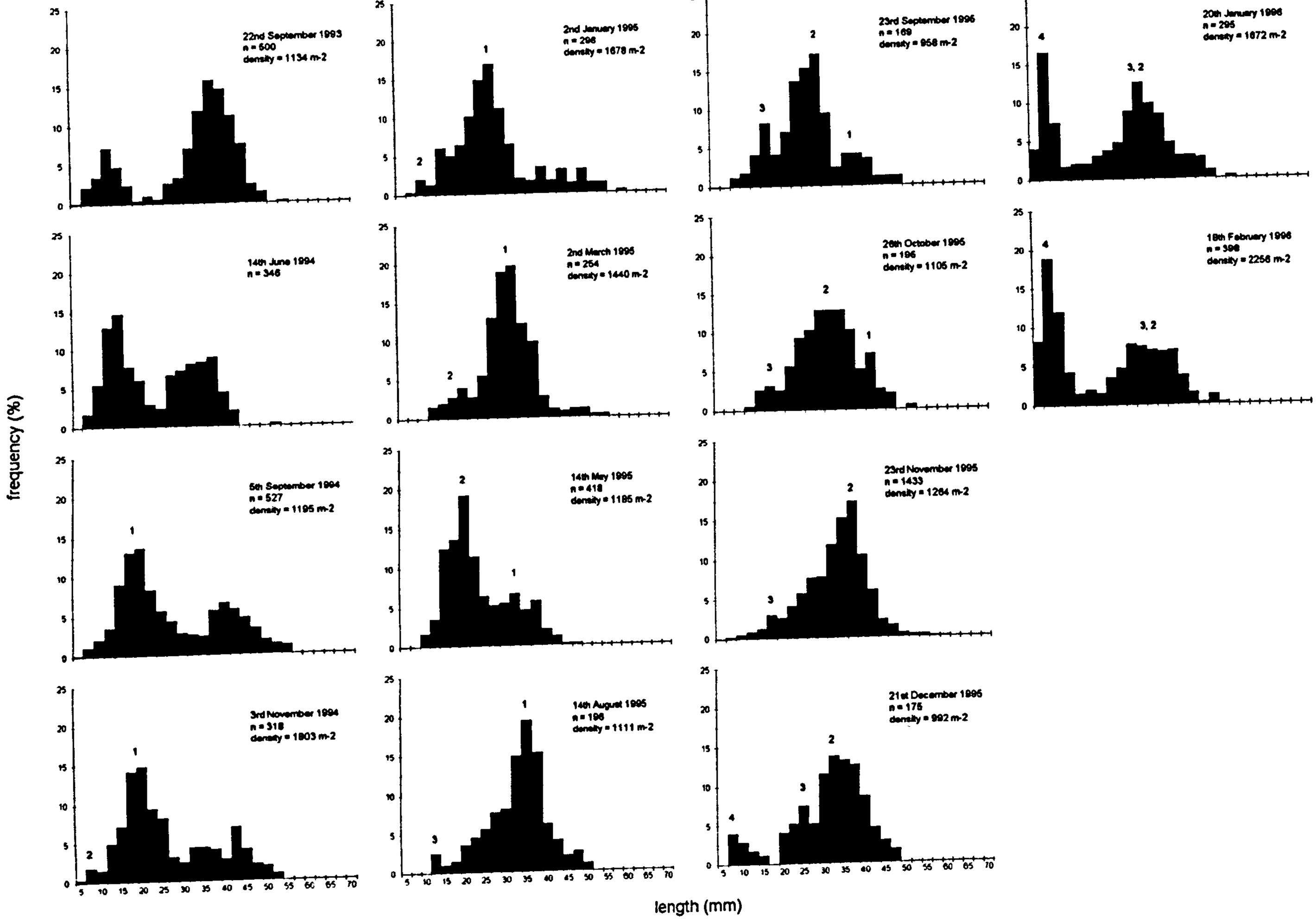

22nd September 1993
n = 600
density = 1134 m-2
2nd January 1995
n = 296
density = 1678 m-2
23rd September 1995
n = 169
density = 958 m-2
20th January 1996
n = 295
density = 1672 m-2
14th June 1994
n = 346
2nd March 1995
n = 254
density = 1440 m-2
26th October 1995
n = 196
density = 1105 m-2
18th February 1996
n = 398
density = 2256 m-2
5th September 1994
n = 527
density = 1195 m-2
14th May 1995
n = 418
density = 1185 m-2
23rd November 1995
n = 1433
density = 1264 m-2
3rd November 1994
n = 318
density = 1803 m-2
14th August 1995
n = 196
density = 1111 m-2
21st December 1995
n = 175
density = 992 m-2
frequency (%)
length (mm)

Figure 4.7 Length frequency distributions of *Mytilus edulis chilensis* from Goose Green. Numbers denote estimated cohorts fitted using the method of Bhattacharya.

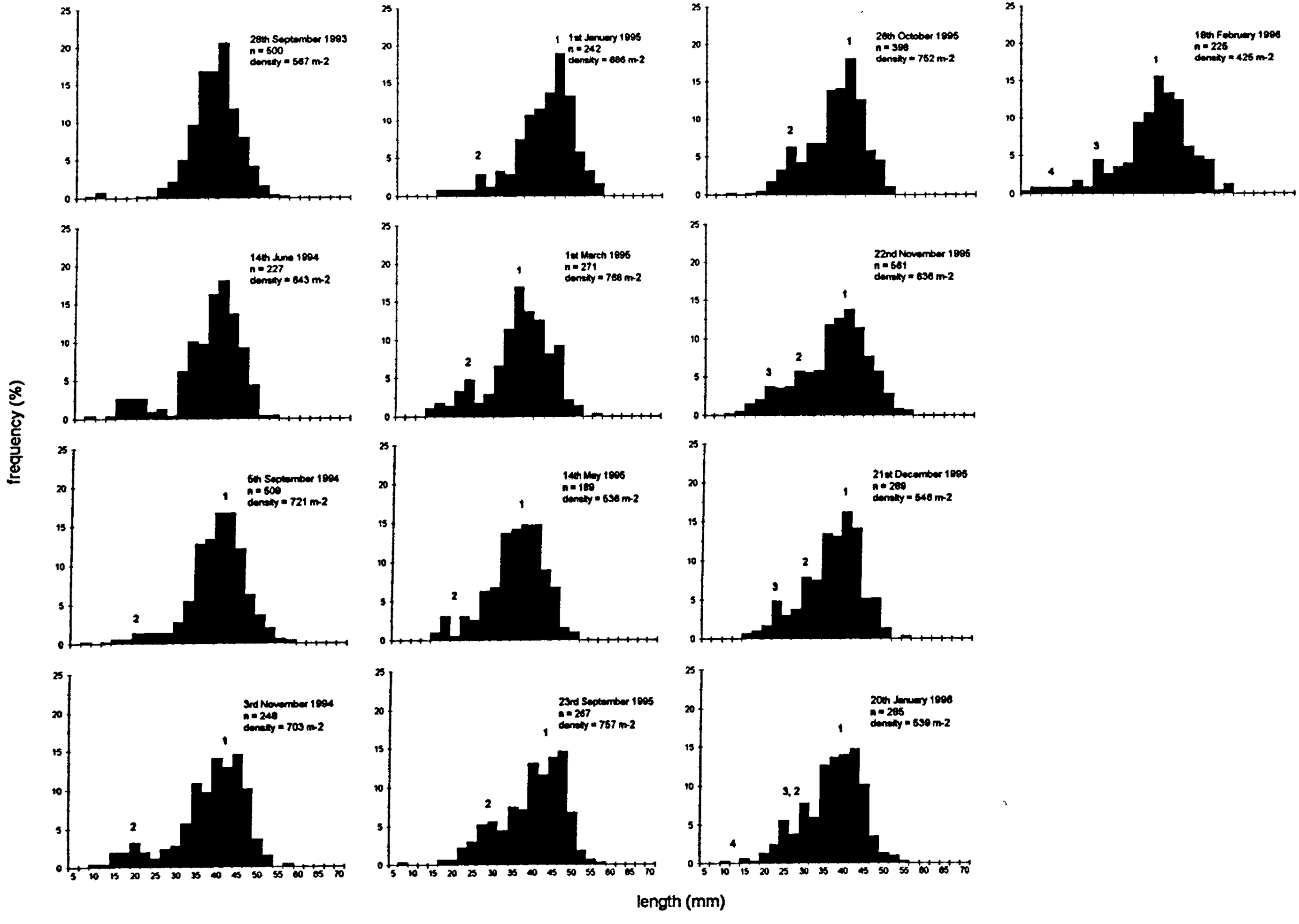
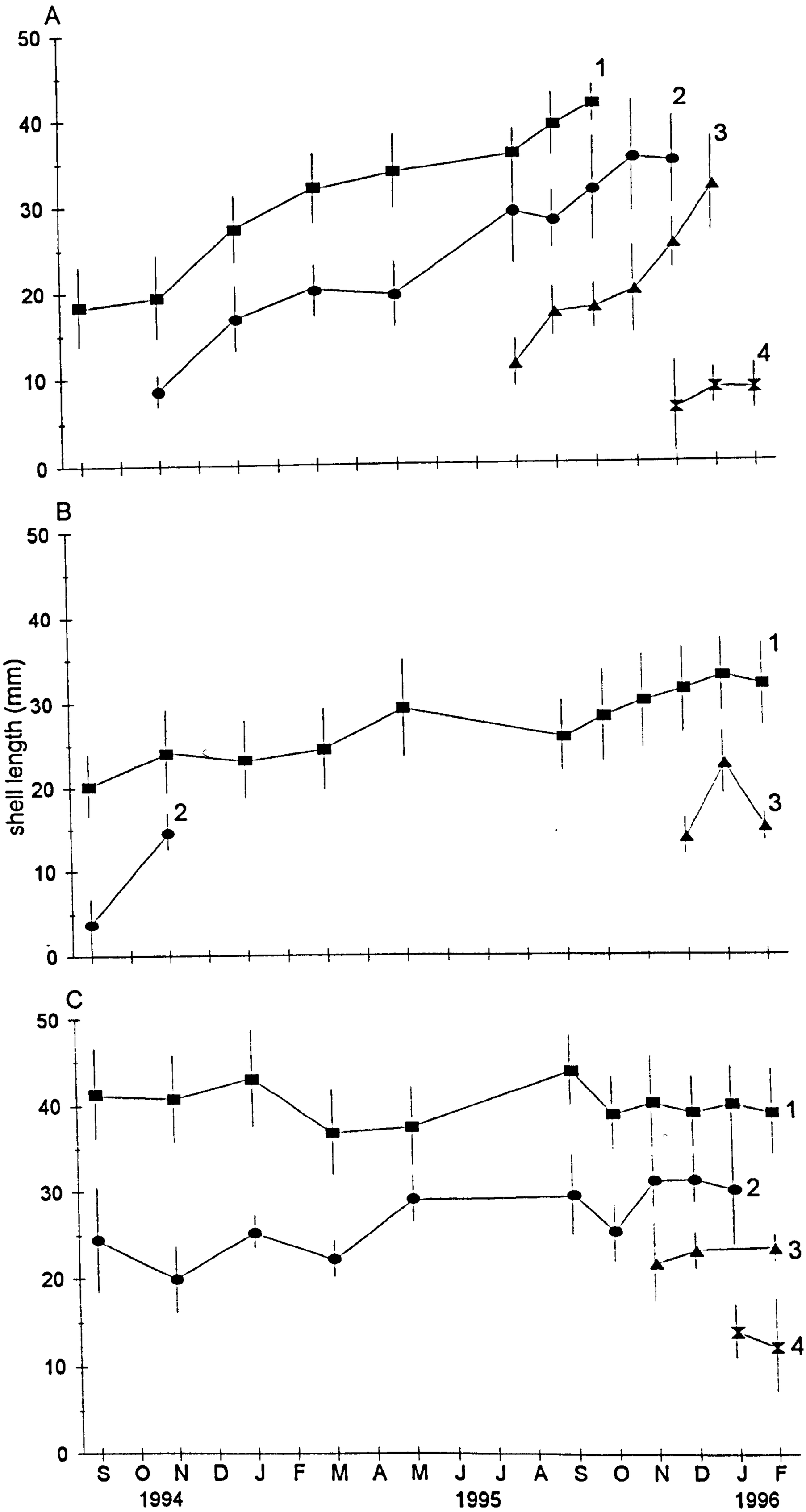

28th September 1993
n = 500
density = 567 m-2
1st January 1995
n = 242
density = 686 m-2
26th October 1995
n = 398
density = 752 m-2
18th February 1996
n = 225
density = 425 m-2
14th June 1994
n = 227
density = 643 m-2
1st March 1995
n = 271
density = 768 m-2
22nd November 1995
n = 561
density = 636 m-2
5th September 1994
n = 509
density = 721 m-2
14th May 1995
n = 189
density = 536 m-2
21st December 1995
n = 269
density = 546 m-2
3rd November 1994
n = 248
density = 703 m-2
23rd September 1995
n = 267
density = 757 m-2
20th January 1996
n = 285
density = 539 m-2
frequency (%)
length (mm)

Figure 4.8 Population growth curves of *Mytilus edulis chilensis* resolved by the Bhattacharya method. Bars represent one standard deviation of the mean size. 1, 2. 3, and 4 represent different cohorts (see Figures 4.5, 4.6 and 4.7).

A. Darwin

B. Camilla Creek

C. Goose Green


A
50
40
30
20
10
0
1
2
3
4
B
50
40
30
20
10
0
shell length (mm)
1
2
3
C
50
40
30
20
10
0
1
2
3
4
S O N D J F M A M J J A S O N D J F
1994
1995
1996

smaller individuals (≈5 mm) were observed in January and February 1996 following the summer spawning, although these recruits were not recognised by Bhattacharya analysis. The average length of the mussels in each cohort is plotted against time in Figure 4.8B.

The cohort of mussels (≈25 mm) and the prominent mode comprising larger individuals (≈37 mm) present in the September 1994 sample from Goose Green were followed until December 1995 and February 1996, respectively, after which it was not possible to separate cohort '2' from subsequent recruits, cohort '3'. (Figure 4.7, 4.8C). Cohort '2' appeared to be joined by further relatively large recruits (15 - 25 mm) during March - May 1995, and a slight decrease in the mean modal size of cohort '2' was observed in October when these slightly different sized mussel groups coalesced. Further recruitment of several larger individuals (≈20 mm) was observed in November 1995 resulting in cohort '3'. However, by January 1996 cohorts '2' and '3' were virtually inseparable. A few small (5 - 10 mm) recruits were observed joining the population in September/October although these were not recognised as a distinct cohort by Bhattacharya and they were subsequently lost in the population. In January/February 1996, further recruitment was observed following the spawning period after which cohort '4' could be identified. Growth curves derived from the mean size of the modal groups are shown in Figure 4.8C.

Estimates of population age and growth rates were made using the progression of individual modes during the study period (Figure 4.8). Modes of *M.e.chilensis* from Darwin grew at rates of between 0.56 and 4.09 mm.month $^{-1}$, depending on which individual cohort was traced. The fastest growth rates were observed amongst the newly recruited cohorts. The maximum number of size (age) classes present in the population at any one time appeared to be three, although the merging of larger size classes suggests that older individuals may in fact be present. At Camilla Creek mussels appear to grow somewhat more slowly at an average rate of 0.67 mm. month $^{-1}$; however this estimate was based on a cohort which was already present in the population rather than the newly recruited modes which were difficult to identify and follow. The rather rapid merging of newly recruited cohorts makes it very difficult to estimate the maximum number of age classes present at Camilla Creek, although at least three year classes could be observed at any one time during the study period. The population at Goose Green exhibited growth rates of between 0.34 and 0.57 mm.month $^{-1}$, the higher rates of growth again being observed in the newly recruited

cohort. Up to three year classes could be observed at any one time, although merging of sizes classes may prevent the separation of the older individuals in the population. With the collection of the last size frequency sample in February 1996, only the most recent arrivals could be clearly observed, even though up to four cohorts may have been present throughout the study period.

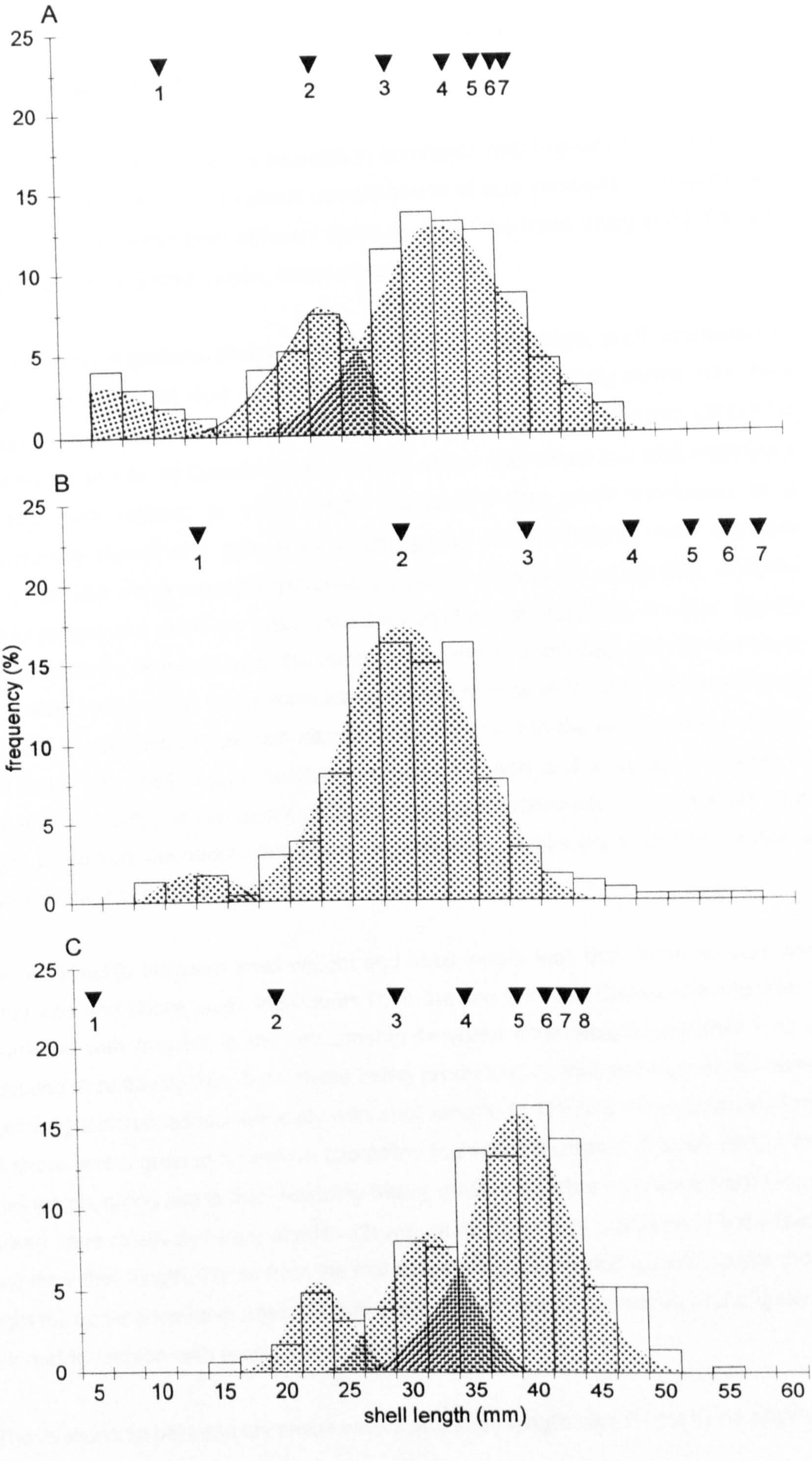
Following the identification of annual growth checks present within the prismatic shell layer of individual mussels from the three study populations (Plate 4.1), age classes estimated from microgrowth band analysis (Table 4.3) and from the modal distributions of length frequency data (Figure 4.8), resolved by the method of Bhattacharya, were compared (Figure 4.9). Considerable discrepancies between the two methods of age determination were evident. Length frequency histograms with superimposed modes predicted by Bhattacharya, provided very different age estimates for mussel populations at Darwin, Camilla Creek and Goose Green, when compared to those estimated using the microgrowth band method. At Darwin the length frequency analyses predicted three year classes of mussels, at 7, 25 and 33 mm in shell length. Examination of internal microgrowth bands, however, revealed the presence of 7 year classes in the same population (Figure 4.9A). The first two year classes broadly coincide, with ≈10% of the population at 1 year old and ≈21% at 2 years. The third length frequency mode appeared to be comprised of 3, 4, 5, 6 and 7 year old mussels, which accounted for ≈69% of the population. The length frequency distribution at Camilla Creek similarly exhibited little agreement with the age estimates derived from internal microgrowth bands. Only the first and second year classes representing ≈3% and ≈88% of the population, respectively, were in agreement; mussels three years and older, ≈3% of the population, occurred in relatively low numbers amongst the larger size classes (Figure 4.8B). The first year class of mussels at Goose Green, ≈5 mm in shell length, was not detected in this length frequency sample. The first, ≈22 mm, and second, ≈32 mm, length frequency modes coincided with the occurrence of two (≈12%) and three (≈20%) year old *M.e.chilensis* as determined from microgrowth band examination. The third and most substantial mode was comprised of 4 - 8 year olds, which accounted for ≈68% of this population (Figure 4.8C).

Figure 4.9 Length frequency distributions of *Mytilus edulis chilensis* together with individual cohorts (stippled) as resolved by the Bhattacharya method, and estimated age in years following microgrowth band analysis (arrowed).

A. Darwin

B. Camilla Creek

C. Goose Green


A
25
20
15
10
5
0
1
2
3
4
5 6 7
B
25
20
15
10
5
0
frequency (%)
1
2
3
4
5
6
7
C
25
20
15
10
5
0
1
2
3
4
5
6 7 8
5
10
15
20
25
30
35
40
45
50
55
60
shell length (mm)

4.3.2. Allometric growth

Tables 4.5, 4.6 and 4.7 show the regression constants and t-values and summarise the relationships between the various combinations of size variables for populations of *Mytilus edulis chilensis* from different shore levels at the three study sites, Darwin, Camilla Creek and Goose Green, respectively.

Shell height was negatively allometric with respect to shell length at all tidal levels at all sites, indicating that shell height increased at a proportionately slower rate than shell length. Shell width, however, exhibited a varied allometric relationship according to tidal level and site. At Darwin the shell width of low shore mussels was negatively allometric with respect to shell length suggesting that width increased at a proportionally slower rate than shell length, giving rise to narrow, more elongate individuals. Mid shore mussels exhibited a positive allometric relationship, whereby width increased at a relatively faster rate than shell length, resulting in wider, slightly elongate mussels. Mussels from the upper region of the zone displayed an isometric relationship, increasing in width at the same relative rate as in length. The populations at Camilla Creek were all positively allometric with respect to the relationship between shell width and shell length, resulting in relatively wide and elongate mussels. At Goose Green only the low shore mussels grew proportionately faster in width than length, those from the middle and upper regions were negatively allometric, resulting in slightly flattened, elongate mussels.

The relationship between shell weight and shell length was also found to vary with study site and shore level. Individuals from the low shore at Darwin are negatively allometric with respect to the relationship between shell weight and shell length, resulting in relatively thin, light shells being produced. At mid and high shore levels shell weight increases isometrically with shell length. At Camilla Creek mussels from all shore levels grew in a positive allometric fashion with regard to shell weight and shell length, giving rise to thick relatively heavy shells. Low shore mussels from Goose Green have relatively heavy shells with weight increasing at a proportionately faster rate than shell length. Those from the mid shore exhibit isometric growth, whilst those from the upper zone have relatively light shells where weight increases in a negatively allometric fashion with respect to shell length.

The relationship between dry tissue weight and shell length was found to be positively

Table 4.5 Allometric relationships for log transformed shell measurements determined for *Mytilus edulis chilensis* from Darwin. Departures from isometry denoted as (+) for positive and (-) for negative allometry.

Zone	Dependent variable[1] (log_{10}) y	Independent variable[2] (log_{10}) x	n	a	b	S.E. of b	r^2	β	t-value	allometry
High	height	length	121	-0.119	0.873	0.018	0.951	1	7.004**	-
	width	length	121	-0.365	0.984	0.025	0.926	1	0.595^{ns}	isometric
	shell weight	length	121	-4.469	3.112	0.063	0.953	3	-1.772^{ns}	isometric
	dry tissue weight	length	121	-5.462	2.849	0.138	0.780	3	1.088^{ns}	isometric
	dry tissue weight	shell weight	121	-1.368	0.908	0.040	0.805	1	2.260*	-
Mid	height	length	660	-0.141	0.891	0.005	0.971	1	18.261**	-
	width	length	660	-0.463	1.048	0.008	0.959	1	-5.316**	+
	shell weight	length	1395	-4.248	2.970	0.017	0.954	3	1.702^{ns}	isometric
	dry tissue weight	length	1395	-5.562	2.992	0.034	0.846	3	0.233^{ns}	isometric
	dry tissue weight	shell weight	1395	-1.269	0.978	0.011	0.838	1	1.844^{ns}	isometric
Low	height	length	169	-0.203	0.934	0.011	0.977	1	5.945**	-
	width	length	169	-0.365	0.923	0.022	0.908	1	3.359**	-
	shell weight	length	169	-4.258	2.862	0.037	0.972	3	3.663**	-
	dry tissue weight	length	169	-5.774	3.116	0.050	0.958	3	-2.321*	+
	dry tissue weight	shell weight	169	-1.132	1.071	0.018	0.954	1	-3.920**	+

[1, 2] the variables x and y in the log transformed allometric equation log y = log a + b.log x. Intercept is denoted by a, β and b (slope of regression line) are the coefficients of isometry and allometry respectively; r^2 is the coefficient of determination; S.E. of b is the standard error of the slope, b; and t - value is the calculated value resulting from the t -test; relationships which depart significantly from isometry, * $p < 0.05$ and ** $p < 0.01$, respectively; [ns] not significant.

Table 4.6 Allometric relationships for log transformed shell measurements determined for *Mytilus edulis chilensis* from Camilla Creek. Departures from isometry denoted as (+) for positive and (-) for negative allometry.

Zone	Dependent variable[1] (log_{10}) y	Independent variable[2] (log_{10}) x	n	a	b	S.E. of b	r^2	β	t-value	allometry
High	height	length	183	-0.189	0.930	0.009	0.981	1	7.189**	-
	width	length	183	-0.528	1.073	0.024	0.915	1	-3.034**	+
	shell weight	length	183	-4.780	3.279	0.044	0.967	3	-6.243**	+
	dry tissue weight	length	183	-5.055	2.625	0.057	0.919	3	6.487**	-
	dry tissue weight	shell weight	183	-1.222	0.777	0.019	0.896	1	11.340**	-
Mid	height	length	690	-0.156	0.905	0.005	0.979	1	18.596**	-
	width	length	690	-0.605	1.110	0.008	0.957	1	-12.335**	+
	shell weight	length	1495	-4.484	3.259	0.018	0.955	3	-14.195**	+
	dry tissue weight	length	1495	-5.051	2.519	0.331	0.794	3	1.447[ns]	isometric
	dry tissue weight	shell weight	1495	-1.304	0.753	0.010	0.791	1	24.536**	-
Low	height	length	210	-0.203	0.928	0.005	0.992	1	12.508**	-
	width	length	210	-0.523	1.038	0.012	0.971	1	-3.122**	+
	shell weight	length	210	-4.734	3.153	0.038	0.970	3	-3.956**	+
	dry tissue weight	length	210	-5.443	2.785	0.0350	0.968	3	6.104**	-
	dry tissue weight	shell weight	210	-1.259	0.857	0.014	0.941	1	9.575**	-

[1, 2] the variables x and y in the log transformed allometric equation log y = log a + b.log x. Intercept is denoted by a, β and b (slope of regression line) are the coefficients of isometry and allometry respectively; r^2 is the coefficient of determination; S.E. of b is the standard error of the slope, b; and t - value is the calculated value resulting from the t -test; relationships which depart significantly from isometry, * $p < 0.05$ and ** $p < 0.01$, respectively; [ns] not significant.

Table 4.7 Allometric relationships for log transformed shell measurements determined for *Mytilus edulis chilensis* from Goose Green. Departures from isometry denoted as (+) for positive and (-) for negative allometry.

Zone	Dependent variable[1] (log_{10}) y	Independent variable[2] (log_{10}) x	n	a	b	S.E. of b	r^2	β	t - value	allometry
High	height	length	129	-0.082	0.854	0.025	0.898	1	5.946**	-
	width	length	129	-0.175	0.876	0.036	0.818	1	3.517**	-
	shell weight	length	129	-3.887	2.789	0.080	0.904	3	2.619**	-
	dry tissue weight	length	129	-5.403	2.916	0.137	0.779	3	0.607ns	isometric
	dry tissue weight	shell weight	129	-1.320	1.002	0.045	0.792	1	-0.050ns	isometric
Mid	height	length	669	-0.098	0.859	0.010	0.914	1	13.727**	-
	width	length	669	-0.331	0.963	0.014	0.869	1	2.487*	-
	shell weight	length	1339	-4.301	3.045	0.024	0.918	3	-1.816ns	isometric
	dry tissue weight	length	1339	-4.851	2.588	0.043	0.722	3	9.382**	-
	dry tissue weight	shell weight	1339	-1.177	0.819	0.013	0.732	1	13.279**	-
Low	height	length	207	-0.080	0.855	0.012	0.957	1	11.438**	-
	width	length	207	-0.686	1.146	0.023	0.920	1	-6.210**	+
	shell weight	length	207	-5.134	3.427	0.050	0.958	3	-8.536**	+
	dry tissue weight	length	207	-5.697	3.053	0.046	0.954	3	-1.149ns	isometric
	dry tissue weight	shell weight	207	-1.107	0.867	0.014	0.945	1	9.063**	-

[1, 2] the variables x and y in the log transformed allometric equation log y = log a + b.log x. Intercept is denoted by a, β and b (slope of regression line) are the coefficients of isometry and allometry respectively; r^2 is the coefficient of determination; S.E. of b is the standard error of the slope, b; and t - value is the calculated value resulting from the t -test; relationships which depart significantly from isometry, * $p < 0.05$ and ** $p < 0.01$, respectively; [ns] not significant.

allometric only in low shore mussels from Darwin, where the overall condition of individual mussels was good. An isometric relationship was observed in mid and high shore mussels from Darwin as well as in individuals from the mid shore at Camilla Creek and the low and high shore at Goose Green. This suggests that body weight increases at the same relative rate as shell length, with overall condition remaining at the same relative level regardless of mussel size. Dry tissue weight was negatively allometric with respect to shell length in mussels from the high and low shore at Camilla Creek and the mid shore at Goose Green, resulting in mussels that are in increasingly poorer condition as they increase in size. Dry tissue weight only increased at a relatively faster rate than shell weight in mussels from the low shore at Darwin. In mid shore mussels at Darwin dry tissue weight and shell weight increased at the same relative rate (isometry), and in the high shore this relationship was one of negative allometry, with dry tissue weight increasing at a relatively slower rate than the shell weight. The poor condition of mussels throughout the population at Camilla Creek was reflected in the consistent negative allometric relationship between dry tissue weight and shell weight. At Goose Green dry tissue weight was negatively allometric with respect to shell weight in mussels at both the lower and mid parts of the shore. Those from the upper region of the mussel bed exhibited an isometric relationship between dry tissue weight and shell weight, with both these variables increasing at the same relative rate.

The above results can be summarised to give an overall description of the shape and appearance of mussels from different shore levels at different sites. Mussels from the low shore at Darwin are relatively elongate, with thin shells and a good body condition relative to shell length and weight; mid shore mussels are wider with slightly heavier shells and a dry tissue weight that increases in proportion to shell length and weight; and those from the high shore are relatively elongate with a slightly reduced body condition. At Camilla Creek all mussels are relatively wide, with heavy shells and an overall poor body condition. Low shore mussels at Goose Green are relatively wide with heavy shells, and thus a relatively low body condition. At the mid shore level mussels are fairly narrow and elongate, with an overall lower body condition relative to shell length and weight. High shore mussels are narrow and elongate, with relatively thin shells and a body condition which increases in proportion to shell length and weight.

A comparison of the slopes and intercepts (Table 4.8 and 4.9) estimated using regression analysis of pairs of size variables, indicated that significant differences in

Table 4.8 A summary of the coefficents of allometry[1] for various combinations of size variables together with mussel zone comparisons[4] for *Mytilus edulis chilensis* from the Falkland Islands

Dependent variable[2] (log_{10}) y	Independent variable[3] (log_{10}) x	Darwin					Camilla Creek					Goose Green				
		low	mid	high	F-values[4] slope	F-values[4] intercept	low	mid	high	F-values[4] slope	F-values[4] intercept	low	mid	high	F-values[4] slope	F-values[4] intercept
Height	Length	0.934*	0.891*	0.873*	4.54†	-	0.928*	0.905*	0.930*	5.06†	-	0.855*	0.859*	0.854*	0.05	7995.96†
Width	Length	0.923*	1.048*	0.984ns	16.09†	-	1.038*	1.110*	1.073*	9.84†	-	1.146*	0.963*	0.876*	36.38†	-
Shell weight	Length	2.862*	2.970ns	3.112ns	4.91†	-	3.153*	3.259*	3.279*	3.58†	-	3.427*	3.045ns	2.789*	38.22†	-
Dry tissue weight	Length	3.116*	2.992ns	2.849ns	1.57	3017.81†	2.785*	2.519ns	2.625*	7.39†	-	3.053ns	2.588*	2.916ns	17.03†	-
Dry tissue weight	Shell weight	1.071*	0.978ns	0.908*	5.81†	-	0.857*	0.753*	0.777*	10.86†	-	0.867*	0.819*	1.002ns	8.38†	-

1 the coefficient b in the allometric equation $y = ax^b$

2,3 the variables y and x respectively in the allometric equation

* relationships which depart significantly from isometry at $p < 0.05$

ns not significant

4 General Linear Modal with covariate; † analysis significant at $p < 0.05$

shell dimensions and soft body tissues between shore level and study sites exist. Almost all, 28 out of 30 comparisons, of the shell dimensions, with shore level and site, showed significant differences ($p < 0.05$) between either the intercepts or slopes of the regression lines.

Table 4.9 F-values from comparisons of allometric relationships between study sites in different zones of the mussel bed

Dependent variable[1] (log_{10}) y	Independent variable[2] (log_{10}) x	Mussel zone: low slope	low intercept	mid slope	mid intercept	high slope	high intercept
Height	Length	19.83*	-	8.40*	-	6.82*	-
Length	Length	24.23*	-	39.81*	-	11.08*	-
Shell weight	Length	27.93*	-	67.19*	-	15.97*	-
Dry tissue weight	Length	18.49*	-	47.96*	-	2.77^{ns}	1911.53*
Dry tissue weight	Shell weight	32.04*	-	102.25*	-	13.13*	-

[1,2] the variables x and y in the log transformed allometric equation log y = log a + b.log x

* General Linear Model with a covariate; analysis significant at $p < 0.05$

ns not significant

4.4 Discussion

Mytilus edulis when grown under intertidal conditions deposited clearly defined growth bands with an almost exact coincidence with the number of emersions (Richardson, 1989). When mussels were continuously immersed the periodicity of band formation was highly correlated with the rate of shell growth, indicating the presence of an innate rhythm which had neither a tidal or daily periodicity. In this study, although there was little correlation between the number of bands observed in the shells of *Mytilus edulis chilensis* and the number of expected tidal emersions, the lack of correlation between the number of bands observed and the increment of growth deposited during the experimental period suggests that band deposition is not endogenously controlled. The poor resolution of the bands in shells grown in the upper zone cages (a result of the slower growth rate), compared to the relatively good resolution of the bands in the

shells of faster growing individuals from the low shore cages, probably contributed to the poor correlation between the expected number and observed number of bands. In slow growing individuals growth bands are deposited close together and are notoriously difficult to distinguish often leading to an underestimation of band numbers, whereas bands deposited during periods of fast growth are typically clear and well defined.

Evidence that band deposition is tidally controlled was provided when the band patterns of relatively fast growing individuals, from the mid - low shore level, were examined (Plate 4.2). A characteristic pattern of weak ill-defined bands deposited during neap tides when the mussels remained continually immersed or are uncovered for a very short period of time, alternating with clear, regular bands resulting from periods of emersion during spring tides, was evident in all *M.e.chilensis* shells examined. Thus *M.e.chilensis* shells are recording evidence of the spring-neap lunar cycle.

The occurrence of an unusually high air temperature during low tide, which was recorded as a clearly defined band in up to 70% of shells examined, provides further evidence that mussels are capable of recording environmental events within their shell structure. Although evidence of changes in environmental conditions such as tidal exposure and temperature have been extensively identified within bivalve shells (Richardson, 1987, 1988, 1989, 1993; Richardson & Walker, 1991; Richardson *et al.*, 1979, 1980, 1981), human disturbances and attacks by predators, as well as spawning breaks and algal blooms are also recorded within the shell structure of bivalves (Richardson *et al.*, 1990a; Richardson, 1993). The ability of bivalves to incorporate a record of environmental change within their shell structure indicates that these shells could be used as environmental chronometers, and could have important potential applications for routine environmental monitoring studies (Richardson *et al.*, 1990a; Richardson, 1993).

Surface growth rings produced during periods of suspended shell growth have previously been used, with varying degrees of success, to determine the age of individual *M.edulis* (Seed & Richardson, 1990). However, periods of suspended shell growth may be associated with seasonal changes in temperature or food availability, prolonged stormy weather, or even with the annual reproductive cycle, and cannot therefore be assumed to be annual in origin (Seed, 1976). Davenport *et al.*, (1984)

estimated the age and growth rate of *M.e.chilensis* obtained from the submerged hull of the 'SS Great Britain' in Stanley Harbour (Falkland Islands), using surface growth rings, which they assumed to be annual in origin. In the present study, surface growth rings over-estimated the age and under-estimated the growth rate of most of the mussels examined. Examination of the microgrowth bands present within the prismatic shell layer of mussels whose surface growth rings were also investigated revealed that many of the surface rings were in fact non-annual. Davenport *et al.* (1984) estimated that *M.e.chilensis* took 7 - 8 years to reach 50 mm in shell length and concluded that mussel growth in the Falkland waters was probably too slow for successful commercial cultivation. Low shore *M.e.chilensis* from Darwin also took 8 years to reach a similar size when surface growth rings were used to age individual mussels. However, when the annual checks present in the prismatic and nacreous layers were examined, these same low shore mussels took just four years to reach ≈ 50 mm. It is not improbable, therefore, that Davenport *et al.* (1984) over-estimated the age, and under-estimated the growth rate of *M.e.chilensis* from the "SS Great Britain" as a result of using surface growth rings. Another possibility is that mussel growth within the hull of the SS Great Britain was unusually slow and therefore not truly representative of mussel growth around the Falklands.

Although the analysis of surface growth rings has been used successfully to determine the age and growth rate of several *M.edulis* populations (Seed, 1969b; 1973; Theisen, 1973), the inherent problems involved in actually quantifying the number of rings, led me to examine the growth patterns within the internal shell structure of *M.e.chilensis* during the present study. Lutz (1976) examined the growth lines present within the nacreous shell layer of *M.edulis* and concluded that these lines had an annual periodicity, being deposited during late spring. In this study the highly significant correlation between the age determined by nacreous lines and that determined from examination of the winter narrowing of the tidal microgrowth bands within the prismatic shell layer suggested that the nacreous lines present within the shell of *M.e.chilensis* were also annual in origin.

Mussels can only feed when immersed, and since food supply is thought to be the single most important factor in determining growth rate (Wallace, 1980; Loo & Rosenberg, 1983; Rodhouse *et al.*, 1984a; Page & Hubbard, 1987; Page & Ricard, 1990), animals located in the lower reaches of the shore might be expected to exhibit the fastest growth rates. Baird (1966) observed increasing growth rates with

decreasing tidal elevation, and thus aerial exposure, in *M.edulis* from the Menai Strait, North Wales. The point of zero growth, ie. the point at which the energy required for metabolism during aerial exposure equals that available during the feeding period, was found to occur at ≈ 55% aerial exposure, the upper limit of the natural population. Seed (1969b) observed a similar trend in growth rates of *M.edulis* on the exposed rocky east coast of England, but found that the point of zero growth was at ≈ 75% aerial exposure, a result of the relatively wider splash zone, within which mussels could survive and grow, albeit very slowly. Wilson (1977) also found that *M.edulis* from low shore sites had considerably higher growth rates than those from the middle of the shore at Carlingford Lough, Northern Ireland. More recently, four mussel species from South Africa were shown to exhibit progressively retarded rates of growth with increasing levels of tidal exposure (van Erkom Schurink & Griffiths, 1993).

Following the determination of growth rates of *M.e.chilensis* from the three geographically close study sites in the Falkland Islands, a clear trend in growth rates with tidal elevation was identified. Mussels from Darwin and Goose Green both exhibited increasing growth rates with decreasing tidal elevation, whilst at Camilla Creek growth rates were similar regardless of tidal elevation. The tidal exposure times experienced by *M.e.chilensis* from Darwin and Goose Green ranged from 18 to 28 % in the low region of the mussel bed, to between 59 and 63 % in the upper region. At Camilla Creek there was very little difference between exposure times for mussels from the low region, ≈ 40%, and those from the upper, 55%, despite being separated by up to 50 m (Chapter 2). Thus feeding time appears to be the main factor controlling growth rate in *M.e.chilensis* populations. Mussels from the upper limit of the zone experience aerial exposure times of between 55 and 63 %, indicating that the point of zero growth is similar to that found in other *Mytilus* populations. It is curious that growth rates of Camilla Creek mussels are considerably higher than those found at sites with similar aerial exposure times. As mentioned in Chapter 2, however, there is considerable silt deposition at Camilla Creek, but not at the other two sites.

Although previous studies have shown that increased levels of silt can have a detrimental effect upon both body condition and plantigrade settlement of *M.edulis* (Bayne, 1964; Dare, 1976; Igic, 1988; Beatty & Aldrich, 1989; McGrorty *et al.*, 1990; McGrorty & Goss-Custard, 1991;1993), other workers have found that the presence of silt can increase ingestion rates and growth in *M.edulis*. Winter (1976) for example, found that when increasingly higher quantities of silt (2.5 - 100 $mg.l^{-1}$) were presented

to *M.edulis* in addition to algal cells, a marked increase in shell weight was observed. This increase was thought to be the result of a substance present within the silt suspension which is necessary for shell formation. Kiørboe *et al.* (1981) similarly observed increased growth and ingestion when silt levels of ≈ 5 $mg.l^{-1}$ were added to water containing *M.edulis*. The presence of silt at Camilla Creek may thus be a contributing factor to the increased growth rates when compared to sites where silt is absent. It is not improbable, however, that the quality and/or quantity of food may be different between the sites. Several authors have identified seasonal and regional variations in the quantity and quality of utilizable food as important determinants of mussel growth. Ceccherelli & Rossi (1984) and Frechette & Bourget (1985) both illustrated how low quality and quantity of food severely affected the growth of *Mytilus*. Although shell growth is relatively fast in mussels from Camilla Creek, tissue growth is poor (Chapter 3), indicating that shell growth is achieved at the expense of tissue growth due either, to low food supply, the high levels of silt or the presence of *E.doellojuradoi*. However, routine water samples are required in order to determine whether or not food availability is responsible for these contrasting growth rates.

Seed (1969b) found that high mortalities occurred in mid and low shore populations of *M.edulis* from the east coast of England, and that the absence of major predators in the upper shore populations resulted in enhanced survival and therefore the occurrence of relatively old individuals, despite the relatively slow growth rates at these tidal levels. Populations in the low shore consisted of mussels under 3 years old, whilst high shore populations often contained twenty or more year classes. *Mytilus edulis chilensis* does not appear to exhibit such a marked trend in longevity with shore level. In fact, populations from the lower reaches of the shore tended to contain some of the oldest individuals. This suggests that mortality was broadly similar in all zones of the mussel bed. During the course of the sampling period the only predators observed on the mussel beds were oystercatchers, which tended to forage at the waters edge, moving with the flood and ebb of the tide. Overall the populations appeared to be relatively long-lived reaching maximum ages of 11 years in the low region of the mussel zone at Goose Green. Several workers have suggested that faster growing mussels may be less long-lived because they will attain the size limit imposed by the environment much more rapidly than those living in habitats where growth rates are much slower (Seed & Suchanek, 1992). When the growth rates and maximum ages of *M.e.chilensis* were compared some of the populations which contained longer-living individuals did in fact exhibit the lowest growth rates. For

example, mussels from the low shore at Goose Green lived for up to 11 years and had k values (von Bertalanffy growth rate constant) of only 0.198, whilst low shore mussels from Darwin lived for up to 8 years and had considerably higher k values (0.396).

Although some authors prefer to use the Gompertz growth equation to describe the growth of *M.edulis* (Bayne & Worrall, 1980), the von Bertalanffy growth equation has been applied far more extensively (see Seed & Suchanek, 1992 and references therein). Size at age data for *M.e.chilensis* were found to be suitable for analysis using the von Bertalanffy growth model, and the resulting L_∞ and k values were noted for comparative purposes. Davenport *et al.* (1984) determined L_∞ and k values of 78 mm and 0.88, respectively, for a subtidal population of *M.e.chilensis* in Stanley harbour (within the submerged hull of the SS Great Britain), but subsequently suggested that the von Bertalanffy growth model may not be a suitable expression for these data which the authors concluded were indeterminate. Using a polynomial growth equation Davenport *et al.* (1984) found that *M.e.chilensis* took approximately 7 - 8 years to reach 50 mm in shell length. As discussed previously, low shore *M.e.chilensis* in the present study grew at considerably faster rates than in the Stanley population, reaching ≈ 50 mm in 4 years at Darwin. The somewhat contrasting results may in part be explained by the use of surface growth rings by Davenport *et al.* (1984), whereby relatively retarded growth rates are determined in a population which does not appear to have an asymptotic size. However, the fact that only large individuals were examined during the present study may have resulted in an over-estimation of population growth rates. Other workers investigating the growth of both commercial and natural populations of *M.edulis*, have noted a wide range of L_∞ and k values (Table 4.10). Kautsky (1982b) found that natural subtidal populations of Baltic *M.edulis* had an asymptotic length of just 32 mm. Sukhotin and Kulakowski (1992) found that the asymptotic length of *M.edulis* populations in the White Sea increased from 33.4 mm to 53.7 mm and the rate at which L_∞ was approached decreased slightly, with decreasing tidal elevation. Commercial populations may exhibit relatively variable L_∞ values, but values of k tend to be high. Dare and Edwards (1976) observed k values of up to 1.02 in *M.edulis* on open plots near low water spring tide mark in the Menai Strait, North Wales. Whilst Dare (1976), observed k values of 0.81 in mussels from a similar tidal level in Morecambe Bay, England.

Growth rate is one of the most important factors determining the suitability of mussel populations for commercial exploitation (Hickman, 1992). Some of the fastest growth

can be observed in cultured populations, particularly those grown on longlines or rafts.

Table 4.10 Values of L_∞ and k for some populations of *Mytilus edulis*

Locality	L_∞ (mm)	k	Authority	Comments
Danish Wadden Sea	77.6	0.561	Thiesen (1968)	Mussels at LWST
Conwy, North Wales	72.7	0.343	Theisen (1968) based on data in Savage (1956)	'Bank mussels'
Greenland	74.4	0.393	Theisen (1973)	'Channel mussels'
	77.5 - 283.9	0.022-0.162		Mussels from 12 different sites
Morecambe Bay, England	62.5	0.810	Dare (1976)	Mussels near LWST
Menai Strait, North Wales	61.8	1.020	Dare & Edwards (1976)	Mussels near LWST (open plots)
Plymouth, England	81.3-93.8	0.222-0.237	Bayne & Worrall (1980)	Between mean LWST and LWNT
Baltic Sea	31.0		Kautsky (1982b)	Subtidal
SS 'Great Britain', Falkland Islands	78.0	0.880	Davenport *et al.* (1984)	Subtidal
Killary Harbour,	89.0	0.139	Rodhouse *et al.* (1984a)	0% exposure
Ireland	69.0	0.152		13% exposure
	67.0	0.972		33% exposure
Gas platform/oil rig,	75.8	0.385	Richardson *et al.* (1990b)	Mussels at MLW
North Sea	70.5	0.345		Subtidal
White Sea	77.1	0.140	Sukhotin & Kulakowski (1992)	Subtidal
	53.7	0.182		low intertidal
	54.8	0.116		mid intertidal
	33.4	0.205		high intertidal

In China, *M.edulis* reach 70 - 80 mm in just 12 months when grown on longlines (Nie, 1991), whilst in Norway longline populations may take 3 years to reach 46 mm (Wallace, 1983). *Mytilus chilensis* in Chile grew to ≈ 54 mm after 14 months in raft cultures (Winter *et al.*, 1984). Although *M.e.chilensis* in the present study achieve sizes suitable for commercial exploitation (> 50 mm), it takes up to 4 years in low shore populations to reach these sizes. Nevertheless, *M.e.chilensis* which were transplanted from relatively slow growing high shore populations into subtidal cages

generally exhibited an increase in growth rate. Individual mussels transplanted to a subtidal cage at Darwin increased by as much as 36 mm in 12 months, after reaching only 22 mm in 3 years prior to transplantation. Such favourable growth rates suggest that the potential for mussel cultivation in the Falkland Islands is good.

Several transplantation experiments in which mussels from relatively poor growing conditions have been placed in more favourable, generally subtidal sites, have documented greatly increased growth rates. Seed (1968) found that when mid and high shore *M.edulis* from the east coast of England were transplanted onto rafts in submerged conditions, growth rates increased dramatically. More recently Kautsky *et al.* (1990) observed that relatively small Baltic *M.edulis* which were transplanted to the North Sea, rapidly increased in size, such that the final shell length was similar to that observed in North Sea mussels. Stirling and Okumus (1994) found that *M.edulis* transplanted between two Scottish Lochs which contained mussel populations with significantly different growth rates, rapidly approached the size imposed by the environment when transplanted to the loch in which the highest growth rates were observed.

Increased growth rates were observed in *M.e.chilensis* which were transplanted to subtidal cages deployed at Darwin and Stanley, whilst mussels transplanted to the cage at Goose Green decreased in growth rate and had all died one year after transplantation. Attempts to identify the possible causes of discrepancies between growth rates of mussels from different cages are documented in Chapter 2. The unreliability of the chlorophyll *a* data together with the lack of any significant differences between the quantity of total suspended solids in the water at Darwin and Goose Green provided little information regarding food supply. However, there were significant differences in water flow at the two sites. The tidal current reached speeds of up to 0.35 $m.sec^{-1}$ at Darwin, whereas at Goose Green no water movement could be detected by a flow meter placed in the water surrounding the mussels. Current speed/water circulation are known to have a significant effect upon the growth of bivalves. As early as 1949 Kerswill reported increased rates of growth in bivalves from areas of enhanced water circulation. Walne (1972) later found that *M.edulis* exhibited significant shell growth when exposed to increased water flow, even though tissue growth was negligible. The lack of tissue growth was thought to be a result of insufficient food in the water. Bayne *et al.* (1983) observed that mussels can buffer their shell growth during short term temporal variations in food availability by utilizing

glycogen reserves accumulated prior to and during gametogenesis. More recently van Erkon Schurink and Griffiths (1993) found that four South African mussel species all grew more rapidly at sites of higher water circulation, regardless of tidal elevation. Increased water flow probably results in an overall increase in food availability for the mussels even if the actual static concentration is relatively low. Routine water samples would be required in order to assess the full extent of food availability at the study sites in the Falkland Islands.

The use of length frequency distributions for determining the age and growth rate of populations is usually confined to those species or populations in which distinct seasonal recruitment and relatively uniform growth rates amongst individuals in each year class, occur (Cerrato, 1980). Although recruitment has often been found to lack seasonality and individual growth rates have been shown to vary considerably in many populations of *Mytilus edulis* (Böetius, 1962; Seed, 1969b; Dare, 1976; Kautsky, 1982b), several workers have successfully used size frequency analysis to estimate the growth rates of mussel populations (eg Thiesen, 1968; Bayne & Worrall, 1980).

Mytilus edulis chilensis in the Falkland Islands develop gametes in spring and have a single spawning period during the summer months (see Chapter 3). Distinct spatfalls occur onto artificial substrate units adjacent to the adult population in the weeks following the spawning period. However, recruitment of individuals ranging between 2 and 20 mm in shell length onto the adult population occurred in relatively low numbers and was not always seasonal. Nevertheless, four cohorts at Darwin and Goose Green and three at Camilla Creek were followed successfully for varying periods of time during the study period. Böetius (1962) followed successfully the modal progression of an unusually heavy spatfall of *M.edulis* in Copenhagen, though variable growth rates and subsequent settlement resulted in the loss of the identity of this cohort after just one year. Seed (1969b), similarly managed to follow the growth of a major settlement of mussels at Filey Brigg on the east coast of England for a period of three years before variable individual growth rates combined with further settlement resulted in the merging of individual size classes. Seed (1969b) also observed that whilst maximum periods of settlement onto the adult beds (secondary settlement) occurred 8 - 10 weeks after spawning, the arrival of mussels from extensive "reservoirs" of temporary attachment (sites of primary settlement), occurred sporadically throughout the year, and the size of these arrivals was highly variable. Dare (1976) studying populations of *M.edulis* in Morecambe Bay, England, similarly

found that secondary settlement of young mussels into the adult population was not easily predicted from a knowledge of the spawning cycle alone. The virtual absence of intertidal settlement derived from the main spawning period and the relatively high winter settlement which occurred long after the end of spawning suggested that factors other than the spawning cycle were influencing settlement in Morecambe Bay. Dare (1976) went on to suggest that these winter settlements may be the result of late autumn spawned plantigrades which had over-wintered on filamentous substrates in the Irish Sea, and which were subsequently released by storms and die-back of algae. Kautsky (1982b), after unsuccessfully identifying cohorts within the length frequency distribution of Baltic *M.edulis*, suggested that the consistently skewed (in favour of small < 5 mm mussels) population structure was a result of large numbers of competitively suppressed small mussels. Furthermore, any mortality within the established population would immediately result in a competitively suppressed mussel taking its place, effectively resulting in "recruitment" throughout the year, thus stabilizing the population and keeping it at the carrying capacity of the area with regard to food and space availability. It is likely that recruitment of *M.e.chilensis* at the three study sites is a combination of settlement from the immediately previous spawning period and also from individuals that have over-wintered on filamentous substrata away from the adult population. The relatively large size range of mussels recruiting at times other than immediately after the spawning period may thus be explained by a period of growth on some filamentous substrata, probably subtidal.

Bayne (1964) found that mussels settling into the adult population in the Menai Strait, North Wales, had only reached 1 - 1.5 mm in shell length approximately 6 weeks after the adults had spawned. *Mytilus edulis chilensis* settling onto the adult beds during December - February 1995/6 (approximately 6 weeks after spawning) ranged between 2 and 15 mm in shell length, considerably larger than that expected if growth rates were assumed to be ≈ 25 $\mu m.day^{-1}$ at mean tide level (Bayne, 1964). It is not improbable, however, that *M.e.chilensis* may have exhibited faster growth rates which could explain the relatively larger sizes of the settling mussels.

Growth rates calculated from the modal progression of cohorts of *M.e.chilensis* indicate that the fastest growth occurred in mussels from Darwin, such that newly recruited mussels (≈ 10 mm) may increase in length by up to 23 mm in the first year of growth. Recruits at Goose Green (15 - 20 mm) appeared to grow much slower (0.57 $mm.month^{-1}$). When growth checks present in the shell structure of *M.e.chilensis*

were observed, the size at the time of formation of the first winter check was found to be at 13 and 6 mm at Darwin and Goose Green respectively. This suggests that some mussels, for example cohort '2' at Darwin (Figure 4.8A), have recruited onto the adult population prior to the austral winter during which they deposited their first growth check; others, for example recruits at Goose Green, have wintered and deposited a growth check for at least one year somewhere other than on the adult population. Recruits at Camilla Creek also appear to have over-wintered elsewhere for at least one year, as the size of the first winter check is ≈ 15 mm and recruits of a similar size were observed in December 1995. The growth rates determined from the Bhattacharya modal progression analysis were considerably higher than those estimated from annual growth checks. Typically, however, new recruits reduce the mean population size resulting in an apparent reduction in population growth rates (Böetius, 1962; Seed, 1969b).

Although previous studies have concluded that length frequency analysis does not provide adequate information regarding the age and growth rate of some *M.edulis* populations (Seed, 1969b; Kautsky, 1982b), there is almost no literature, apart from Richardson *et al.* (1990b), in which the merging of size classes has been verified using a reliable age determination technique such as the analysis of microgrowth patterns within the prismatic layer. There, the authors concluded that length frequency distributions for *M.edulis* from offshore production platforms were of little use in assessing the age of mussels, since the analysis of winter growth checks within the prismatic shell layer revealed that only the first age class was supported by the presence of a distinct mode within the population, and all subsequent age classes were impossible to separate, again due to the variability in individual growth rates. In the present study, age classes as determined from winter growth checks were only represented by obvious modes in the first three years. At Darwin the first and second year classes have clear modes within the length frequency distributions, but animals three years and over were represented by a single, large cohort comprising several overlapping age classes. At Camilla Creek the first and second year classes are represented by obvious cohorts, but animals three years and over are very few and no distinct mode could be identified using the Bhattacharya method. No one year old mussels were present at Goose Green, perhaps further confirming that the small mussels spend the first year of life elsewhere. The second and third year classes were represented by obvious modes, but mussels four years and above were again represented by a single large mode. It is curious that in the populations at Darwin and

Goose Green merging of the older age classes results in a prominent, broad mode, whilst at Camilla Creek, these large, older individuals are particularly sparse. The main cohort present at Camilla Creek appears to consist of two year old mussels, suggesting either that there was an extremely good recruitment two years previously, or that some error is involved. Since only larger animals were selected for growth band analysis, some degree of error may have been introduced, consequently the prominent cohort present at Camilla Creek may in fact contain some relatively small, yet old mussels; only by analysing the growth bands in some of these smaller individuals could this have been confirmed. Thus, in conclusion, length frequency distributions have limited application for the determination of age and growth rates of *M.e.chilensis*, apart from the first 2 - 3 years following recruitment. Variable individual growth rates and sporadic recruitment are probably the main factors which result in the merging of size classes.

The allometric growth of field populations of *M.edulis* is known to exhibit marked variation. Seed (1968) attempted to explain some of these variations in shell morphology by identifying some key controlling factors. He observed that variation in shell form within any given population could be attributed to differences in age, old mussels having proportionately heavier shells, where width often exceeds shell height. Thus, the age structure of a population will have a potentially marked effect upon shell morphology. Growth rate and density also had a marked influence upon shell morphology. Fast growing mussels in relatively densely packed populations often had a more elongate form due to high physical compression from neighbouring mussels, whereas slow growing low density populations tended to be more triangular as a result of low compression. Brown *et al.* (1976) went on to show that in intertidal *M.edulis* shell weight is typically isometrically related to dry tissue weight, whereas in subtidal *Modiolus modiolus* populations dry tissue weight increased at a relatively faster rate than shell weight. The authors hypothesised that this was probably a result of the sheltered subtidal conditions compared to the more exposed intertidal conditions in which *M.edulis* were found.

The populations of *M.e.chilensis* observed during the present study exhibited a wide range of allometric relationships between shell and tissue parameters. In populations where mussels were relatively long lived, for example in the low shore at Goose Green, shells were generally heavy and relatively wide. At Camilla Creek both shell length and shell weight increased at a significantly faster rate than dry tissue weight

regardless of shore level. However, these mussels were not especially long-lived with maximum ages ranging between 5 and 10 years. Some other factor may therefore be responsible for these generally heavier shells and poor body condition. As previously mentioned, Kiørboe *et al.* (1981) suggested that increased shell growth in *M.edulis* when exposed to silt levels of ≈ 5 $mg.l^{-1}$ could be the result of some stimulation of shell growth induced by the silt. Poor body condition in mussels from Camilla Creek is possibly a result of food availability, the high levels of silt and/or the presence of *Edotia doellojuradoi*, which is known to have a detrimental effect upon the reproductive condition of those individuals infested by this valviferan isopod (see Chapter 5). The ability of *M.edulis* to buffer shell growth during short term temporal variations in food by utilising stored nutrients (Bayne *et al.*, 1983) may also contribute to these allometric relationships.

Mytilus edulis chilensis from the low shore at Darwin were exceptional in their relationship between dry tissue weight and both shell length and shell weight, whereby a significant positive allometric relationship was observed, indicating relatively high body condition. However these mussels are not as long-lived as their low shore counterparts from Goose Green and do not have particularly wide or heavy shells. High shore mussels, which have the shortest feeding times, from both Darwin and Goose Green were relatively elongate with thin shells and poor body condition, indicating that food supply is a principal factor influencing shell morphology.

Seed (1968) observed that along with increased growth rates, mussels transplanted to more favourable conditions also showed marked changes in their relative shell proportions, for example, individuals exhibited a more rapid increase in shell height relative to shell length following transplantation. Transplantation experiments carried out on several *M.edulis* populations indicated that variation in shell morphology is essentially phenotypic (Seed, 1968). Recently, however, electrophoretic techniques have revealed that genetic differentiation might account for differences in growth rate and morphological features in several mussel populations, as for example in the Canadian Maritimes (Gartner-Kepkay *et al.*, 1980), in eastern North America (Koehn *et al.*, 1984) and between the North and Baltic Seas (Johannesson *et al.*, 1990; Kautsky *et al.*, 1990). Stirling and Okumus (1994) found that the growth rates of transplanted *M.edulis* populations were controlled, at least partially, by environmental factors such as chlorophyll *a* levels and currents, but that persistent morphological differences between populations were possibly a result of genetic variation.

Overall allometric growth in populations of *M.e.chilensis* from the Falkland Islands varies considerably with both shore level and site. These variations appear to be under the control of factors such as age and food quantity and/or quality, although genetic control cannot be entirely dismissed. Further transplantation experiments and measurements of food availability are required in order to determine the major controlling factors.

4.4.1. Conclusions

The deposition of microgrowth bands within the prismatic shell layer of *Mytilus edulis chilensis* appears to be regulated by the spring-neap lunar cycle, which occasionally reflects the diurnal component of the mixed semi-diurnal regime of the area. The lack of correlation between the number of bands observed and the expected number of tidal emersions was the result of low resolution of the growth banding, caused by slow growth.

Surface growth rings were unsuitable for determining the age and growth rate of individual mussels. The presence of non-annual disturbance rings resulted in the over-estimation of age and the under-estimation of growth rates. Growth lines present within the inner nacreous layer and umbone were annual in origin.

Mussel growth increases with decreasing tidal elevation, a direct consequence of immersion and hence feeding time. Longevity of *M.e.chilensis* exhibited no relation with tidal elevation and the oldest mussel (11 years old) was observed in the low shore population at Goose Green. Longer-living populations generally exhibited lower k values than those that were shorter-lived. The point of zero growth occurred at between 55 and 63% aerial exposure, similar to that documented for populations of *M.edulis* elsewhere. The von Bertalanffy growth model was suitable for describing the growth of *M.e.chilensis* populations with L_∞ and k values generally increasing and decreasing, respectively, with decreasing tidal elevation. *Mytilus edulis chilensis* transplanted to favourable conditions in submerged cages exhibited growth rates which would be acceptable for mussel cultivation.

The use of population length frequency distributions had limited applications in determining the age and growth rate of *M.e.chilensis* populations largely as a result of sporadic recruitment and variable individual growth rates. Although the method of

Bhattacharya successfully resolved length frequency distributions into individual modes, when these separated modes were compared to the size at age estimated from prismatic winter growth checks, only the first and second year classes were in close agreement. Mussels 3 years and older were generally represented by a single large mode within the length frequency distribution. Growth rates estimated by following the progression of individual modes were consistently higher than those estimated by analysis of winter growth checks. Comparisons of size at age using length frequency distributions and prismatic winter growth checks confirmed that some juvenile mussels must overwinter away from the established populations in their first year.

Various allometric relationships were exhibited by the populations of *M.e.chilensis* from different tidal levels and study sites. Mussel age and food supply appear to be the principal controlling factors determining shell morphology and the allometric relationships between the different growth variables, although control by genotype cannot be discounted.

Chapter 5

Ecological relationships between the valviferan isopod *Edotia doellojuradoi* Giambiagi, 1925, and its host *Mytilus edulis chilensis*

5.1. Introduction

Routine monthly samples revealed the presence of a valviferan isopod living within the mantle cavity of *Mytilus edulis chilensis*. Although marine bivalve molluscs are used extensively as hosts for a wide range of commensal and symbiotic organisms, such as copepods, brachyuran crabs and polychaetes (for reviews see Cheng, 1967 and Bower, 1992) there is a paucity of information on the association between marine bivalves and isopods. Wagele (1991) described *Edotia doellojuradoi* within mytilids from Tierra del Fuego and Jaramillo *et al.* (1981) and Gonzalez & Jaramillo (1991) documented the presence of *Edotia magellanica* in the mantle cavity of both the mussel, *Mytilus chilensis*, and the clam, *Mulinia edulis*, from the Strait of Magellan and the Lingue and Queule estuaries (Southern Chile), respectively. The valviferan isopod found in this study was identified as *Edotia doellojuradoi*.

Following the initial discovery of this association, studies were carried out during October 1994, November/December 1995 and April 1996 at three sites on East Falkland. Infestation and abundance relative to host size and density as well as tidal elevation and salinity are described, and the relative sizes of male, female and juvenile isopods recorded. Relationships between brood size, size of adult females and host size are investigated, and the possible effects of the isopod upon the body and reproductive condition of the host mussel determined.

5.2. Material and Methods

5.2.1. Sample collection and treatment

In October 1994, November/December 1995 and April 1996 samples of mussels and

sediment were removed to a depth of 5 cm from between three and five replicate quadrats (≈0.17 m^2) from the upper, mid and lower regions of the mussel bed at the three study sites (see Chapter 2 for site descriptions). The samples were washed and sieved through a 1 mm mesh sieve and all the mussels and any free living isopods were removed. The isopods were preserved in 4% buffered formalin until required. The maximum anterior-posterior dimension (length) of each mussel was measured to 0.1 mm using vernier calipers and sorted into one of six 10 mm size categories. Each mussel was then opened and the number, length (the maximum distance between the frontal margin of the cephalon and distal tip of the telson in flattened animals) and width (the point of maximum carapace width) of each isopod present in the mantle cavity measured to 0.1 mm. All isopods present in a sub-sample of thirty infested mussels from each mussel size class, at each site and tidal level were preserved in 4% buffered formalin for further analysis.

In order to study the effect of salinity and host mussel density on the distribution of isopods at Camilla Creek, mussels were collected from five replicate quadrats from seven stations at low shore down the estuarine creek. Each mussel was opened and the number of isopods recorded. Salinity measurements were made at low tide using a refractometer.

5.2.2. Isopod examination

Each isopod was examined for the presence of any distinguishing sexual characteristics. Males were identified by the paired penes on the sternum of the seventh thoracic segment and the male appendices on the second pleopod, whilst females were identified by the presence of marsupial oostegites on the second-fourth thoracic segments and the absence of a penis (Plate 5.1). Juvenile isopods were classified as those individuals not displaying obvious male or female characteristics. Brood size was defined as the number of juvenile isopods accompanying a mature female within the mantle cavity of a given host mussel.

The sizes of male and female isopods from three zones of the mussel bed at Camilla Creek were compared using a two-sample t-test. Any possible effect of tidal elevation on the size of male and female isopods was tested for by applying the non-parametric Kruskal-Wallis test. The Dunn statistic (Whitaker, 1990) for multiple comparisons of non-parametric data was used to identify the origin of any significant differences

Plate 5.1 *Edotia doellojuradoi*,

A. Male, dorsal view

B. Female, dorsal view

C. Juvenile, dorsal view

D. Male, ventral view

E. Female, ventral view

F. Juvenile, ventral view

P, position of penis; O, indicates one of three pairs of marsupial oostigites on the second - fourth thoracic segments. Scale bar = 1 mm.


A
B
C
D
E
F
d
o

between mean ranks.

Length and width measurements were log transformed and allometric relationships determined (see Chapter 4, section 4.2.2. for method). Any effects due to tidal elevation or site on male or female isopods were identified before the allometric relationships of length on width for male and female *E.doellojuardoi* were compared using the general linear model with a covariate.

5.2.3. Effect on host

The effect of the isopod upon the body condition of the host was determined from the analyses of thirty infested and thirty uninfested mussels from each 10 mm size class from each tidal level and site. Dry flesh weights were determined after drying for 3 days at 65°C. These data were then log-transformed and the dry flesh weights of infested and uninfested mussels compared using the general linear model with a covariate.

The reproductive condition of host mussels was determined by histological examination of the excised mantles of ten infested and ten uninfested mussels (see Chapter 3, section 3.2.1. for method). Mussels were collected when peak reproductive condition was expected (see Chapter 3, section 3.3.2.). The volume fraction of all gametes (developing and ripe) present in four random point counts of sectioned mantle tissue from each animal was determined and differences between infested and uninfested animals identified by applying the non-parametric Kruskal-Wallis test.

5.3. Results

5.3.1. Occurrence and abundance of *Edotia doellojuradoi*

Table 5.1 shows the overall occurrence of isopods at the three study sites. Only at Camilla Creek did isopods occur throughout the entire vertical range of the host; at Darwin and Goose Green infested mussels were largely confined to the lower shore. Overall infestation appears to have increased between 1994 and 1995 except amongst mussels from the high shore at Camilla Creek. Maximum infestation occurred at the estuarine location, Camilla Creek, where in the low shore in 1995 59% of the mussel population were infested; minimum infestation was recorded at Darwin where only 2%

of the low shore population in October 1994 were infested. Infestation generally increased with decreasing tidal elevation except in 1994 at Camilla Creek, where only small differences were recorded. Infestation rates determined from examination of routine monthly samples (Figure 5.1A), confirms the increase in infestation between 1994 and 1995 noted above, but does not provide any obvious evidence of a seasonal pattern.

No significant correlation between infestation and salinity was found along the estuary at Camilla Creek (r_s = -0.288, $p > 0.05$) despite salinities ranging from 16 ppt upstream to 36 ppt at the seaward limit of the estuary (Figure 5.1B). There did, however, appear to be a negative relationship between infestation rate and host mussel density (Figure 5.1C), although this was not statistically significant (r_s = -0.714, $p > 0.05$).

Table 5.1 Overall percentage occurrence of *Edotia doellojuradoi* within *Mytilus edulis chilensis* at the 3 study sites; the number of mussels examined in brackets.

Shore level	Darwin		Camilla Creek		Goose Green	
	1994	1995	1994	1995	1994	1995
High	0(842)	0(0)	51(268)	24(315)	0(344)	0(106)
Mid	0(2257)	0(0)	48(1599)	55(703)	0(893)	6(572)
Low	2(1075)	24(687)	46(1559)	59(617)	18(768)	39(505)

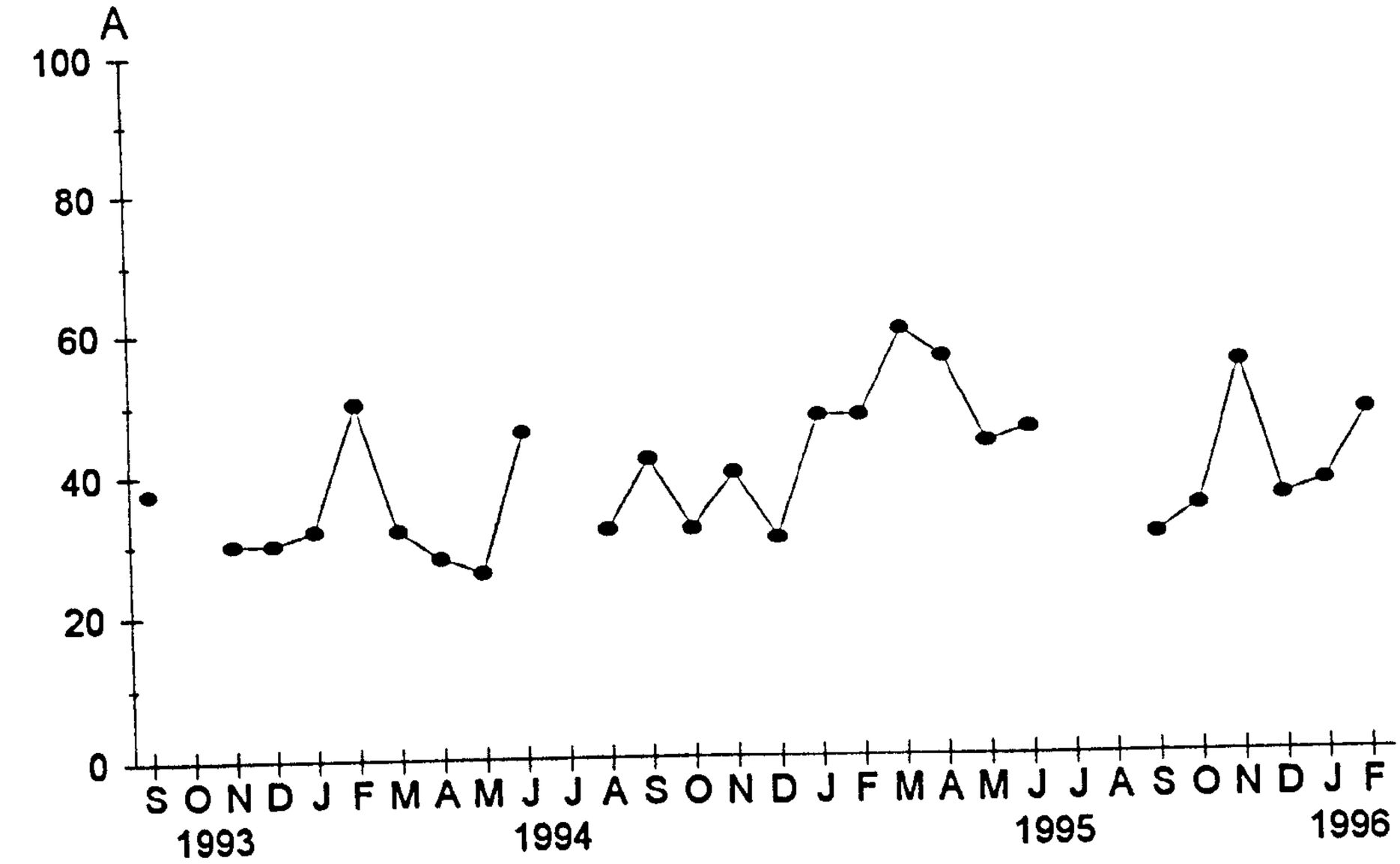
Isopods occurred in all mussel size classes with the smallest infested mussel measuring 8.7 mm in shell length. Figure 5.2A illustrates the size distribution of mussels from three tidal levels at Camilla Creek. Here the population is dominated by mussels in the 15 - 35 mm size categories although individuals range from 7.3 mm to 58.0 mm in shell length. A surprising feature of these populations is the scarcity of small mussels (<15 mm). Figures 5.2B and 5.3 illustrate how infestation varies with host size. Apart from relatively low incidences of the isopod amongst the smallest size classes of mussels, there is a general tendency amongst the larger size categories for infestation to decline with host size. Nevertheless, infestation often remains high even within those size classes where mussels are only poorly represented. Isopod abundance, however, shows a general tendency to increase with host size (Figure 5.2C). The maximum number of isopods recorded within any individual mussel during this investigation was 146, in a mussel measuring 69 mm in shell length from the low shore at Goose Green

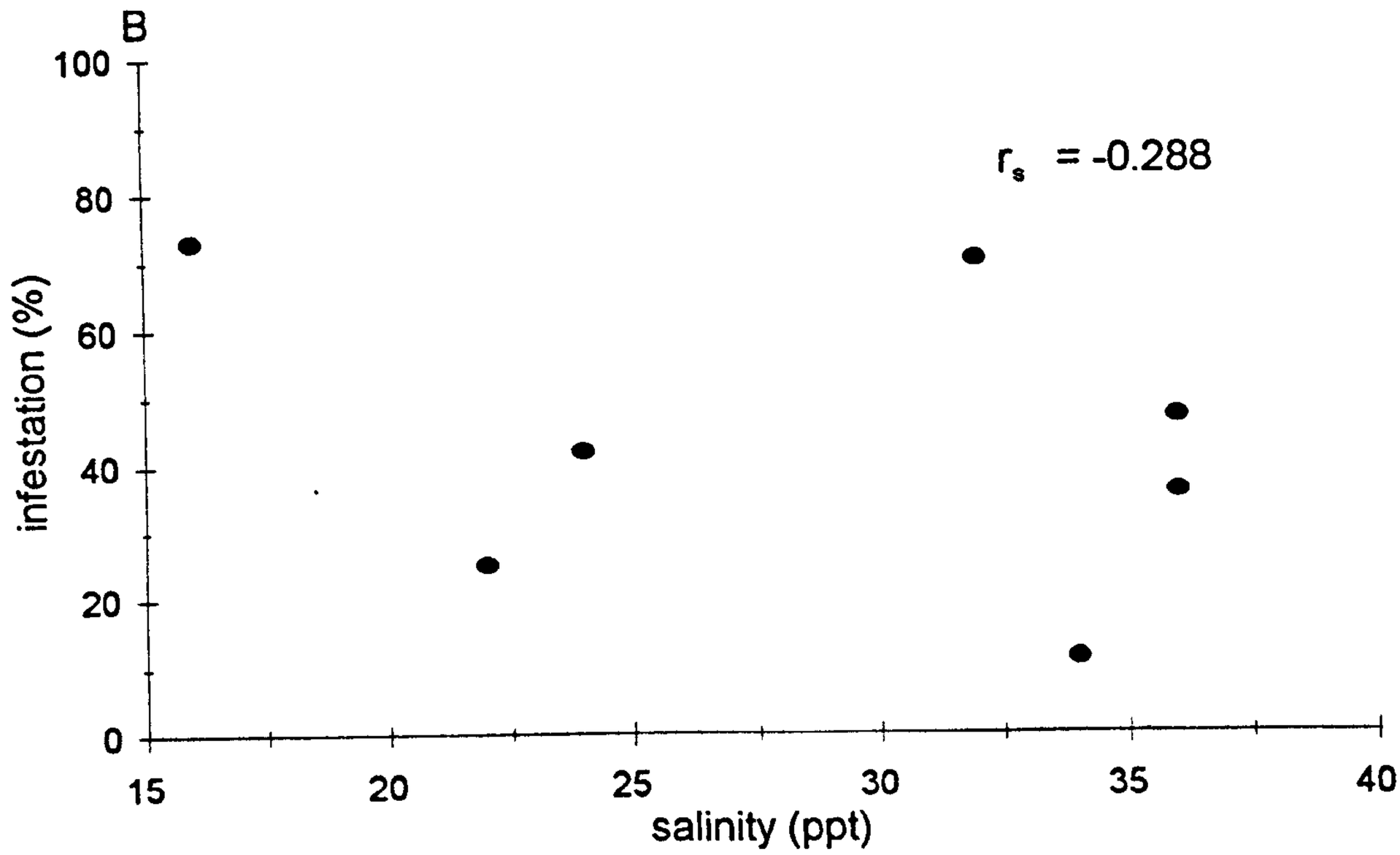
Figure 5.1 Infestation of *Mytilus edulis chilensis* by *Edotia doellojuradoi*

A. throughout the study period,

B. relationship with salinity,

C. relationship with host mussel density.


A
100
80
60
40
20
0
S O N D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996


B
100
80
60
40
20
0
infestation (%)
r_s = -0.288
15
20
25
30
35
40
salinity (ppt)

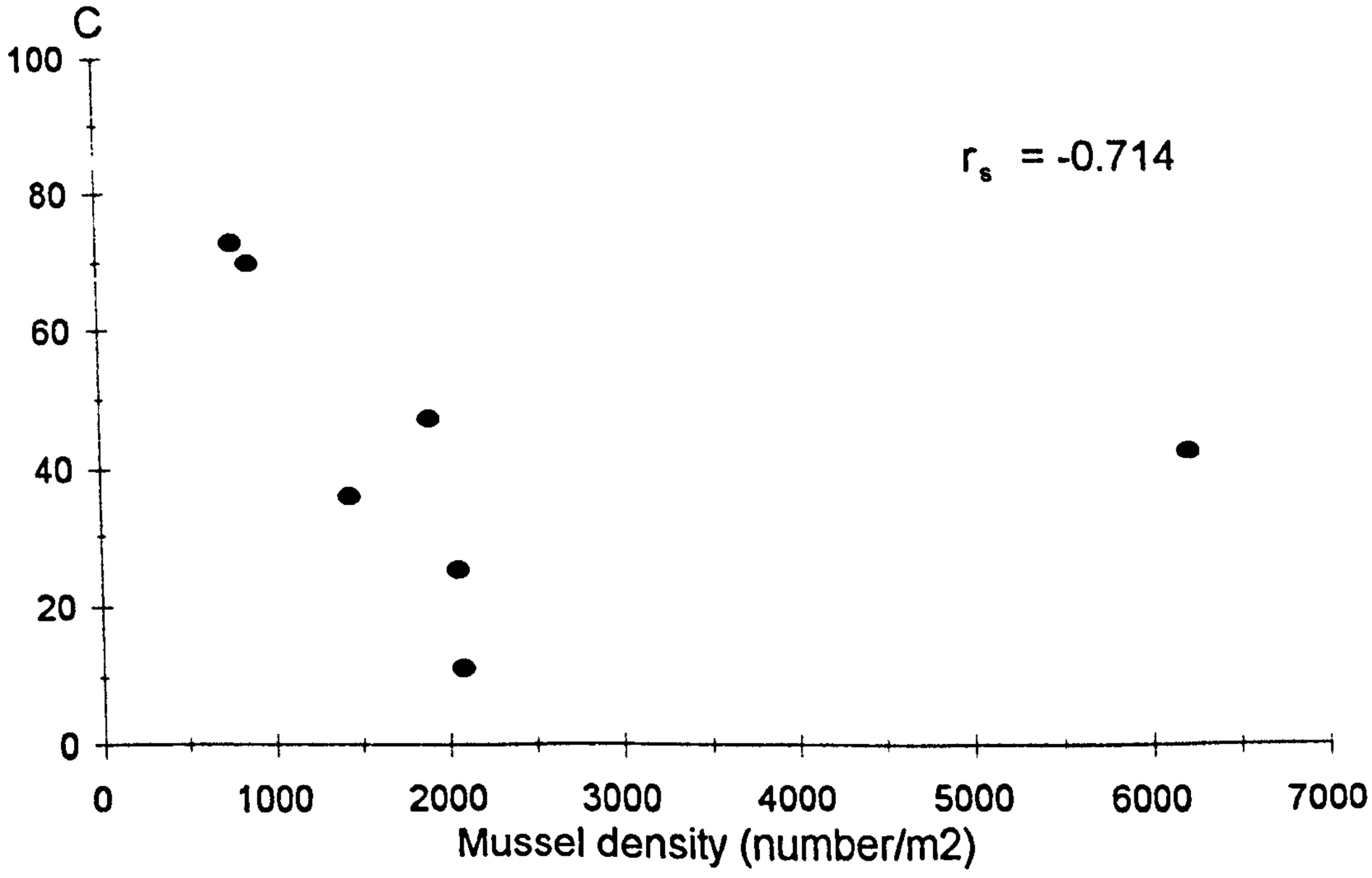

C
100
80
60
40
20
0
r_s = -0.714
0
1000
2000
3000
4000
5000
6000
7000
Mussel density (number/m2)

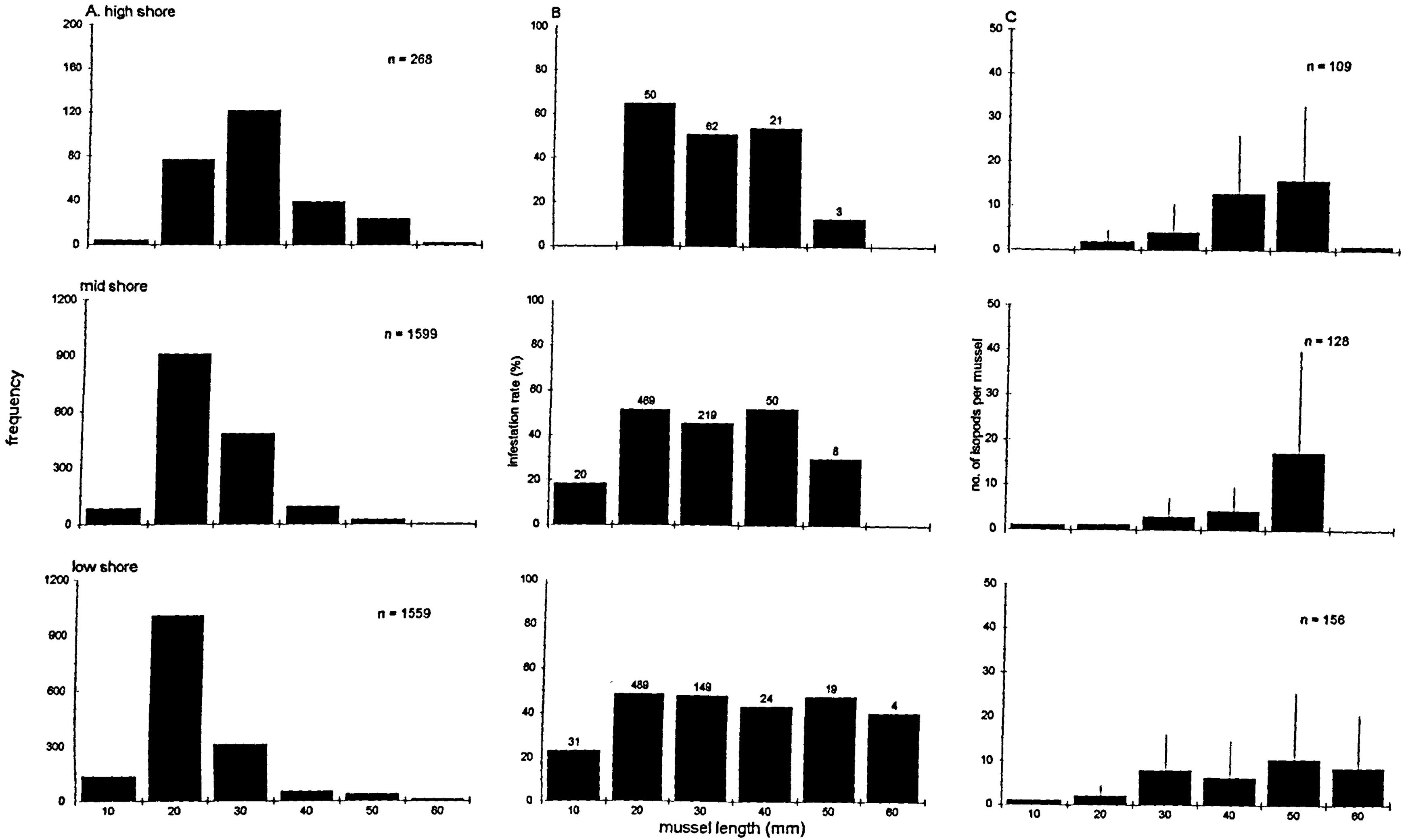
Figure 5.2

A. Size frequency distributions of host, *Mytilus edulis chilensis*, populations;

B. Infestation rates by *Edotia doellojuradoi*;

C. Average numbers of *Edotia doellojuradoi* within *M.e.chilensis*

from three tidal levels at Camilla Creek, October 1994. Vertical bars indicate + 1 SD. Numbers accompanying histogram bars are the number of infected mussels.


A. high shore
n = 268
B
50
62
21
3
C
n = 109
mid shore
n = 1599
frequency
infestation rate (%)
20
469
219
50
8
no. of isopods per mussel
n = 128
low shore
n = 1559
31
489
149
24
19
4
n = 156
mussel length (mm)

during 1994.

5.3.2. Size and distribution of *Edotia doellojuradoi*

Plate 5.1 shows the dorsal and ventral view of male, female and juvenile *E.doellojuradoi*, which have a smooth and soft carapace that tends to be light brown/orange in colour. The size ratio between male and female *E.doellojuradoi* is 0.42 indicating that on average males are only about half as long as the females. The population structure of *E.doellojuradoi* is essentially bimodal, the first and most prominent mode (0.5 - 5.5 mm) consisting of juveniles and males, and the second much smaller mode (5.5 - 10.5 mm) comprising a few juveniles together with females (Figure 5.4A). Juvenile specimens range from 1.2 to 9.0 mm in length whilst males and females measured 3.3 - 5.7 mm and 5.5 - 13.4 mm respectively.

Table 5.2. Comparison of sizes in mm (mean ± s.d.) of male and female *Edotia doellojuradoi* from 3 zones of the mussel bed at Camilla Creek.

	All zones	High	Mid	Low	H - value
Male	3.83 ± 0.23	3.86 ± 0.23	3.88 ± 0.23	3.78 ± 0.22	5.82^{ns}
Female	9.05 ± 1.57	8.62 ± 1.31	9.86 ± 1.69	8.81 ± 1.48	16.85^{**}
t - value	61.00^{**}	36.38^{**}	35.61^{**}	38.16^{**}	

** significant at $p < 0.01$

ns not significant

Table 5.2 contains the average sizes of male and female *E.doellojuradoi* from high, mid and low zones of the mussel bed. Females were consistently significantly larger than males. Female isopods from within mid shore mussels were significantly larger than those from either the high or low shore. Males on the other hand did not differ in size between shore levels. Figure 5.5A illustrates the lack of correlation between the length of male isopods and the host mussel length ($r_s = -0.109$, $p > 0.05$). In contrast female isopods (Figure 5.5B) exhibit a strong positive correlation with host size ($r_s = 0.886$, $p < 0.05$).

No differences in the allometry of male or female *E.doellojuradoi* were detected either with tidal elevation ($F = 2.74$, $p > 0.05$ and $F = 0.06$, $p > 0.05$, respectively) or with site

Figure 5.4 Size frequency distributions of *Edotia doellojuradoi* at

A. Camilla Creek

B. Goose Green

empty bars = juvenile isopods; filled bars = male isopods; hatched bars = female isopods.

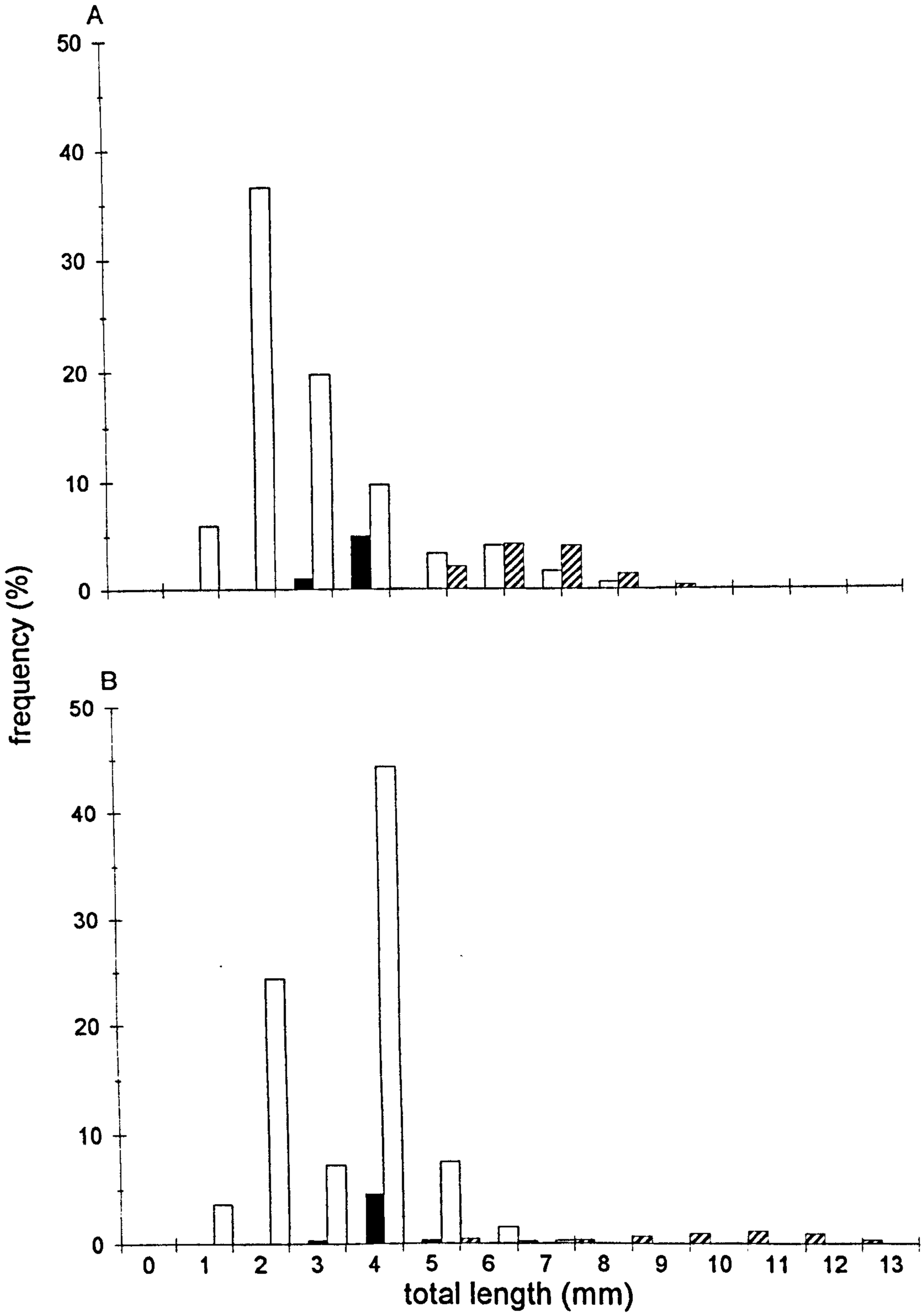

A
50
40
30
20
10
0
B
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13
frequency (%)
total length (mm)

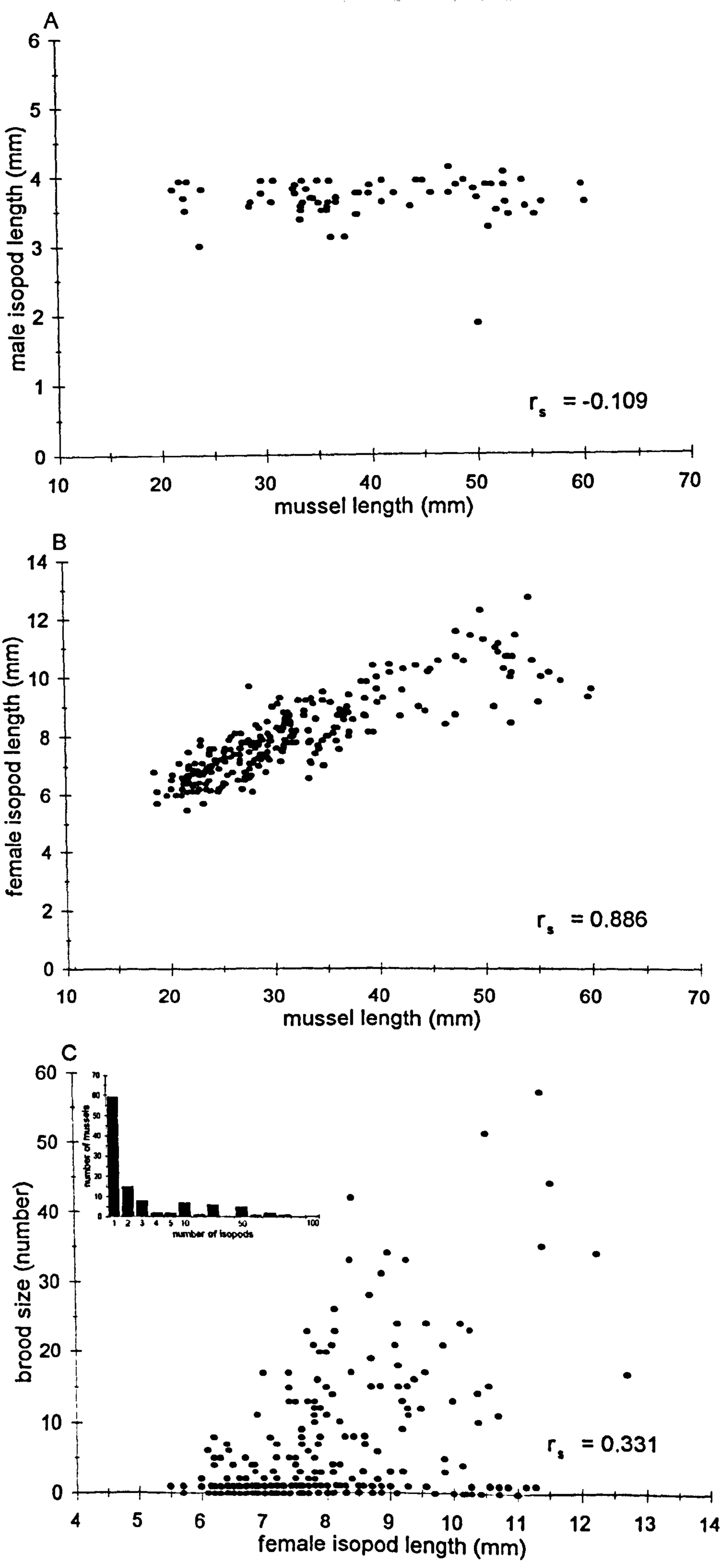
Figure 5.5 The relationship between

A. *Mytilus edulis chilensis* length and male *Edotia doellojuradoi* length,

B. *M.e.chilensis* length and female *E.doellojuradoi* length,

in *M.e.chilensis* from Camilla Creek;

C. Brood size and female length of *E.doellojuradoi* from Camilla Creek, (inset) the frequency of *M.e.chilensis* containing different numbers of *E.doellojuradoi* at Goose Green.


A
male isopod length (mm)
0
1
2
3
4
5
6
10
20
30
40
50
60
70
mussel length (mm)
r_s = -0.109
B
female isopod length (mm)
0
2
4
6
8
10
12
14
10
20
30
40
50
60
70
mussel length (mm)
r_s = 0.886
C
brood size (number)
0
10
20
30
40
50
60
4
5
6
7
8
9
10
11
12
13
14
female isopod length (mm)
r_s = 0.331
number of mussels
number of isopods
1
2
3
4
5
10
50
100

(F = 1.16, $p > 0.05$ and F = 1.50, $p > 0.05$, respectively). Therefore length and width data for male and female *E.doellojuradoi* from all sites and tidal levels were pooled and the allometric relationships determined (Table 5.3) .

Table 5.3. Allometric relationships between the log transformed parameters of length (y, independent variable) and width (x, dependent variable) for male and female *Edotia doellojuradoi*.

Sex	n	a	b	s.e. of b	r^2	β	t	allometry
Male	310	-0.439	0.836	0.049	0.48	1	3.30*	negative
Female	274	-0.440	1.022	0.020	0.90	1	-1.11^{ns}	isometric

variables x and y in the log transformed equation log y = log a + b.log x; a = intercept; β and b (slope of regression line) are the coefficients of isometry and allometry respectively; r^2 = coefficient of determination; s.e. of b = standard error of the slope b; t = calculated value resulting from the t-test; relationships which depart significantly from isometry, * significant, $p < 0.05$; ^{ns} not significant

Males displayed significant negative allometric growth, ie. they increased in width at a relatively slower rate than in length, becoming progressively more slender as they increased in size. Females on the other hand exhibited isometric growth, ie. parameters of length and width increased at the same relative rate. Allometry of male and female *E.doellojuradoi* differed significantly (F = 12.09, $p < 0.05$), indicating that males were consistently more elongate than the rather rotund females.

Sex ratios (males : females) varied with both collection time and site. A maximum ratio of 3:1 was observed at Goose Green in 1994 and a minimum ratio of 1:2 found at Camilla Creek in 1994, subsequent samples collected from Camilla Creek in 1996 showed a slight increase in the ratio of males : females, 1.25:1. Never more than one female was found within the mantle cavity of any given mussel, whereas up to 13 males could be found accompanying a single female in mussels from Goose Green in 1994. Whilst large numbers of mussels contained between one and five isopods, relatively few mussels contained more than 50 individuals (Figure 5.5C inset). Brood size was significantly correlated with the size of the attendant female ($r_s = 0.331$, $p < 0.05$) strongly suggesting that fecundity is size dependent (Figure 5.5). The juvenile isopod population from Goose Green was distinctly bimodal (Figure 5.4B). This bimodal

Table 5.4 Constants for log-transformed regressions of dry flesh weight (y) against shell length (x) for infested and uninfested *Mytilus edulis chilensis*; a; intercept, b; slope in the allometric equation $y = ax^b$

Site (shore level)	1994				1995			
	a	b	F-value		a	b	F-value	
			a	b			a	b
Darwin (low shore)								
infested	-5.630	3.012			-	-		
			0.76^{ns}	0.89^{ns}			-	-
uninfested	-5.860	3.174			-	-		
Camilla Creek (high shore)								
infested	-5.075	2.606			-4.974	2.686		
			0.05^{ns}	0.43^{ns}			0.02^{ns}	0.01^{ns}
uninfested	-5.109	2.672			-4.943	2.704		
(mid shore)								
infested	-5.398	2.790			-4.748	2.445		
			0.00^{ns}	0.02^{ns}			5.38*	7.23*
uninfested	-5.403	2.801			-5.139	2.750		
(low shore)								
infested	-5.290	2.65			-5.12	2.63		
			1.68^{ns}	3.24^{ns}			1.31^{ns}	1.60^{ns}
uninfested	-5.404	2.751			-5.294	2.762		
Goose Green (low shore)								
infested	-5.540	2.951			-	-		
			2.56^{ns}	2.90^{ns}			-	-
uninfested	-5.823	3.128			-	-		

* significant , $p < 0.05$, ^{ns} not significant

distribution could be observed even within individual mussels, suggesting that two cohorts may be present simultaneously within the host.

5.3.3. Effect on host

When the regressions of log-transformed dry flesh weights and shell lengths of infested and uninfested mussels were compared using the general linear model no significant differences could be discerned, except amongst mussels from the mid shore population at Camilla Creek during 1995. Here, infested mussels below 19 mm in shell length were heavier than uninfested individuals whilst those above 19 mm had lower flesh weights than the uninfested individuals of comparable size (Table 5.4). Taken overall these results strongly suggest that the isopod has little or no effect on the body condition of mussels at these sites. However, when gamete volume fractions of infested and uninfested mussels were compared, they were found to be significantly different ($H = 8.85$, $p < 0.05$), suggesting that the isopod has a detrimental effect upon the reproductive condition of its host.

5.4. Discussion

Relationships between isopods and bivalves appear to be rather unusual although *Edotia magellanica* has previously been reported in mussels from the northern side of the Strait of Magellan and the clam *Mulinia edulis* in the Lingue and Queule estuaries in southern Chile (Jaramillo *et al.*,1981 and Gonzalez & Jaramillo, 1991). Mussels in the Falkland Islands are extensively infested by *Edotia doellojuradoi.* Whilst *E.doellojuradoi* occurs abundantly within the mantle cavity of its host mussel, no free-living individuals of this species were observed. However, significant numbers of another edotiid, possibly *Edotia tuberculata*, were observed living freely within the sediment and shell fragments associated with the mussel matrix.

At present it is difficult to explain differences in infestation of mussels at the three study sites, in particular why isopod infestation occurs throughout the mussel zone at Camilla Creek but is restricted to the low shore at Goose Green and Darwin. However, Camilla Creek is generally more sheltered from severe weather than either Goose Green or Darwin and has very high levels of silt deposition. The increase in infestation with decreasing tidal elevation may, however, be associated with the degree of algal cover

at the three study sites. Algae, which could provide a suitable refuge for isopods, occurred throughout the mussel zone at Camilla Creek, increasing in density towards the low-water mark, whereas at Goose Green and Darwin it was present only in the lower reaches of the zone. Increasing infection rates of bivalves by the brachyuran crab *Pinnotheres* with decreasing tidal elevation has been well documented (Houghton, 1963; Seed, 1969c; Kruczynski, 1972; Haines *et al.*, 1994). Longer submersion times of the bivalves living at lower shore levels are thought to increase the chance of the crab finding a host.

Although infestation appears to have increased at most sites between 1994 and 1995 (Table 5.1) samples were not collected at precisely the same time each year. These differences were initially thought to reflect variation in an annual cycle of occurrence. However, observations of infestation from routine monthly samples (Figure 5.1A) do not provide any convincing evidence of such a cycle, although there does appear to be a slight increase in infestation with time. The negative relationship between infestation and host mussel density, although not significant, suggests that in areas where mussel numbers are low, all available habitat space is utilised, whereas a surplus of habitat space appears to occur if mussel numbers are high. High levels of infestation occurred across a broad range of mussel size categories, and infestation remained high even in size classes that were poorly represented within the population (Figure 5.2A and B). Apart from relatively low infestation amongst small mussels there was evidence of a slight but consistent decrease in infestation with increasing host size. Tablado and Gappa (1995) have suggested that a similar decrease found with infection rates in *M.edulis* by *Tumidotheres maculatus* could result from differential mortality of the pea crab. This trend is rather surprising since the infestation of most parasites/commensals usually increases with host size. Infestation of *M.edulis* by the pea crab, *Pinnotheres pisum*, for example, increased with increasing host size (Seed, 1969c), and was probably the result of increased exposure time to the source of infection, and/or a response to the relative amount of water filtered by mussels of different sizes. The actual abundance of isopods within individual mussels, on the other hand, shows a distinct increase with mussel size (Figure 5.2C). This increase probably reflects the larger brood sizes produced by large females which tend to be associated with the larger size categories of mussel; larger size categories of mussels will also have been available for colonization for proportionately longer periods of time.

The size ratio between male and female *Edotia* varies between species. In *E.corrugata*

and *E.oculata* (Sheppard, 1957) and *E.transversa* (Menzies, 1962) male individuals are larger than the females, a result similar to that found within the free-living edotiid population within the mussel matrix at Camilla Creek. In *E.magellanica* (Jaramillo *et al.*, 1981) and *E.bilobata* (Sheppard, 1957) however, females are larger than males, as recorded for *E.doellojuradoi* in this investigation; no size differences were detectable between male and female *E.oculopetiolata* (Sheppard, 1957). Differences between the size of female *E.doellojuradoi* at different shore levels, although significant, does not show any particular pattern. A trend reflecting differences in the host mussel size distribution might have been expected, especially since the size of female isopods is positively correlated with host size and there are increasingly higher numbers of large mussels with increasing tidal elevation. The size of male *E.doellojuradoi*, on the other hand, appears to be independent of host size and does not differ significantly with tidal elevation. In the brachyuran crab *Pinnotheres*, females are typically larger than males with only a slight overlap in size (Seed, 1969c) and the relationship between host size and size of male and female crabs is similar to that found in the present study (Seed, 1969c; Haines *et al.* 1994; Tablado & Gappa, 1995).

Differences in the allometric relationship of length on width between male and female *E.doellojuradoi* are such that the ratio may be used to identify the sex without checking for the presence of secondary characteristics. The greater width of female compared to male isopods is probably a direct consequence of reproduction, where the females are responsible for brooding eggs within the marsupium.

Various characteristic features of the life cycle of *E.magellanica* as suggested by Jaramillo *et al.* (1981) and further discussed by Gonzalez & Jaramillo (1991) have been observed amongst *E.doellojuradoi* in the present study. Firstly, the occurrence of large numbers of mussels containing less than five isopods and relatively few containing more than fifty; Jaramillo *et al.* (1981) found that only 1 out of 74 mussels contained more than 50 isopods. Secondly, never more than one adult female isopod was found in any given host strongly suggesting that multiple infestation is avoided by some as yet unexplained mechanism. This has also been observed in bivalves that are inhabited by pea crabs (Seed, 1969c; Haines *et al.*, 1994; Tablado & Gappa, 1995). Several mature male *E.doellojuradoi* may be present accompanying a single adult female (a maximum of 13 were found in a host mussel from Goose Green in 1994) although it was difficult to identify the progenitor male, it was assumed that the majority would have been part of a brood which has reached maturity before leaving the host. Thirdly, the occurrence

of a bimodal distribution of juveniles within mussels (Figure 5.4B) suggests that a single mature female is capable of rearing two broods simultaneously. In contrast to *E.magellanica* from South America which appear to have very small broods, *E.doellojuradoi* from the Falkland Islands has much larger broods, suggesting either that mortality is much lower or that fecundity is higher. The brood size of *E.doellojuradoi* was significantly correlated with female size, which is generally typical for marine isopods (Naylor, 1972); however, in *E.magellanica* from South America no such correlation was evident (Gonzalez & Jaramillo, 1991).

Initial observations indicating that *E.doellojuradoi* has no apparent effect upon the hosts body condition (see also Gonzalez & Jaramillo, 1991) led to the suggestion that this isopod was probably not feeding on the particulate material filtered by the host, but that it may have been consuming the pseudo-faeces which accumulate in the mantle cavity. Alternatively feeding may be suspended during reproduction as has been observed in other marine isopods such as *Arcturella sawayae* (Moreira, 1973). However, results from histological examination of the gonad indicate that the isopod appeared to have a detrimental effect upon the reproductive condition of the host. Thus, although the overall tissue weight was not altered by the presence of the isopod, the proportion of gametes produced was, suggesting that reproductive effort is somewhat lower in mussels harbouring isopods. Curiously, at the two other study sites, Darwin and Goose Green, where mussels from the mid part of the zone were not infested with *E.doellojuradoi,* the reproductive effort was much higher than that found at Camilla Creek (see Chapter 3). It is possible that the technique used to assess the body condition of host mussels is not sufficiently sensitive to detect any effect which the isopods may be having, particularly since reproductive effort at this site is so low, suggesting changes in tissue weight could also be correspondingly small.

The fact that reproductive effort in mussels is highly dependent upon food supply (Seed and Suchanek, 1992) would suggest that if *E.doellojuradoi* is utilising food particles filtered by the host then a reduction in growth would be observed. Kent (1979) found that the presence of the shell boring polychaete *Polydora ciliata* in *M.edulis* significantly lowers the body condition of the host, and more specifically the mantle tissue (dry weight), suggesting that heavy infestations might lower fecundity. Typically any effect an occupant such as *Pinnotheres* has on its bivalve host is reflected by a reduction in body condition and possibly reproductive condition (Sandoz & Hopkins, 1947; Haven, 1958; Andrews *et al.*, 1968; Seed, 1969c; Kruczynski, 1972; Tablado & Gappa, 1995),

although effects on shell growth both allometrically (shell shape) and absolute (shell length) have also been observed (Bierbaum & Ferson, 1986; Tablado & Gappa, 1995). Other negative effects such as morphological changes in skull bones and gill rakers of fish which are host to parasitic isopods belonging to the family *Cymothidae* have been observed by Trilles (1964) and Romestand (1978) even when differences in the size-weight relationship are not always evident.

In the schematic illustration of the probable life cycle of *E.magellanica* provided by Jaramillo *et al.*(1981) mature males, followed by juvenile females, leave the host mussel and become 'free-living' individuals before re-infesting a new host. Although Jaramillo *et al.* (1981) did not actually record the presence of free-living individuals in the Strait of Magellan, when *E.magellanica* was first described (see Nordenstam, 1933), these were reported burrowing in shell gravel and coarse sand beneath stones in the intertidal zone. The absence of free-living *E.magellanica* from clam (*Mulinia edulis*) beds situated on sand bars outside the Lingue and Queule estuaries of Southern Chile was thought to be due to the soft and more transient nature of the substratum, where free-living stages could more easily be washed away (Gonzalez & Jaramillo, 1991). Although this study too has failed to locate any free-living stages of *E.doellojuradoi*, another closely related edotiid (possibly *Edotia tuberculata*, G. Poore pers.comm.) did occur in abundance within the mussel matrix, but only at Camilla Creek. This isopod, however, was generally larger, darker in colour and more heavily sculptured than *E.doellojuradoi* (Plate 5.2). Moreover, the size ratio between males and females was 1.43 compared with only 0.42 for *E.doellojuradoi*. The failure to find free-living stages of *E.doellojuradoi* when infesting new hosts remains unexplained. It is possible, however, that *E.doellojuradoi* only moves between hosts at high tide and thus remains undetected during low tide sampling. Further work is clearly required in order to resolve the life cycle of this interesting edotiid isopod.

5.4.1. Conclusions

Whilst *Edotia doellojuradoi* occurred abundantly within the mantle cavity of host mussels, *Mytilus edulis chilensis*, no free-living individuals were observed. Infestation varied between study sites, with tidal elevation and with host size. Factors such as host density, algal cover, immersion time and differential isopod mortality are thought to exert some influence upon the degree of infestation. The abundance of *E.doellojuradoi*, which increases with host size, reflects the larger brood sizes produced by large

females which tend to be associated with larger size categories of mussel. Male, *E.doellojuradoi* are approximately half the length of females and exhibit no relation with the size of their host. The relatively rotund body shape of female isopods compared to the somewhat elongate shape of males is probably related to reproduction, since females are responsible for brooding eggs in the marsupium.

Several aspects of the relationship between *E.doellojuradoi* and *M.e.chilensis* were found to be in agreement with the probable life cycle of *Edotia magellanica* as proposed by Jaramillo *et al.* (1981).

Although *E.doellojuradoi* appears to have no deleterious effect upon the body condition of its host, the significantly reduced reproductive condition amongst infested mussels implies otherwise. More sensitive measurements of condition are clearly required in order to determine the exact nature of this interesting relationship.

Plate 5.2 *Edotia tuberculata*,

A. Male, dorsal view

B. Female, dorsal view

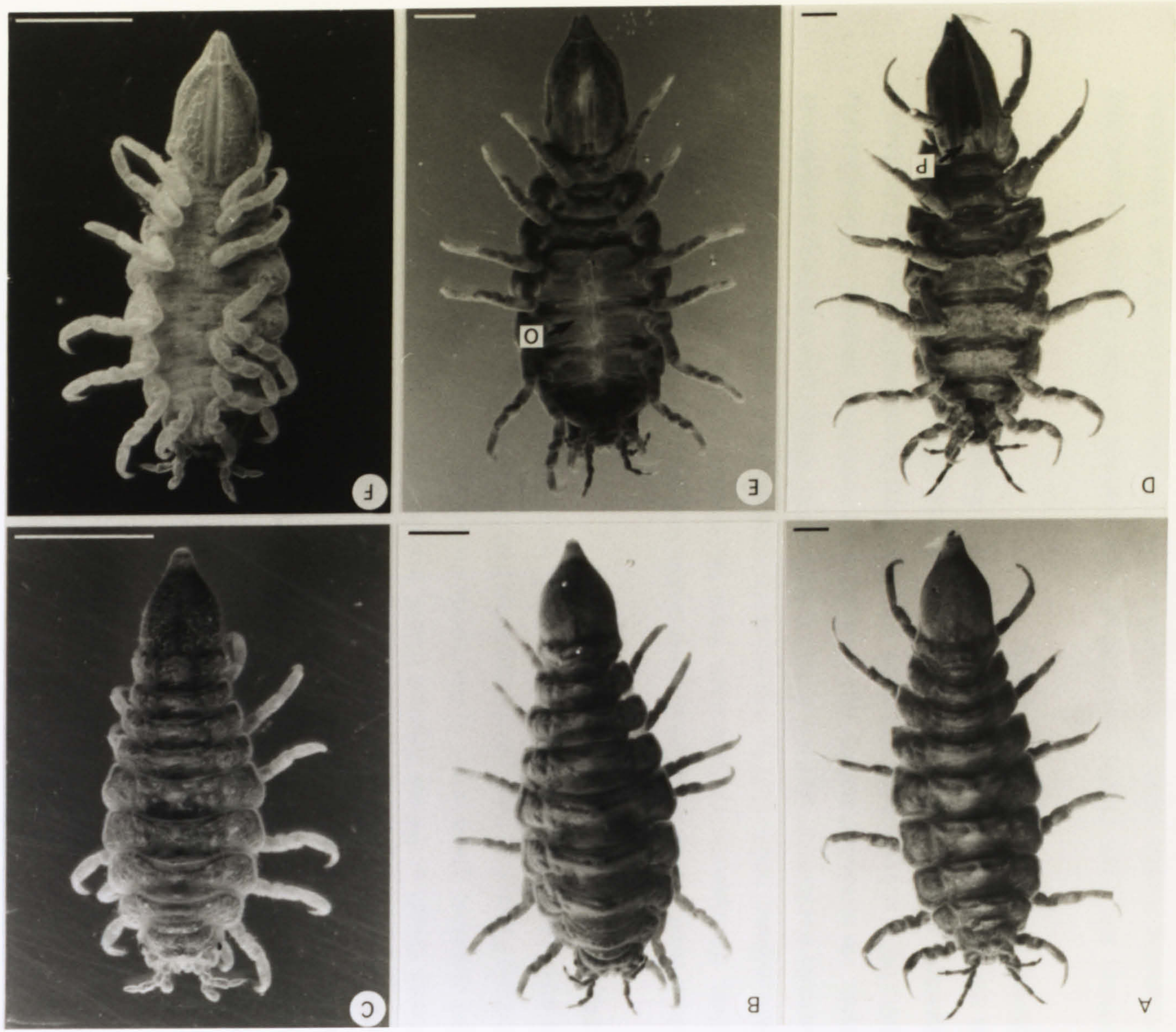
C. Juvenile, dorsal view

D. Male, ventral view

E. Female, ventral view

F. Juvenile, ventral view

P, position of penis; O, indicates one of three pairs of marsupial oostigites on the second - fourth thoracic segments. Scale bar = 2 mm.


A
B
C
D
E
F
O
P

Chapter 6

Coccomyxa parasitica (Chlorococcales, Coccomyxaceae) and its relationship with *Mytilus edulis chilensis*

6.1. Introduction

The infestation of bivalves by unicellular algae appears to be relatively common. 'Green oysters' and 'green-gilled clams' for example have been described by Lankaster (1886), Mitchell and Barney (1917), Medcof (1945) and Kerswill (1946). There are, however, relatively few reports of algae occurring in the soft tissue of the blue mussel, *Mytilus edulis*. Kerswill (1946) observed green gills in *M.edulis* growing in the intertidal zone of Williams Creek on Prince Edward Island, but could not identify the cause. Meixner (1984) observed 'green spots' in the mantle and adductor tissues of *M.edulis* from the Flensburg Fjord (Denmark), and suggested that this was due to a parasitic endobiotic alga which he identified as a blue-green 'alga', cyanobacterium, belonging to the genus *Microcystis*.

Naidu & South (1970) found green areas distributed within the mantle tissue of the giant scallop, *Placopecten magellanicus,* from the shallow waters off the west coast of Newfoundland. Naidu (1971), subsequently observed that this green colouration was caused by a unicellular alga which had a detrimental effect upon the body condition of its host. He concluded that the alga could be considered to be parasitic. Stevenson and South (1974) subsequently identified and described this parasitic alga as *Coccomyxa parasitica*, a new member of the Coccomyxaceae, Chlorococcales. They speculated that the parasitic relationship may be one of a facultative nature after successfully culturing the alga on inorganic media.

Hartman and Pratt (1976) identified an alga present on the siphonal tissues and surrounding mantle areas of the heart cockle *Clinocardium nuttallii* as a facultative parasite of the genus *Chlorella*. Lauckner (1983) speculated that Hartman and Pratt (1976) were unaware of the descriptions of the parasitic *Coccomyxa* spp. and suggested that the presumed *Chlorella* spp. may well have been a member of the *Coccomyxaceae*.

During this ecological study of *Mytilus edulis chilensis* from the Falkland Islands, green areas were observed in the soft body parts of mussels from Goose Green, but not in mussels from the other two study sites, Darwin and Camilla Creek. The following chapter attempts to identify and describe the causative agent of these green areas; the occurrence and distribution of the algae within the tissue of the host are also described.

6.2. Materials and methods

6.2.1. Sample collection and treatment

In November 1995 mussels from within five to nine random quadrats (≈0.17 m^2) were collected from the high, mid and low areas of the mussel zone at Goose Green (see Chapter 2 for site description). The shell length of all mussels in each sample were measured (maximum anterior - posterior dimension) to 0.1 mm using dial vernier calipers. Each mussel was subsequently opened and the distribution and abundance of algal patches within four areas of the flesh noted. These were :

1) mantle edge
2) external mantle surface; a) posterior and b) anterior
3) posterior adductor muscle
4) visceral mass; a) posterior and b) anterior.

The abundance of the alga was categorised into :

1) light - occasional, small spots
2) moderate - larger, common patches
3) heavy - tissues almost totally covered.

In addition to the samples collected in November 1995, further monthly samples of mussels were collected from the middle part of the mussel zone (≈ 0.66 m above chart datum) at Goose Green and the infection rates calculated for the period September 1993 to February 1996.

6.2.2. Preparation and observations of infected mussel tissue

Pieces (≈1 mm^2) of infected mussel tissue were excised and fixed using 2.5% glutaraldehyde in phosphate buffer (ph 7.2) for 12 hours at 5°C, followed by further fixation in 2% osmium tetraoxide in the buffer for one hour. The samples were thoroughly washed after each fixation with phosphate buffer. Samples were then dehydrated sequentially at 15 minute intervals in 30%, 50%, 70%, 90% and absolute alcohol, before being embedded in 'SPURR' resin (Spurr, 1969). Mounted resin blocks were then sectioned and thick (≈ 500 nm) and thin (≈ 50 nm) sections prepared. Thick sections were placed onto glass microscope slides and stained with toluidine blue (1%) before being mounted in DPX; these were then photographed using a light microscope. Thin sections were collected onto 'celloidin' coated grids, then stained with uranyl acetate and lead citrate prior to examination in a Philips transmission electron microscope where images were recorded photographically.

6.2.3. Effect on host

Samples of 50 mussels over the size range of the population at Goose Green were collected monthly throughout 1994. The length of each mussel was measured to 0.1 mm using dial vernier calipers and the dry tissue weight recorded after oven drying for 3 days at 65°C. In order to identify any possible effect that the alga might have on the mussel, the slopes and intercepts of log transformed dry tissue weights on shell length of infected and uninfected mussels were compared using the general linear model with a covariate (Minitab).

6.3. Results

6.3.1. Description of the alga *Coccomyxa parasitica*

The following description is based on light microscope and transmission electron microscope *in situ* observations of the alga within the host tissue.

Low power observations using the light microscope provide clear evidence of colonisation of the host mussel tissue by the infecting alga. The alga is aggregated into large patches (≈400 µm in diameter), apparently encapsulated, within the connective

tissue of the host (Plate 6.1A). High power examination using a T.E.M. revealed that the patches consisted of cells that were spherical to ovoid in shape and ranged between 1 and 4 µm in diameter. One or two chloroplasts were usually visible within the cell cytoplasm, containing stacks of between two and seven thylakoids whilst some starch grains could be observed (Plate 6.1B,C). There were no obvious pyrenoids. Mitochondrial profiles were observed and a central nucleus, with an obvious nucleolus was present (Plate 6.1C). Distinct ribosomes, rough endoplasmic reticulum and electron dense vesicles, possibly storage products, were visible within the cytoplasm (Plate 6.1B,C). All cells were enclosed by two membranes (7 - 10 nm thick) and in some instances these appeared to be relatively thick (20 - 40 nm) with a darkened region between the membranes (Plate 6.2A). The outer membrane appeared to be animal (host) in origin and the inner one algal. This could be seen clearly in the dividing cells (Plate 6.2A,B). No flagella or flagellar bases were observed and reproduction appeared to be achieved by cell division into either two or four daughter cells (1.7 - 2.5 µm) before the parental membrane ruptured. It is not known whether division occurred prior to or during infestation of the host mussel tissue. Leukocytes could be identified (Plate 6.2C) containing one or more intact algal cells in the dense cytoplasm. Leukocyte vacuoles had a double membrane indicating that the algal cells remain separate from the mussel tissue. Evidence of algal cell digestion was provided by the presence of lamellar bodies (Plate 6.2C), where concentric layers of cell membranes remain from partially digested cells; and multi-vesiculate bodies, which typically contain enzymes and are characteristic of the presence of lysosomes. It is not clear whether the leukocytes were ingesting algal cells at random or only those showing signs of breakdown.

6.3.2. Occurrence and abundance of *Coccomyxa parasitica*

Infection rates were highest (23%) in mussels from the middle of the mussel zone (Table 6.1) and lowest in mussels from the upper part of the zone (0%). Approximately 30% of those mussels infected in the mid and low zones were lightly infected, 10% moderate and only 4% were heavily infected. Despite the occurrence of high levels of infection in mussels from Goose Green, routine monthly sampling (Chapter 3, September 1993 to February 1996) of mussel populations from two other sites, Darwin and Camilla Creek, provided no evidence of algal infection.

Plate 6.1

A. A photomicrograph of a section of *Mytilus edulis chilensis* mantle tissue showing the presence of a 'colony' of *Coccomyxa parasitica* within the connective tissue. Scale bar = 100 µm.

B. and C. Transmission electron microscope images of *C.parasitica* cells within *M.e.chilensis* connective tissue. c, chloroplast; m, mitochondrial profiles; s, starch grain; v, electron dense vesicle; rer, rough endoplasmic reticulum; n, nucleus. Scale bar = 500 nm.

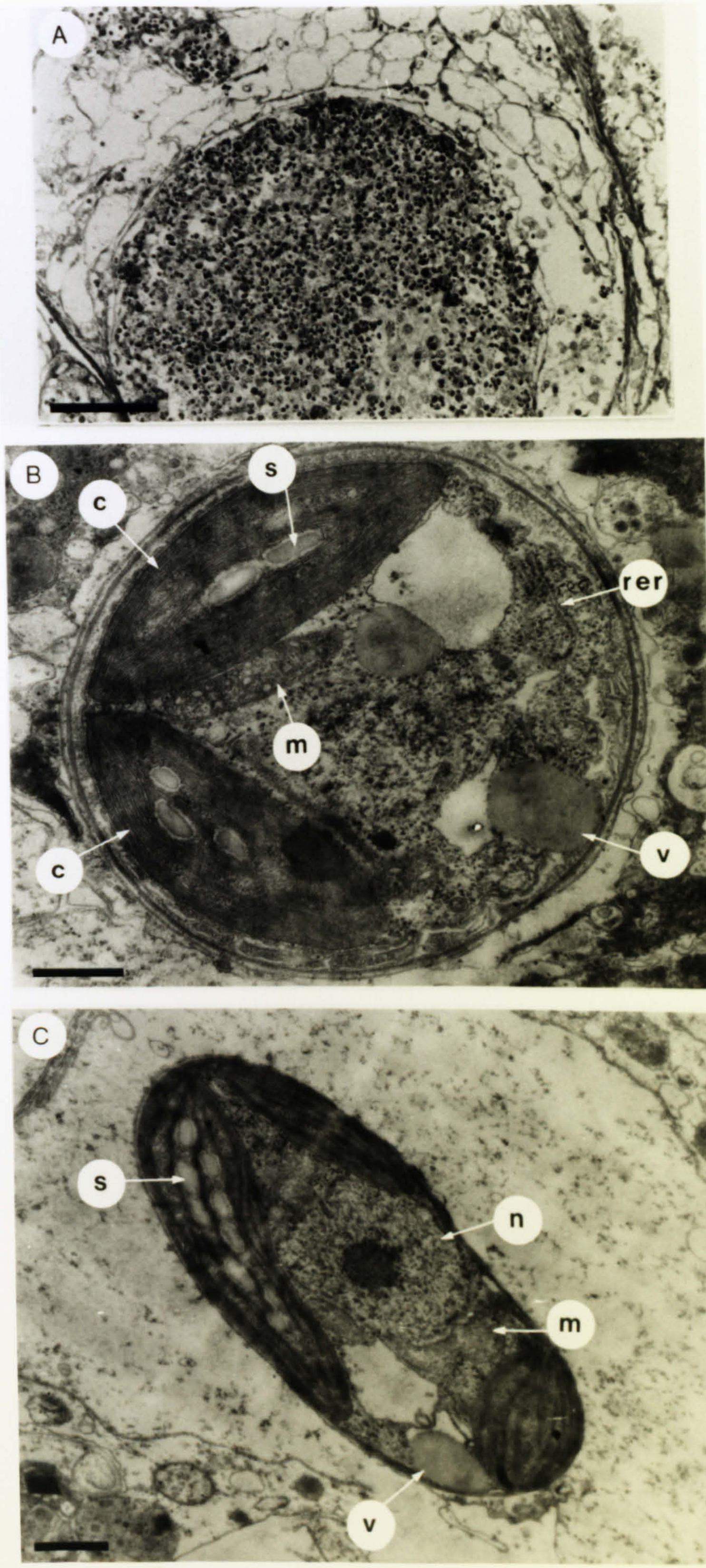

A
B
c
s
rer
m
c
v
C
s
n
m
v

Plate 6.2

A. Transmission electron microscope images of a dividing *Coccomyxa parasitica* cell. The large double arrows highlight the animal cell membrane; the single large arrow indicates the algal cell parental membrane; and the small double arrows show the algal daughter cell membrane.

B. and C. Low power transmission electron microscope images of the molluscan tissue with leukocytes containing algal cells. mv, multi-vesiculate body; ml, multi-lamellar body; l, leukocyte. Scale bar = 500 nm.

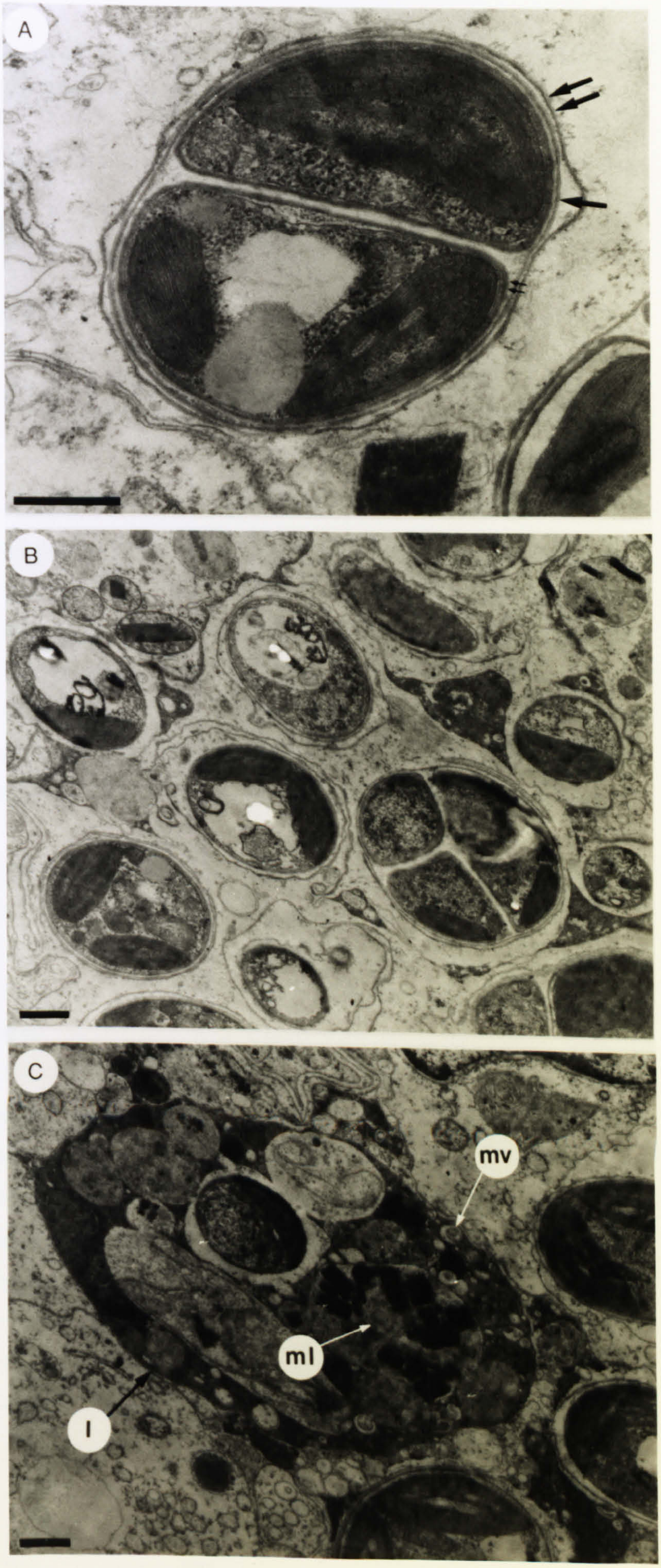

A
B
C
mv
ml
I

Table 6.1. The occurrence of *Coccomyxa parasitica* within *Mytilus edulis chilensis* from Goose Green, Falkland Islands. Bracketed numbers denote % infection.

Zone	Number examined	Number infected	Occurrence (number infested) none	light	moderate	heavy
High	106	1 (<1)	105 (99)	1 (1)	0	0
Mid	572	134 (23)	438 (77)	93 (16)	33 (6)	8 (<1)
Low	245	12 (5)	233 (95)	11 (5)	1 (<1)	0
Total	923	147 (16)	776 (84)	105 (11)	34 (<1)	8 (<1)

Coccomyxa parasitica was particularly abundant along regions of the mussel tissue most exposed to light. The preferred sites in order of decreasing abundance were 1) the mantle edge; 2) the posterior area of the visceral mass; 3) the posterior area of the outer mantle surface; and 4) the posterior adductor muscle. Very low infections were observed in the anterior areas of the mussel (Table 6.2). Large and often densely packed algal colonies were present principally at the mantle edge, becoming increasingly more sparse and scattered toward the anterior of the host. Whilst posterior infections were light through to heavy, anterior tissues were only lightly infected.

The average size of mussels infected with *C.parasitica* was 25 mm in the upper part of the mussel zone and 61 mm in the low part of the zone, corresponding to ages of between 4 and 6 years (Chapter 4). However, in almost every case the shells of infected mussels were seriously damaged, either by erosion over the whole valve surface or by impaired shell growth at the posterior shell growing margin. Mussels which exhibited little or no obvious evidence of shell damage, were rarely infected. Length frequency distributions of the mussel populations from the low, mid and upper parts of the mussels zone, together with the infection rates of *C.parasitica* are illustrated in Figure 6.1. Very few small mussels are infected by *C.parasitica*. In mussels from the mid and lower regions of the mussel zone, algal infection rates remain relatively high despite the declining frequency of the host mussels in the larger size classes. Monthly infection rates collected during routine monthly sample collections at Goose Green (Figure 6.2) provide no evidence for a seasonal pattern, although considerable variation over the two and a half year study period was clearly evident.

Table 6.2 The occurrence of *Coccomyxa parasitica* in different areas of the soft body parts of *Mytilus edulis chilensis* at Goose Green.

A. mantle edge

	None	Light	Moderate	Heavy
Number	21	48	48	30
Percent	14	33	33	20

B. anterior of outer mantle surface

	None	Light	Moderate	Heavy
Number	137	8	1	0
Percent	94	6	<1	0

C. posterior of outer mantle surface

	None	Light	Moderate	Heavy
Number	68	55	21	2
Percent	47	38	14	1

D. posterior adductor muscle

	None	Light	Moderate	Heavy
Number	75	62	8	1
Percent	51	42	6	<1

E. anterior area of visceral mass

	None	Light	Moderate	Heavy
Number	141	5	0	0
Percent	97	3	0	0

F. posterior area of visceral mass

	None	Light	Moderate	Heavy
Number	64	65	16	1
Percent	44	45	11	<1

Figure 6.1 Length frequency distributions (filled bars) of *Mytilus edulis chilensis* together with infection rates (hatched bars) by *Coccomyxa parasitica* in mussel populations from Goose Green

A. high zone

B. mid zone

C. low zone

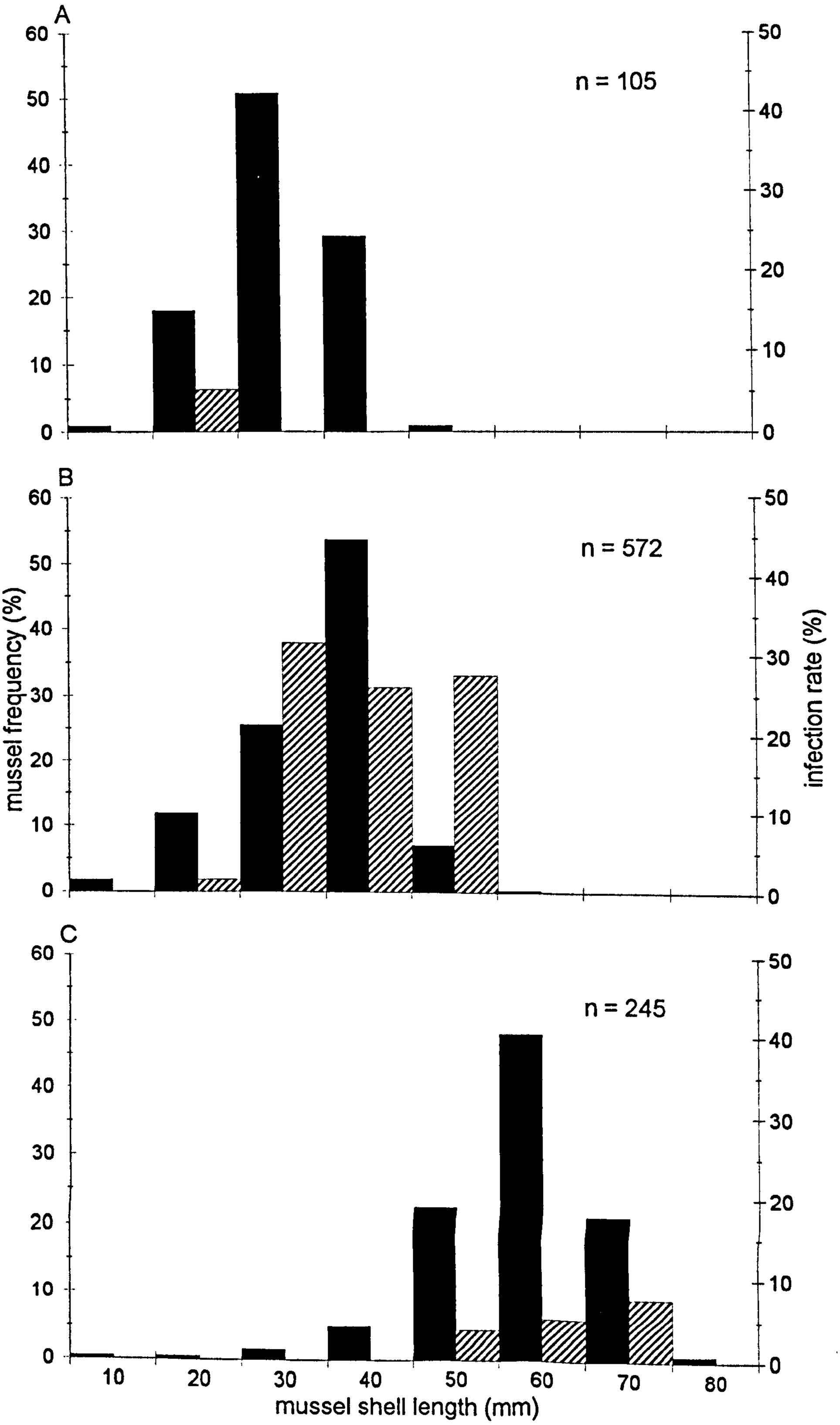
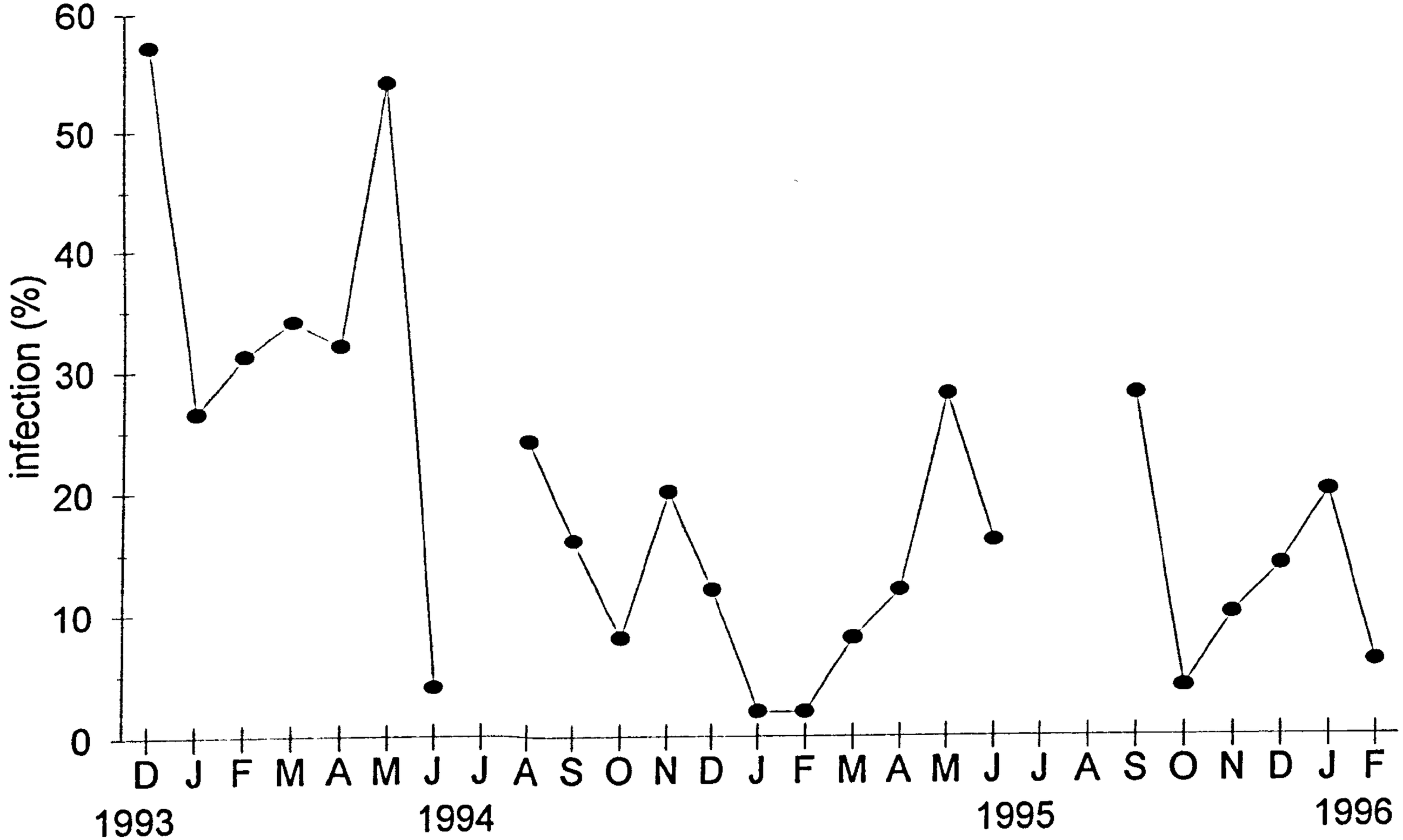

A
n = 105
B
n = 572
C
n = 245
mussel frequency (%)
infection rate (%)
mussel shell length (mm)
0
10
20
30
40
50
60
70
80

Figure 6.2 Seasonal variation in the infection rate of *Coccomyxa prasitica* within the host mussel, *Mytilus edulis chilensis,* from the mid region of the mussel population at Goose Green.


60
50
40
30
20
10
0
infection (%)
D J F M A M J J A S O N D J F M A M J J A S O N D J F
1993
1994
1995
1996

6.3.3. Effect of *Coccomyxa parasitica* upon the host mussel

Initial observations of moderate to heavily infested mussels indicated that some detrimental effect was incurred as a result of the presence of *C.parasitica*. The mantle tissue was slippery and translucent and the adductor muscle appeared weak and stringy. When regressions of dry tissue weight on shell length of infected (log dry tissue weight = log shell length x 2.91 - 5.44) and uninfected (log dry tissue weight = log shell length x 2.95 - 5.49) mussels were compared the slopes did not differ significantly ($F = 0.04$, $p > 0.05$), and a common slope was therefore assigned to the regression equations for infected and uninfected mussels :

infected : log dry tissue weight = log shell length x 2.93 - 5.44
uninfected : log dry tissue weight = log shell length x 2.93 - 5.49.

The intercepts were significantly different ($F = 919.39$, $p < 0.05$), the intercept of infected mussels being significantly lower than that of non infected mussels.

6.4. Discussion

The alga described in the present study, which occurs within the tissues of *Mytilus edulis chilensis* from the Falkland Islands, was identified as *Coccomyxa parasitica* following its marked similarities (Table 6.3) with *C.parasitica* observed in *Placopecten magellanicus* from Newfoundland, Canada (Naidu and South, 1970; Naidu, 1971; and Stevenson and South, 1974; 1975).

The occurrence of *Coccomyxa parasitica* in mussels from Goose Green but not the other two study sites, Darwin and Camilla Creek, is difficult to explain. The shells of mussels from Goose Green were generally in a poorer condition than at either of the other two sites; shell valves had also suffered considerable abrasion and several individuals appeared to have been subjected to severe growth impairment at the posterior shell margin, which may have made them more vulnerable to infection. Infection rates were not related to tidal elevation although there was some correlation with host age and shell condition. Mussels approximately 4 - 6 years of age with damaged or eroded shells tended to be more susceptible to infection than younger mussels with intact shells. Naidu (1971) similarly found that larger, older *Placopecten*

magellanicus, especially those with damaged or deformed shells, were particularly susceptible to infection by *C.parasitica*. Meixner (1984) also noted that colonies of the blue-green alga *Microcystis* only occurred in *Mytilus edulis* with damaged shells.

Table 6.3 Characteristic features of *Coccomyxa parasitica* from the Falkland Islands and Newfoundland.

Character	Falkland Islands	Newfoundland
Size	1.2 - 4.1 µm	1.0 - 11.0 µm
Shape	spherical - ovoid	spherical, ovoid, rod-shaped
Colour	green	green
Chloroplast	1 or 2	1, 2 or occasionally 3
Pyrenoid	none	none
Flagella	none	none
Reproduction	2 or 4 daughter cells	4, 8 or 16 daughter cells

The distribution of *C.parasitica* within the host tissues suggests that there is a relationship between the site of infection and light. The highest abundances occur on the mantle edge and in tissues located in the posterior territory of the shell, areas where light would normally penetrate during periods of gaping when the animal is immersed, although infection is typically observed in individuals which have experienced impaired shell growth. *Coccomyxa parasitica* is also relatively abundant within the posterior adductor muscle, a site where light penetration would not usually be expected; however, where the adductor was heavily infested the shell valves were eroded leaving a paper-thin, almost transparent shell layer. Conversely, in areas where the shell is intact and available light minimal, for example within the tissues located in the anterior territory of the shell, the abundance of the alga is considerably reduced. Naidu (1971) similarly suggests that the availability of light within the scallop *P.magellanicus* may be important in controlling the distribution of *C.parasitica* within the host tissues. In these scallops the highest levels of abundance occur on the mantle lobes, which are obvious sites for exposure to maximum light.

From the description, distribution and abundance of *C.parasitica* in the present study it may be hypothesised that the algal cells are taken up as part of the normal mussel

diet and then phagocytosed by leukocytes, which are subsequently distributed throughout the host tissues via the circulatory system of the host. A combination of shell damage, and thus increased levels of available light, as well as the possible resistance of *C.parasitica* to digestion could result in the establishment, growth and survival of this alga within the host mussel tissues. Naidu (1971) suggested that initial host - algal contact was associated with shell damage in *P.magellanicus* and that the alga gains entry through the host mantle after which it spreads along the mantle edge and into other uninfected tissues. Stevenson and South (1975), however, suggest that *C.parasitica* might enter *P.magellanica* via the normal process of feeding and digestion and that host phagocytosis, which is possibly increased due to an acceleration in shell deposition (a result of shell damage), could increase the chance of infection and contribute to the spread of this digestion-resistant alga.

The reduction in body condition of infected mussels suggests that *C.parasitica* is possibly a parasite of *M.e.chilensis*. However, the fact that mussels infected by *C.parasitica* also tend to have damaged shells, could imply that the decrease in condition is not due solely to the presence of the alga. Shell damage could have resulted in an overall reduction in the body condition of *M.e.chilensis*, which in turn may leave the animal more vulnerable to infection by a facultative parasitic alga such as *C.parasitica*. The host mussel may be directing some of the energy normally destined for tissue growth into shell secretion in order to repair damaged shell. Kent (1979) suggested that the reduction in body condition of *Mytilus edulis* infected by the shell boring polychaete, *Polydora ciliata*, could be due to an energy imbalance, whereby proportionately more energy than normal is provided for shell secretion, thus reducing that available for tissue growth. Energy partitioning is affected by several factors, for example, food supply, temperature, aerial exposure. Rodhouse *et al.* (1984a, 1986) found that naturally occurring intertidal mussels in Killary Harbour, western Ireland, allocated a greater proportion of their energy budget to reproduction compared to mussels cultivated on ropes which channelled most of their energy into somatic growth. Mussels from the Baltic, where predation is very low, produce relatively thin shells with small adductor muscles and allocate a significantly greater proportion of their energy budget to reproduction compared to mussels of similar size from fully marine areas (Kautsky *et al.*, 1990). Although Naidu (1971) concluded that *C.parasitica* was a parasite of *P.magellanicus* following his observations of a reduction in the body condition of infected scallops, Stevenson and South (1974) thought that at best *C.parasitica* was a facultative parasite after they successfully isolated the alga and grew

it on an inorganic media.

6.4.1. Conclusions

Coccomyxa parasitica occurs within the soft tissues of *Mytilus edulis chilensis* from Goose Green. Individuals which appeared to be particularly susceptible to infection were 4 - 6 years old with abraded shell valves and/or severe growth impairment at the posterior shell margin. The distribution of *C.parasitica* within the host tissues suggests that there is some relationship between the site of infection and light. Thus, *C.parasitica* is abundant within the posterior tissues where light would normally penetrate during periods of gaping, and within the posterior adductor muscle, where the overlying shell has been severely eroded.

It is hypothesised that the algal cells are taken up as part of the diet of the mussel and then phagocytosed by leukocytes which are subsequently distributed throughout the host tissue via the circulatory system of the host. A combination of shell damage, and thus increased levels of available light, as well as the possible resistance of *C.parasitica* to digestion could result in the establishment, growth and survival of this alga within the host mussel tissues.

Although the reduced body condition of infected mussels suggests that the alga is parasitic, the damaged shells of these individuals may also be contributing to this low body condition with the result that proportionately more energy is allocated to shell repair at the expense of tissue growth.

Chapter 7

General Discussion

Although *Mytilus edulis chilensis* is widely distributed throughout the Falkland Islands there is a paucity of information regarding the ecology of this animal in this part of its geographic range. As outlined in Chapter 1 representatives of the genus *Mytilus*, in particular *Mytilus edulis*, have been highly successful in their colonisation of many temperate shores around the world largely due to their abilities to withstand wide fluctuations in environmental factors (Seed, 1976; Seed & Suchanek, 1992 and references therein). The zonal distribution of *M.edulis* on a given shore is known to be under the control of several, sometimes interacting, factors. The upper limit is usually controlled by physical factors, such as desiccation and temperature (Suchanek, 1985), and the lower limit by biological factors, such as predation and competition (Paine, 1974). *Mytilus edulis chilensis* has an upper distributional limit similar to that recorded for populations of *M.edulis* (Baird, 1966; Seed, 1969b), typically occurring at around 60% aerial exposure, suggesting that the same physical factors are responsible. However, the apparent absence of predators in the Falkland Islands, with the exception of oystercatchers and seagulls, results in the lower limit of distribution being set by physical factors such as the type of substrata and sediment deposition. At Goose Green the mussel bed extends in a more or less continuous cover to a number of small offshore islands (see Chapter 2), whilst at Camilla Creek the mussel bed covers much of the estuary bottom. Only at Darwin is there a clear demarcation of the lower limit, and this is further highlighted by a change in substrate type from shingle, where mussels are present, to fine mud and shell debris, where there is a clear absence of mussels.

The ability to reproduce successfully and ensure the survival of the species is clearly important for any organism. Molluscs such as *Macoma balthica* (Gilbert, 1973) and *Modiolus modiolus* (Seed & Brown, 1978) begin to breed only after their somatic growth has become increasingly reduced, whereas *Mytilus edulis* (Bayne, 1975; 1976) and *Cerastoderma edule* (Seed & Brown, 1978) continue to grow during and between breeding seasons. The ability of *M.edulis* to survive, grow and reproduce in highly variable environments is well-known (see Seed & Suchanek, 1992 for review). Several important life history traits including a high growth rate, the ability to reach sexual

maturity at a relatively young age and a high fecundity (see Chapter 3 & 4) all contribute to the success of the species. *Mytilus edulis chilensis* has the ability to reproduce over several seasons and has a planktotrophic development, a 40 mm female discharging up to 1.92×10^6 eggs. Growth is maximal in the first year following settlement into the established population (see Chapter 4), during which sexual maturity is usually reached (< 25 mm in shell length; Chapter 3). Although there appears to be little predation pressure from either natural predators or humans, mortality resulting from severe weather conditions during the winter months appears to be high (see Chapter 2 & 3). Thus the need to reproduce efficiently is of considerable importance. Although the ability of a species to reproduce successfully throughout the year may be conducive to its survival, particularly if exposed to detrimental conditions which frequently result in high mortalities, the ability to reproduce continually is not necessarily controlled by endogenous factors, such as genotype and hormone cycles alone. In fact environmental variables such as temperature and food supply are known to exert considerable control over the reproductive cycle (Lubet & Aloui, 1987; Newell *et al.*, 1982; Kautsky, 1982a).

Mytilus is known to exhibit a wide range of reproductive strategies depending upon the particular environmental regime to which the organisms are subjected (Newell *et al.*, 1982). The timing and duration of the gametogenic cycle have been extensively used as the means for comparing these strategies (Seed & Suchanek, 1992 for review). A single relatively short cycle of gametogenesis occurs in *M.e.chilensis* in the Falkland Islands, broadly corresponding with increased seawater temperatures (12 - 14°C) during the austral summer. Temperature has been recognised as an important factor exhibiting broad control over the gametogenic cycle of mussels for some time. In fact geographical trends in the timing of gametogenesis further confirm this conclusion. In the northern hemisphere *M.edulis* from warmer more southerly populations generally spawn earlier than those from the cooler northern waters (Seed & Suchanek, 1992 and references therein), whilst in the southern hemisphere *Mytilus* from warmer, northern waters spawn earlier than those in the cooler southern regions (Wilson & Hodgkin, 1967; Kennedy, 1977; Dix & Ferguson, 1984; Gray *et al.*, in press). However, despite the broad control that temperature appears to have on the gametogenic cycle, it is the quality and quantity of food that are the principle controlling factors (Newell *et al.*, 1982; Kautsky, 1982a). The fact that the cycle of gametogenesis in *M.e.chilensis* is relatively short and occurs just once a year, strongly suggests that food availability is a major factor controlling the timing and duration of reproductive events. Despite the

apparent build up of nutrient reserves during the autumn months, a period when food remains available, further gametogenic cycles do not occur. In fact stored nutrients are quickly depleted during the winter months, when food availability is thought to be negligible, thus removing the source for any immediate process of redevelopment (see Chapter 3).

Reproductive output, a measure of energy available for reproduction, may account for a substantial proportion of the total production and standing crop of mussel populations (Griffiths & Griffiths, 1987), as well as representing a significant energy subsidy to the pelagic system (Kautsky, 1982a). Population reproductive output is largely controlled by the population size structure, since smaller individuals allocate considerably lower levels of energy to reproduction than their larger conspecifics which may eventually allocate virtually all of their surplus energy to reproductive development (Bayne & Worrall, 1980; Kautsky, 1982a; Sprung, 1983; Thompson, 1984; Rodhouse *et al.*, 1986). The reproductive output of populations of *M.e.chilensis* at the three study sites in the Falkland Islands were comparable to those documented for populations of *M.edulis* elsewhere (Bayne & Worrall, 1980; Kautsky, 1982a; Thompson, 1984), with up to 60% of the soft body weight being allocated to reproduction (see Chapter 3). This sizeable contribution to reproduction will serve to maintain the standing crop and provide a valuable food source for pelagic species in and around the coastal waters of the Falkland Islands.

The ability of a sessile species to locate and select a habitat compatible with adult survival is particularly important since once attached such species are not able to move elsewhere should conditions subsequently prove to be unsuitable. Organisms such as barnacles and mussels are highly gregarious and have evolved efficient attachment systems enabling them to grow and survive and be dominant space occupiers on many wave-exposed rocky shores (Crisp, 1984; Yonge, 1976; Morton, 1992). The highly gregarious nature of *Mytilus*, settling if not directly, then indirectly, into established mussel beds, is thought to be adaptive since *Mytilus* occurs predominantly in the intertidal or shallow subtidal zones and will therefore be subjected to mechanical forces of water movement. The reduced surface area exposed to such forces by mussels living in dense clusters makes clumps of mussels better able to withstand these forces than isolated individuals (Harger, 1972; Paine, 1974). The neotenic retention of the larval byssus system also plays an important role in the survival and success of *Mytilus* (Yonge, 1976). *Mytilus* post-larvae (individuals

that have settled and metamorphosed, usually at sites of temporary attachment) have the ability to enter a secondary pelagic phase (bysso-pelagic migration, drifting aided by the secretion of long fine byssus-like threads) prior to final settlement at sites of permanent attachment, usually the established population (Bayne, 1964).

Although settlement of plantigrades is highly seasonal and directly related to the reproductive cycle in some populations of *M.edulis* (Bayne, 1964; King *et al.*, 1989), in others settlement is often sporadic and may bear little relation to the reproductive cycle (Seed, 1969a; Dare, 1976). Considering that *M.e.chilensis* has a single, short, relatively well-defined spawning period, it might be expected that settlement/ recruitment would be similarly brief. However, settlement onto both artificial filamentous substrata as well as into the established population occurred more or less throughout the year (see Chapter 3). The apparently sporadic settlement into established populations can be explained by a period of over-wintering at sites of primary settlement away from the mussel beds (Seed, 1969a; Dare, 1976). This period of over-wintering, for which evidence was further provided in Chapter 4, has considerable adaptive implications. Firstly, it effectively reduces intraspecific competition, and secondly, it prevents the small, vulnerable postlarval stages from entering the strong inhalant currents of larger mussels (Seed & Suchanek, 1992), thus increasing the chances of survival of the new generation. Following the severe winter of 1995, during which large areas of mussel bed in the Falkland Islands were lost due to ice scouring and storms, settlement onto the remaining established mussel beds increased dramatically when compared to the previous year. This increase in settling plantigrades may be explained either by an increase in the energy allocated to reproduction, and thus increased numbers of larvae, or by an increase in food availability (see Chapter 3). However, regardless of the exact reasons for this increased settlement, the recovery of the population disturbed during the previous winter will be facilitated, in that any gaps within the mussel bed will be quickly recolonised as a result of the increased numbers of plantigrades.

Energy which is surplus to metabolic requirements is available for somatic growth and/or for gamete production (Seed & Suchanek, 1992). The relative quantities of energy allocated to growth and reproduction, however, vary according to age or body size (Bayne & Worrall, 1980; Kautsky, 1982a; Sprung, 1983; Thompson, 1984; Rodhouse *et al.*, 1986). Young mussels grow rapidly and direct little or no energy into reproduction, but with increasing size there is a gradual transition from somatic growth

to reproduction, so that in the largest mussels almost all this surplus energy is channelled into gamete synthesis. From investigations carried out during the course of this study into the growth and reproduction of *Mytilus edulis chilensis*, a similar pattern of energy partitioning appears to occur. The typically asymptotic pattern of growth which occurs in all of the populations studied, illustrates how young mussels grow rapidly in their first two or three years (particularly in the relatively faster growing populations) quickly approaching the asymptotic length imposed by the environment; thereafter growth slows considerably as the mussels gradually approach their maximum size (see Chapter 4). Further evidence for this relatively fast growth in young mussels is provided from the changes in the population length frequency distributions (see Chapter 4). Associated with this change from fast to relatively slow growth (in length) with increasing size (age) is the change in fecundity, which is relatively low in small mussels but much higher in larger individuals (see Chapter 3). Although the reproductive condition of mussels less than 25 mm in shell length was not investigated, the reproductive condition of 'small' mussels (25 - 35 mm in shell length) at Goose Green was significantly lower than both medium and large individuals.

The strategy of allocating energy initially to somatic growth and subsequently to reproduction has important ecological implications. Newly recruited mussels need to grow fast in order to minimise the effects of competition and any size-related predation. Moreover, the ability to allocate as much as 50 or 60% of the soft tissues to reproduction (see Chapter 3; Thompson, 1979; Kautsky, 1982a) results in a high reproductive output, thus contributing to the success of mussels as dominant space occupiers of the intertidal zone in many coastal areas.

The deposition of microgrowth bands present within the prismatic shell layer of *Mytilus edulis chilensis* is controlled by the spring-neap lunar cycle, which occasionally reflects the diurnal component of the mixed semi-diurnal tidal regime of the area. These growth bands exhibit a pattern whereby clear, regular bands are deposited during spring tides and weak ill-defined bands are formed during neap tides. These patterns were consistent with those described in *Mytilus edulis* (Richardson, 1989). The distance between individual and/or groups of tidal bands has been related to environmental conditions such that in temperate waters mussels grow slowly with narrow increments between individual bands during the cold winter months, and quickly in the warmer spring and summer months when growth increments are widely

spaced (Richardson *et al.*, 1990a; Richardson, 1993). As well as providing a detailed record of growth both short term, by means of tidally induced bands, and long term, by the seasonal narrowing of microgrowth bands within the prismatic shell layer, *M.e.chilensis* also has the ability to record the occurrence of anomalously high air temperatures to which mussels may be subjected during low water of spring tides (see Chapter 4).

The use of bivalve shells as chronometers of environmental change has been extensively investigated (Lutz & Rhoads, 1980; Richardson *et al.*, 1990a; Richardson, 1993). As well as providing evidence for changes in environmental conditions such as tidal exposure and temperature, human disturbance or attacks by predators, spawning breaks and algal blooms have all been identified within the shell structure of bivalves as periods when the normal pattern of shell deposition is temporarily interrupted (Richardson *et al.*, 1990a; Richardson, 1993). The ability of bivalves to incorporate within their shells a record of environmental change has useful applications both in the assessment of growth rates and longevity in natural and cultured populations, and for studying the effect on shell growth of anthropogenic inputs from industrial outfalls situated in environmentally sensitive areas such as harbours, estuaries and coastal embayments (Richardson *et al.*, 1990a; Richardson, 1993).

Shell shape in mytilids is of considerable ecological value (see Chapter 1). The heteromyarian form, coupled with the neotenic retention of the larval byssus apparatus, ensures that representatives of the Mytilidae are frequently the dominant space occupiers within the intertidal zone of many coastal areas. Although the basic heteromyarian form is retained in all mytilid species, there are several different morphs within each genus and even within each species. The factors responsible for producing these different shell morphologies are both genotypic and phenotypic. Although genotype is now thought to exert considerable control over shell shape (Kautsky *et al.*, 1990; Stirling & Okumus, 1994), the ability of other factors such as age, growth rate, population density and wave exposure have also been shown to have a significant effect upon shell shape (Seed, 1968). *Mytilus edulis chilensis* also exhibits a variety of shell morphologies (see Chapter 4), and these too appear to be related to age and growth rates as well as to environmental factors such as aerial exposure, food supply and possibly siltation.

Although the natural populations of *M.e.chilensis* investigated during this study did not

exhibit remarkably high growth rates, particularly when compared to commercial populations (see Hickman, 1992 and Table 4.10), the ability of individuals to increase by as much as 36 mm in shell length after just 12 months following transplantation to a more favourable site, indicates that the potential for using these animals for culture is high (Chapter 4). As well as an ability to grow quickly, mussels must also possess certain other attributes if they are to be suitable for culture. Fecundity must be relatively high in order to provide an adequate natural source of seed. *Mytilus edulis chilensis* is relatively fecund at Darwin and Goose Green, providing an annual supply of early plantigrades during the summer months which can be successfully collected on artificial filamentous substrates (see Chapter 3). Although the timing of early plantigrade settlement and peak reproductive activity were reasonably well correlated, occurring at similar times each year, settlement of late plantigrades was somewhat sporadic, in some cases bearing little relation to the timing of peak reproductive condition (see Chapter 3). The ability to forecast the timing of mussel settlement so that the supply of mussel seed is both regular and reliable is of paramount importance in mussel farming. However, despite extensive investigation into mussel settlement and its relationship with factors such as season, water depth, temperature and salinity, the ability to forecast settlement times has proved rather difficult, largely due to environmental variability (Cheong & Lee, 1984; King *et al.*, 1989; Perez-Camacho *et al.*, 1991). In areas where seed supply is either non-existent or limited, mussel seed are transported from areas where they are naturally abundant or where they are hatchery reared (Hickman, 1989; Nie, 1991).

The presence of the valviferan isopod, *Edotia doellojurdoi*, within the mantle cavity of *M.e.chilensis,* at all of the study sites investigated, could have implications for any future mussel cultivation in the Falkland Islands. The isopods are confined to host mussels from the lower reaches of the shore at Darwin and Goose Green, but occur at all shore levels within mussels from Camilla Creek. Although the presence of *E.doellojuradoi* does not appear to have any significant effect upon the host's body condition (dry flesh weight), it does seem to have a detrimental effect upon the hosts reproductive condition (see Chapter 3 and 5). Mussels farmed using on-bottom cultures may be more susceptible to infestation by these isopods, and although there may be no detrimental effect upon flesh content, the presence of the isopods would be unsightly and if not removed prior to sale the value of the product could be considerably reduced. Although the pathogenicity of organisms such as polychaetes (Kent, 1979), copepods (Paul, 1983) and pea cabs (Bierbaum & Ferson, 1986)

remains to be clarified, it is apparent that mussels cultured using off-bottom methods are generally less susceptible to infestations by these and probably other pathogens (Hickman, 1992). The entire life cycle of *E.doellojuradoi* is yet to be confirmed but the sequence of events proposed by Jaramillo *et al.* (1981) for *Edotia magellanica* infecting *Mytilus chilensis*, appears to provide an acceptable hypothesis which is consistent with the findings of this study. However, the means by which juvenile isopods leave their host mussels and find new, uninfected hosts needs to be resolved.

The presence of an apparently parasitic green alga, *Coocomyxa parasitica,* within the soft tissues of up to 16% of *Mytilus edulis chilensis* has further ecological as well as potential commercial implications. The alga appears to have a significantly detrimental effect upon the body condition (dry flesh weight) of the host mussel, and may therefore be causing a reduction in the reproductive ability of infected mussels. Although the occurrence of *C.parasitica* is confined solely to mussels within populations from Goose Green (see Chapter 6), infected individuals usually exhibit some degree of shell damage, indicating that shell damage is a significant factor influencing the infection of these mussels. The fact that there are no records of this alga infecting cultured mussels, is probably a direct result of the short growing times and the relatively sheltered subtidal habitats in which they are grown. Cultured mussels are generally harvested at between 6 months and 3 years old, when they have achieved market size (Hickman, 1992). However, populations of *M.e.chilensis* that are particularly susceptible to infection appear to be between 4 and 6 years old and live in the middle part of the intertidal zone (Chapter 6). In Newfoundland up to 43% of *Placopecten magellanicus* are infected by *C.parasitica*; infected scallops are in poorer condition and have dark and stringy meats that render them unfit for market (Naidu, 1971).

Until recently the sparsely populated, relatively non-industrial Falkland Islands have been subjected to little or no pollution. However, the increased shipping in both the fishing and cargo industries, as well as the rather rapid expansion of the capital Stanley, where there is no treatment of raw sewage, may well have resulted in increased levels of contaminants within adjacent coastal waters. More immediately, the potential development of a hydrocarbon industry, which will not only further increase the input of anthropogenic substances, but will also introduce the possibility of oil spills during the transfer of crude oil from the drilling platforms and the input of potentially toxic drilling muds used during the extraction process. Mussels, which are

locally abundant and widely distributed around the coast of the Falkland Islands, and which have already proved to be suitable biomonitors of coastal regions elsewhere around the world (see Widdows & Donkin, 1992 and Livingstone & Pipe, 1992 for reviews and Chapter 1), could be used for monitoring the levels of contaminants, both of human and hydrocarbon origin, and assist in regulating the input of any potentially harmful substances into the coastal regions of these Islands.

7.1 Further work

With regard to the subject matter of this thesis, there is clearly a need for further investigation in many areas. Firstly, more information regarding the quantity and quality of food in and around the study areas is required. This has obvious implications both for the reproductive strategy and growth of *M.e.chilensis*. The fact that gametogenesis occurs only once each year strongly suggests that food may be a limiting factor. Whilst the marked differences in reproductive condition and fecundity of mussels from the three study sites may be related at least partly to the presence of *E.doellojuradoi* and silt deposition, it could also result from local differences in food availability. Consequently, the location of areas of relatively high food supply would enable sites for potential mussel culture, where growth needs to be relatively fast, to be located. Secondly, longer term observations of settlement times and the density of mussel spat would provide valuable information for any potential mussel culture; such information would also prove central to any future studies of mussel biofouling. Thirdly, the exact nature of the relationship between the valviferan isopod, *E.doellojuradoi*, and its host needs to be resolved. In particular the source of the food supply of the isopod needs to be identified and the detrimental effects upon the host mussel further examined. Further details of the life cycle of this isopod are also required. Finally, the precise relationship between the green alga, *Coccomyxa parasitica*, and its host, *M.e.chilensis* is needed, in particular how and why the alga infects some mussels and not others, and how the alga is able to survive in what can only be considered as an unusual habitat.

Thus, in conclusion, despite the extensive information regarding the ecology of mussels, in particular *M.edulis*, from many parts of the world, this study has, hopefully, provided a valuable insight into the ecology of *Mytilus* from a part of its geographical range where, hitherto, it has been only poorly documented. The ecological information gained during this study should provide a background for further work not only on the

ecology of this important species, but also for any future mussel cultivation and/or use of mussels as biomonitors in the management of the coastal waters of the Falkland Islands.

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Appendix

1. Air and seawater temperatures (°C) recorded by TinyTalk temperature loggers situated in the middle of the intertidal zone (≈ + 0.7 m chart datum) at A. Darwin, B. Camilla Creek and C. Goose Green. Records from January 1995 to February 1996.

2. Seasonal volume fraction data for different tissue components of male and female *Mytilus edulis chilensis* from Darwin, Camilla Creek and Goose Green.

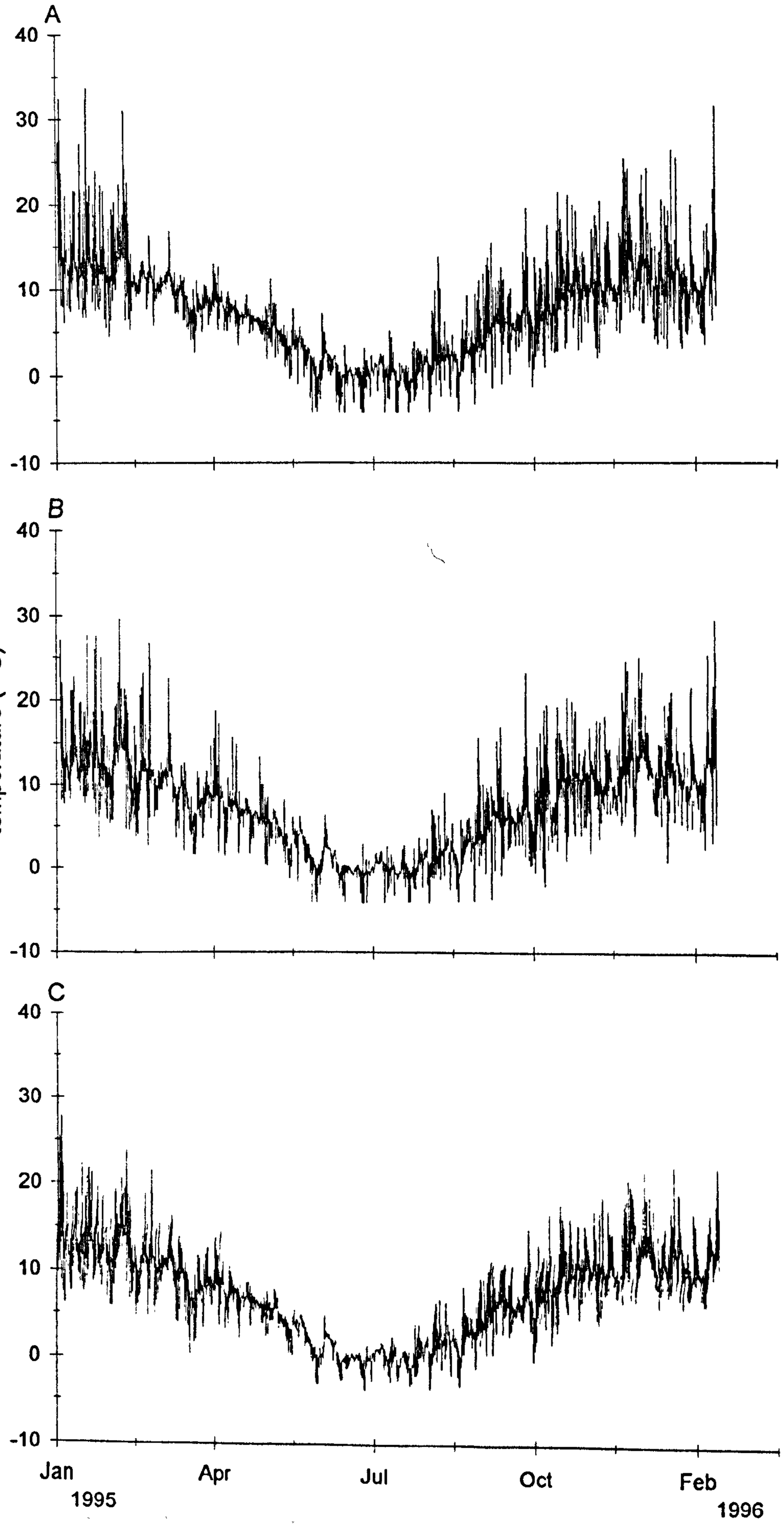
3. Seasonal variation in the settlement (mean number ± S.E. m^{-2}) of *Mytilus edulis chilensis* spat onto artificial substrate units at three study sites in the Falkland Islands.

Appendix 1 Air and seawater temperatures (°C) recorded by TinyTalk temperature loggers situated in the middle of the intertidal zone (≈ + 0.7 m chart datum). Records from January 1995 to February 1996.

A. Darwin

B. Camilla Creek

C. Goose Green


A
40
30
20
10
0
-10
B
40
30
20
10
0
-10
temperature (°C)
C
40
30
20
10
0
-10
Jan
1995
Apr
Jul
Oct
Feb
1996

Appendix 2.A Seasonal volume fraction data for different tissue components of male and female *Mytilus edulis chilensis* from Darwin

Date	n	Connective tissue		Developing gametes		Ripe gametes		Spent follicles	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
21/09/93	39	66.59	2.67	25.83	2.66	0.19	0.38	6.67	1.03
16/10/93	39	54.80	3.05	32.48	3.12	0.96	0.43	11.74	1.94
14/11/93	40	27.75	3.14	27.33	1.99	36.09	3.19	8.83	1.59
11/12/93	40	30.42	2.90	0.00	0.00	57.42	2.43	12.17	2.14
15/01/94	39	40.07	4.05	0.00	0.00	41.41	4.92	18.52	3.43
12/02/94	40	64.00	3.78	0.00	0.00	9.23	1.78	26.77	2.87
13/03/94	39	71.73	3.35	0.00	0.00	5.30	1.76	22.97	2.15
09/04/94	39	88.62	1.11	2.71	0.48	0.16	0.09	8.48	1.10
15/05/94	39	82.70	1.91	3.61	0.56	1.02	0.52	12.66	1.45
14/06/94	40	81.55	1.55	9.16	0.88	0.72	0.43	8.55	0.98
06/08/94	28	78.87	3.64	7.71	1.13	0.00	0.00	13.51	2.66
05/09/94	40	82.30	1.61	9.07	1.01	0.00	0.00	8.62	1.08
06/10/94	40	50.78	2.52	30.48	1.89	4.28	0.87	14.48	1.50
03/11/94	39	37.72	2.90	30.48	2.29	15.73	1.79	16.06	1.37
08/12/94	39	29.78	1.94	7.05	1.61	48.86	2.96	14.30	1.23
02/01/95	40	30.03	2.43	3.82	1.06	33.17	2.37	32.97	2.51
01/02/95	40	62.84	3.73	0.00	0.00	9.23	2.62	27.93	3.08
02/03/95	39	86.27	2.33	0.05	0.05	1.76	0.87	11.91	1.66
14/04/95	39	88.86	1.83	0.90	0.32	0.56	0.36	9.66	1.84
13/05/95	39	88.53	1.21	8.04	0.98	0.00	0.00	3.40	0.59
18/06/95	39	90.92	1.09	6.27	0.75	0.00	0.00	2.79	0.67
14/08/95	30	79.73	2.02	17.53	1.64	1.11	0.69	1.63	0.71
23/09/95	40	68.69	2.16	20.78	2.11	1.55	0.66	8.97	1.66
26/10/95	38	21.37	1.76	39.19	3.31	24.71	1.54	14.72	2.50
24/11/95	40	13.44	0.94	11.89	2.19	59.67	1.89	15.00	2.02
21/12/95	36	28.01	2.05	27.84	4.27	27.40	3.38	16.73	2.65
20/01/96	40	85.34	1.70	0.00	0.00	1.21	0.39	13.44	1.72
18/02/96	39	86.38	1.33	0.00	0.00	0.00	0.00	13.62	1.3

Appendix 2B Seasonal volume fraction data for different tissue components of male *Mytilus edulis chilensis* from Darwin

Date	n	Connective tissue		Developing gametes		Ripe gametes		Spent follicles	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
21/09/93	12	57.23	5.44	37.58	6.08	2.40	1.04	2.78	1.94
16/10/93	19	51.11	3.38	42.93	4.48	0.35	0.35	5.61	1.91
14/11/93	20	28.61	4.45	34.56	2.66	34.83	4.92	1.99	0.92
11/12/93	20	38.40	4.14	0.00	0.00	59.83	4.18	1.77	0.61
15/01/94	19	37.92	6.65	0.00	0.00	60.91	6.71	1.16	0.57
12/02/94	12	47.41	2.48	0.00	0.00	23.71	2.23	28.91	1.96
13/03/94	8	38.34	3.43	0.00	0.00	25.84	2.41	35.84	2.57
09/04/94	15	89.21	1.06	3.83	1.00	0.44	0.23	6.49	1.24
15/05/94	20	79.78	3.22	4.88	0.93	1.99	0.97	13.34	2.36
14/06/94	20	79.67	2.33	9.32	1.40	1.44	0.84	9.56	1.47
06/08/94	12	65.38	6.16	12.40	1.78	0.00	0.00	22.21	4.79
05/09/94	20	83.06	1.94	9.60	1.23	0.00	0.00	7.34	1.11
06/10/94	20	46.95	2.90	38.37	1.84	4.89	1.13	9.78	1.80
03/11/94	19	35.90	3.54	40.58	2.23	12.27	2.43	11.23	1.49
08/12/94	15	34.07	3.69	18.34	1.89	34.51	4.72	13.09	1.67
02/01/95	20	27.62	3.31	7.64	1.76	41.11	2.60	23.62	1.84
01/02/95	12	54.69	8.18	0.00	0.00	28.76	5.56	16.57	3.44
02/03/95	4	50.03	6.63	0.00	0.00	17.20	2.62	32.80	6.50
14/04/95	8	88.07	2.52	2.50	1.29	2.76	1.61	6.66	1.39
13/05/95	19	85.93	2.21	10.09	1.79	0.00	0.00	3.97	0.95
18/06/95	19	90.96	1.82	6.68	1.39	0.00	0.00	2.34	0.95
14/08/95	23	77.26	2.39	19.36	1.98	1.44	0.90	1.93	0.92
23/09/95	20	63.55	2.94	29.67	2.29	3.11	1.24	3.66	1.09
26/10/95	18	18.27	1.21	57.41	2.71	24.31	2.25	0.00	0.00
24/11/95	20	15.79	1.34	23.78	2.19	54.99	2.47	5.45	1.67
21/12/95	20	27.99	2.50	47.67	3.55	19.67	4.91	4.67	0.94
20/01/96	8	88.62	2.64	0.00	0.00	4.99	1.17	6.40	1.90
18/02/96									

Appendix 2C Seasonal volume fraction data for different tissue components of female *Mytilus edulis chilensis* from Darwin

Date	n	Connective tissue		Developing gametes		Ripe gametes		Spent follicles	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
21/09/93	27	70.75	2.71	20.60	2.13	0.24	0.24	8.41	1.09
16/10/93	20	58.31	4.97	22.57	3.05	1.55	0.75	17.57	2.79
14/11/93	20	26.88	4.53	20.11	1.91	37.34	4.16	15.66	2.14
11/12/93	20	22.43	3.26	0.00	0.00	55.01	2.50	22.57	2.64
15/01/94	20	42.12	4.85	0.00	0.00	22.89	4.11	35.01	4.02
12/02/94	12	45.31	4.09	0.00	0.00	7.07	1.69	47.64	3.68
13/03/94									
09/04/94	20	87.33	1.92	2.42	0.48	0.00	0.00	10.23	1.82
15/05/94	19	85.77	1.81	2.27	0.48	0.00	0.00	11.95	1.68
14/06/94	20	83.44	2.01	8.99	1.11	0.00	0.00	7.55	1.31
06/08/94	16	88.82	2.29	4.20	0.60	0.00	0.00	6.99	1.75
05/09/94	20	81.54	2.61	8.55	1.62	0.00	0.00	9.90	1.84
06/10/94	20	54.60	4.00	22.58	2.18	3.67	1.34	19.17	1.93
03/11/94	20	39.44	4.61	20.89	2.44	19.01	2.46	20.66	1.75
08/12/94	24	27.10	2.01	0.00	0.00	57.83	2.43	15.06	1.71
02/01/95	20	32.44	3.56	0.00	0.00	25.23	3.09	42.33	3.63
01/02/95	8	43.87	5.14	0.00	0.00	3.03	0.83	53.05	5.68
02/03/95	4	88.87	4.06	0.55	0.55	0.00	0.00	10.55	3.89
14/04/95	8	93.90	1.57	1.92	0.49	0.00	0.00	4.18	1.91
13/05/95	20	90.99	0.81	6.10	0.70	0.00	0.00	2.87	0.72
18/06/95	20	90.88	1.28	5.89	0.69	0.00	0.00	3.22	0.97
14/08/95	7	87.84	1.28	11.51	0.95	0.00	0.00	0.67	0.43
23/09/95	16	74.51	2.92	14.86	2.11	0.00	0.00	10.63	1.86
26/10/95	20	24.16	3.08	22.79	2.16	25.07	2.16	27.97	1.88
24/11/95	20	11.11	1.12	0.00	0.00	64.35	2.49	24.56	2.08
21/12/95	16	28.04	3.49	3.06	1.40	37.07	3.25	31.80	2.88
20/01/96	4	78.35	2.08	0.00	0.00	2.20	0.89	19.45	1.65
18/02/96									

Appendix 2.D Seasonal volume fraction data for different tissue components of male and female *Mytilus edulis chilensis* from Camilla Creek

Date	n	Connective tissue		Developing gametes		Ripe gametes		Spent follicles	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
15/09/93	39	69.14	3.30	16.65	2.22	2.22	0.78	11.97	1.64
16/10/93	37	50.38	4.34	27.57	3.41	13.58	2.92	8.47	1.60
14/11/93	40	70.56	4.15	13.99	2.49	8.64	2.39	6.80	1.11
11/12/93	39	42.19	3.76	4.27	1.22	47.78	3.89	5.75	1.16
15/01/94	40	57.17	3.76	0.00	0.00	22.94	3.30	19.89	3.23
12/02/94	40	72.50	3.99	0.00	0.00	15.67	3.29	11.84	1.27
13/03/94	38	82.86	2.98	0.05	0.05	5.55	1.88	11.52	2.10
09/04/94	20	82.53	4.86	2.33	0.69	7.78	3.63	7.34	1.30
15/05/94	22	74.76	5.49	1.55	0.55	12.73	3.81	10.93	2.27
14/06/94	39	82.03	2.55	4.95	1.03	0.00	0.00	13.02	1.79
06/08/94	39	85.24	3.09	2.79	0.67	6.78	2.90	5.17	0.70
05/09/94	35	91.47	1.32	2.31	0.65	0.00	0.00	6.21	1.30
06/10/94	40	73.69	2.58	17.61	2.39	1.55	0.60	7.14	0.97
03/11/94	38	52.55	4.42	22.19	2.46	10.52	1.69	14.75	1.80
08/12/94	40	58.58	4.06	16.16	2.43	12.10	2.42	13.16	2.53
03/01/95	38	53.57	3.36	7.72	1.51	9.27	1.34	29.44	3.34
01/02/95	38	88.96	1.43	0.00	0.00	0.87	0.38	10.16	1.22
02/03/95	40	81.22	2.71	0.00	0.00	3.22	1.58	15.56	1.64
14/04/95	40	94.17	0.80	1.43	0.40	0.00	0.00	4.38	0.85
13/05/95	40	90.30	2.46	1.38	0.35	1.86	0.94	6.44	1.63
18/06/95	38	85.34	1.81	8.26	1.15	1.92	0.69	4.46	1.14
23/09/95	39	84.74	2.17	10.42	1.63	0.00	0.00	4.81	0.79
26/10/95	39	55.30	3.55	24.48	2.85	4.39	1.25	15.83	1.59
24/11/95	40	28.50	2.46	30.28	2.68	21.11	2.48	20.12	1.92
22/12/95	36	34.80	3.60	12.52	1.94	33.87	3.78	18.81	2.78
20/01/96	40	68.18	3.49	0.83	0.32	12.29	2.27	18.69	2.26
18/02/96	40	81.15	2.63	0.00	0.00	5.57	1.70	13.28	1.88

Appendix 2.E Seasonal volume fraction data for different tissue components of male *Mytilus edulis chilensis* from Camilla Creek

Date	n	Connective tissue		Developing gametes		Ripe gametes		Spent follicles	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
15/09/93	16	73.81	4.52	17.80	4.01	1.80	0.88	6.55	1.41
16/10/93	19	40.12	5.78	38.95	4.91	17.31	5.11	3.62	1.17
14/11/93	12	44.81	5.81	33.71	2.43	20.55	5.75	0.91	0.42
11/12/93	20	31.34	4.70	8.00	2.05	60.56	4.50	0.11	0.11
15/01/94	16	48.62	4.24	0.00	0.00	43.20	3.84	8.19	1.53
12/02/94	16	45.41	3.95	0.00	0.00	38.90	3.24	15.70	1.67
13/03/94	8	59.99	4.93	0.00	0.00	25.83	3.75	14.15	3.00
09/04/94	4	42.20	6.61	4.98	2.29	38.90	3.80	13.90	3.66
15/05/94	15	67.03	7.20	2.28	0.74	18.67	4.89	11.99	3.17
14/06/94	8	83.64	3.11	7.21	2.37	0.00	0.00	9.16	2.64
06/08/94	20	76.77	5.31	4.01	1.17	13.22	5.32	6.00	0.82
05/09/94	3	86.23	6.47	7.77	5.32	0.00	0.00	5.97	1.83
06/10/94	20	65.75	3.01	28.19	2.90	1.00	0.57	5.05	1.34
03/11/94	19	48.14	4.95	32.46	2.83	8.68	2.14	10.74	1.16
08/12/94	20	47.26	4.72	28.76	2.53	17.20	4.50	6.77	1.41
03/01/95	19	68.57	2.66	13.81	2.16	6.69	1.67	10.93	1.33
01/02/95	4	72.80	2.47	0.00	0.00	7.22	1.07	20.00	2.01
02/03/95	4	39.45	6.12	0.00	0.00	32.22	3.80	28.33	3.79
14/04/95	12	95.56	0.98	4.05	0.90	0.00	0.00	0.36	0.36
13/05/95	12	77.66	6.90	1.47	0.68	6.20	2.86	14.64	4.57
18/06/95	23	81.50	2.52	9.43	1.74	3.19	1.08	5.90	1.77
23/09/95	23	81.41	3.26	12.54	2.43	0.00	0.00	6.04	1.22
26/10/95	20	44.20	3.66	37.84	3.13	0.78	0.54	17.18	2.24
24/11/95	20	25.11	2.79	43.44	2.50	19.66	4.49	11.78	1.91
22/12/95	19	20.73	2.77	21.67	1.52	49.18	2.97	8.42	1.66
20/01/96	12	68.45	4.89	0.00	0.00	20.05	3.86	11.49	2.25
18/02/96	8	67.09	7.18	0.00	0.00	20.93	5.55	11.97	2.40

Appendix 2.F Seasonal volume fraction data for different tissue components of female *Mytilus edulis chilensis* from Camilla Creek

Date	n	Connective tissue		Developing gametes		Ripe gametes		Spent gametes	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
15/09/93	20	63.89	5.04	18.22	2.59	2.89	1.35	14.99	2.44
16/10/93	18	61.21	5.59	15.57	2.69	9.64	2.46	13.58	2.58
14/11/93	16	72.10	5.55	9.69	2.54	6.18	2.79	12.02	1.78
11/12/93	19	53.62	4.75	0.35	0.35	34.32	4.86	11.69	1.42
15/01/94	16	45.98	2.92	0.00	0.00	14.16	2.49	39.87	4.51
12/02/94	4	82.80	5.63	0.00	0.00	1.10	0.63	16.10	5.70
13/03/94	4	57.78	6.55	0.55	0.55	1.10	1.10	40.55	7.78
09/04/94	12	92.93	1.33	2.22	0.72	0.00	0.00	4.82	1.02
15/05/94									
14/06/94	15	70.07	4.89	9.03	1.63	0.00	0.00	20.89	3.54
06/08/94	8	93.06	1.66	3.58	0.72	0.00	0.00	3.31	1.57
05/09/94	17	93.84	0.79	3.38	0.74	0.00	0.00	2.75	0.70
06/10/94	16	79.26	4.04	8.78	2.00	2.64	1.30	9.32	1.48
03/11/94	15	46.07	6.86	15.11	2.23	15.65	2.71	23.18	3.13
08/12/94	20	69.90	5.64	3.55	1.06	7.00	1.07	19.55	4.48
03/01/95	19	38.57	3.78	1.64	0.75	11.84	1.96	47.95	2.46
01/02/95	4	91.10	3.74	0.00	0.00	1.10	0.63	7.80	3.22
02/03/95									
14/04/95	4	95.03	2.92	2.20	1.27	0.00	0.00	2.77	2.11
13/05/95	16	93.89	1.06	2.35	0.62	0.00	0.00	3.73	0.80
18/06/95	15	91.23	1.55	6.48	1.09	0.00	0.00	2.26	0.76
23/09/95	16	89.54	2.02	7.38	1.70	0.00	0.00	3.05	0.64
26/10/95	19	66.99	4.99	10.42	1.66	8.18	2.21	14.41	2.28
24/11/95	20	31.88	3.98	17.11	2.21	22.56	2.22	28.45	2.06
22/12/95	11	40.66	4.34	3.55	2.18	25.89	5.15	29.89	5.92
20/01/96	12	44.26	3.88	2.76	0.87	20.92	3.92	32.04	4.15
18/02/96	12	70.56	3.51	0.00	0.00	4.62	1.41	24.83	4.17

Appendix 2.G Seasonal volume fraction data for different tissue components of male and female *Mytilus edulis chilensis* from Goose Green

Date	n	Connective tissue		Developing gametes		Ripe gametes		Spent gametes	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
27/09/93	33	72.26	3.03	21.11	2.65	1.40	0.50	5.22	0.92
16/10/93	39	25.97	2.30	29.99	2.61	39.24	2.97	4.80	1.14
14/11/93	40	13.25	2.15	11.21	1.81	67.99	2.30	7.55	1.61
11/12/93	40	30.73	4.43	6.02	1.17	51.10	3.66	12.14	1.80
15/01/94	39	42.67	3.57	0.06	0.06	27.30	3.55	26.96	2.35
12/02/94	40	66.28	4.02	0.42	0.25	10.73	2.79	22.56	2.75
13/03/94	40	82.96	2.12	0.89	0.36	2.75	0.89	13.40	1.60
09/04/94	38	65.60	3.29	7.68	0.84	0.00	0.00	26.72	2.96
15/05/94									
14/06/94	40	70.86	2.64	14.16	1.23	1.38	0.53	13.60	2.49
06/08/94	32	79.25	2.65	6.85	1.17	0.00	0.00	13.90	2.18
05/09/94	39	76.03	2.08	15.36	1.71	0.00	0.00	8.60	1.39
06/10/94	40	42.28	2.44	35.56	1.89	8.80	1.15	13.34	2.07
03/11/94	40	21.19	2.57	18.68	2.47	38.55	2.33	21.57	2.30
08/12/94	40	20.60	2.35	5.39	0.91	64.34	2.27	9.66	2.02
01/01/95	40	29.29	2.20	2.53	0.80	38.79	2.83	29.39	2.51
01/02/95	40	82.57	1.68	0.00	0.00	0.55	0.31	16.87	1.66
02/03/95	40	86.03	1.47	0.00	0.00	0.85	0.47	13.11	1.45
14/04/95	39	91.68	0.96	4.26	0.64	0.22	0.15	3.80	0.68
13/05/95	28	85.19	1.49	8.45	1.34	3.17	1.03	3.19	0.80
18/06/95	40	90.53	1.12	7.85	0.94	0.44	0.34	1.16	0.29
23/09/95	39	73.83	1.95	22.34	1.72	1.48	0.72	2.33	0.60
26/10/95	40	29.06	1.90	42.31	1.78	23.40	1.63	5.22	1.07
24/11/95	40	17.18	1.78	17.07	2.84	49.97	2.52	15.78	1.76
21/12/95	40	14.83	1.94	12.55	2.47	51.63	3.23	20.97	3.01
20/01/96	40	31.36	3.09	0.16	0.16	35.60	3.55	32.89	3.61
18/02/96	40	41.39	4.11	0.00	0.00	29.51	4.95	29.11	3.40

Appendix 2.H Seasonal volume fraction data for different tissue components of male *Mytilus edulis chilensis* from Goose Green

Date	n	Connective tissue		Developing gametes		Ripe gametes		Spent follicles	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
27/09/93	17	73.38	4.50	20.36	4.23	2.73	0.86	3.53	1.15
16/10/93	20	29.40	3.36	41.82	2.33	28.68	3.47	0.11	0.11
14/11/93	20	8.69	1.75	21.17	1.70	68.89	2.42	1.24	0.44
11/12/93	20	13.56	2.69	12.04	1.34	65.87	2.52	8.52	2.15
15/01/94	16	43.66	6.86	0.15	0.15	33.62	5.02	22.55	2.77
12/02/94	12	63.73	7.35	1.40	0.80	22.44	6.03	12.42	2.36
13/03/94	16	85.26	1.91	2.23	0.81	3.66	1.20	8.84	1.69
09/04/94	20	53.09	3.50	10.22	1.16	0.00	0.00	36.70	3.32
15/05/94									
14/06/94	20	63.28	4.32	16.65	2.10	2.77	0.97	17.31	4.65
06/08/94	8	63.71	5.25	12.31	2.99	0.00	0.00	23.95	5.99
05/09/94	19	68.96	3.17	21.08	2.54	0.00	0.00	9.96	2.69
06/10/94	20	45.44	3.56	42.62	2.19	8.43	1.79	3.49	1.02
03/11/94	20	26.87	3.73	31.80	2.31	30.72	2.67	10.59	1.48
08/12/94	20	26.58	3.74	10.12	0.97	62.79	3.47	0.49	0.29
01/01/95	20	29.42	2.77	5.07	1.40	45.97	3.25	19.55	2.11
01/02/95									
02/03/95	4	82.88	4.39	0.00	0.00	8.57	2.64	8.55	2.03
14/04/95	20	92.34	1.26	4.44	0.83	0.44	0.30	2.77	0.64
13/05/95	13	81.65	1.84	8.95	2.27	6.84	1.74	2.56	1.15
18/06/95	20	89.93	1.96	8.72	1.55	0.88	0.68	0.44	0.25
23/09/95	19	70.03	2.18	28.66	2.07	0.58	0.23	0.70	0.41
26/10/95	20	22.80	1.80	50.28	1.69	26.91	2.40	0.00	0.00
24/11/95	20	19.91	2.10	33.57	2.06	39.47	2.84	7.05	1.54
21/12/95	20	14.33	2.84	25.10	2.92	55.67	5.39	4.87	1.44
20/01/96	20	35.00	4.97	0.33	0.33	50.44	3.72	14.24	2.20
18/02/96	16	20.92	2.43	0.00	0.00	65.63	2.93	13.47	1.57

Appendix 2.I Seasonal volume fraction data for different tissue components of female *Mytilus edulis chilensis* from Goose Green

Date	n	Connective tissue		Developing gametes		Ripe gametes		Spent follicles	
		mean	S.E.	mean	S.E.	mean	S.E.	mean	S.E.
27/09/93	16	71.07	4.17	20.90	3.23	0.00	0.00	7.03	1.37
16/10/93	19	22.37	3.02	17.54	2.58	50.36	3.41	9.74	1.72
14/11/93	20	17.81	3.70	1.25	0.35	67.08	3.98	13.86	2.50
11/12/93	20	47.91	6.50	0.00	0.00	36.33	5.08	15.76	2.71
15/01/94	19	39.43	4.16	0.00	0.00	27.72	5.14	32.84	3.15
12/02/94	8	31.45	4.47	0.00	0.00	20.00	6.91	48.52	5.54
13/03/94	4	59.20	14.00	0.00	0.00	12.88	5.34	28.00	8.70
09/04/94	15	83.07	2.95	5.54	0.90	0.00	0.00	11.39	2.29
15/05/94									
14/06/94	20	78.44	1.96	11.68	1.06	0.00	0.00	8.89	1.57
06/08/94	20	83.10	2.57	6.04	1.04	0.00	0.00	10.88	1.96
05/09/94	20	82.75	1.74	9.93	1.54	0.00	0.00	7.32	0.92
06/10/94	20	39.12	3.28	28.50	2.14	9.17	1.48	23.19	2.53
03/11/94	20	15.51	3.12	5.55	1.27	46.38	2.94	32.54	2.64
08/12/94	20	14.63	2.23	0.67	0.32	65.88	2.98	18.83	2.79
01/01/95	20	29.16	3.49	0.00	0.00	31.62	4.13	39.22	3.36
01/02/95									
02/03/95									
14/04/95	15	91.55	1.85	5.17	1.12	0.00	0.00	3.25	1.14
13/05/95	15	88.26	2.01	8.01	1.61	0.00	0.00	3.74	1.14
18/06/95	20	91.12	1.13	6.99	1.08	0.00	0.00	1.88	0.49
23/09/95	20	77.44	3.02	16.33	1.94	2.34	1.39	3.88	1.00
26/10/95	20	35.33	2.74	34.34	1.88	19.88	1.97	10.44	1.36
24/11/95	20	14.45	2.79	0.57	0.57	60.47	2.54	24.50	1.53
21/12/95	20	15.34	2.69	0.00	0.00	47.60	3.48	37.06	2.81
20/01/96	20	27.72	3.63	0.00	0.00	20.75	3.85	51.53	3.46
18/02/96	16	42.41	4.58	0.00	0.00	8.14	2.00	49.44	4.59

Appendix 3 Seasonal variation in the settlement (mean number ± S.E. m^{-2}) of *Mytilus edulis chilensis* spat onto artificial substrate units at 3 study sites in the Falkland Islands

Date	Darwin	Camilla Creek	Goose Green
03/11/94	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
08/12/94		0.00 ± 0.00	41.11 ± 16.81
03/01/95	679.52 ± 77.05	0.00 ± 0.00	453.01 ± 193.89
01/02/95	4674.24 ± 1519.40	20.59 ± 16.81	473.60 ± 67.25
02/03/95	947.20 ± 177.93	0.00 ± 0.00	267.69 ± 102.27
14/04/95		0.00 ± 0.00	164.73 ± 16.81
14/05/95		0.00 ± 0.00	20.59 ± 16.81
18/06/95	61.77 ± 29.12	0.00 ± 0.00	41.18 ± 16.81
16/07/95	195.62 ±46.80	0.00 ± 0.00	34.32 ± 14.82
14/08/95	195.62 ± 46.80	0.00 ± 0.00	34.32 ± 14.82
23/09/95		0.00 ± 0.00	32.32 ± 14.82
26/10/95	41.18 ± 33.63	0.00 ± 0.00	102.96 ± 16.81
24/11/95	82.37 ± 44.48	0.00 ± 0.00	0.00 ± 0.00
22/12/95	20.59 ± 16.81		20.59 ± 16.81
20/01/96	185.32 ± 0.00	0.00 ± 0.00	20.59 ± 16.81
18/02/96	102.96 ± 16.81	20.59 ± 16.81	164.73 ± 84.06