Ecology and Ecophysiology of *Katelysia scalarina* (Bivalvia: Veneridae), a commercially exploited clam.

(Maree)
Lynda Bellchambers B.Sc. Hons (Tas)

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, University of Tasmania (February, 1998)

Aquaculture

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of the author's knowledge and belief the thesis contains no copy or paraphrase of material previously published or written by other persons except when due reference is made in the text of the thesis.

This thesis may be made for loan and limited copying in accordance with the Copyright Act, 1968.

Lynda Bellchambers

"But as for certain truth no man has known it,

Nor ever shall.

And even if he were by chance to utter the final truth,

he would himself not know it.

For all is but a woven web of guesses"

Xenophanes

Abstract.

The general aim of this study was to increase knowledge of *Katelysia scalarina* an intertidal suspension feeding bivalve common in many of the sheltered bays and estuaries of southern Australia. Specifically, attention has been focused on the factors that may determine the distribution and abundance of natural populations. Secondly, information was gathered to help ensure effective management of natural populations to prevent over-exploitation and provide a basis for future aquaculture developments.

The effects of density and tidal level on the survival, growth and meat ratio of *K. scalarina* were examined using a series of caged manipulation trials. Both density (171.5-686.1 clams/m^2) and tidal position had significant effects on the survival and growth of *K. scalarina* with a decrease in survival and growth evident with increasing distance from the low tide mark. Shell growth at high tidal positions was approximately half of that lower on the shore, which may be due to the depletion of food resources. In contrast, meat ratio displayed a direct relationship with tidal position, as a result of suppressed shell growth at high tidal positions.

In contrast to the above trial, the effects of stocking density were much smaller than that of tidal level. None of the measured parameters displayed a significant response to density manipulations of *K. scalarina* grown at a single tidal position. The failure of *K. scalarina* to respond to density treatments up to thirty times that of the natural population may in part be due to the location of the experimental treatments. Experimental cages situated low in the intertidal zone may provide suspension feeders access to a very abundant food source, negating the effects of artificially enhanced densities.

The salinity tolerance of adult and juveniles was investigated using a series of acute (21d) toxicity trials (5-55‰). Results indicate that adults are intolerant of low salinities (<25‰), while juveniles have a wide salinity tolerance range. Despite differences in the salinity tolerance of adults and juveniles, there were no significant differences in their ability to osmoregulate. *K. scalarina* is essentially an osmo- and ionic-conformer, with the possible exception of K^+, that relies on the mechanism of shell valve closure to isolate the body tissues from unfavourable salinities. Results indicate that *K. scalarina* relies on regulation of the free amino acid pool to cope with fluctuations in the external medium.

Although *K. scalarina* is capable of surviving a salinity range of 25-50‰, the zone for optimal growth may be a narrower band within this range. Respiration and algal clearance trials were conducted to determine whether irregular valve closure patterns limit oxygen consumption and algal clearance which may in turn limit growth potential. Oxygen consumption was depressed in salinities outside 35‰ even though these salinities were within the tolerance range. However, evidence from algal clearance trails was not so clear cut. Juveniles display a decrease in algal consumption in salinities > 45‰ and < 30‰.

The potential for *K. scalarina* to be grown as a by-crop on existing oyster farms in Tasmania promoted the investigation of the natural food sources of the species. Besides using the existing infrastructure of established marine farms these areas offer a number of additional food sources due to organic enrichment from pseudofaeces and biodeposition. The stomach contents of clams situated both below existing oyster racks and away from oyster racks indicated that *K. scalarina* is a suspension feeder that relies primarily on phytoplankton present in the water column for nutrition. This indicates that potential exists for competition for food resources if *K. scalarina* is grown in oyster growing areas in southern Australia. However, *K. scalarina* exhibited poor survival in cages when grown on several commercial oyster leases.

Despite commercial exploitation of *K. scalarina*, this study is the first comprehensive investigation of the species and therefore provides a valuable resouce for the management of wild populations and future aquaculture ventures. Finally, this study contributes significantly to the existing knowledge of a dominant component of the southern Australian estuarine fauna.

Acknowledgements

"I have only reached this happy state after having for many years suffered every possible kind of toil"
Sindbad the Sailor, Tales from the Arabian Nights

However this happy state was not reached without the assistance of many people to whom I would like to extend my sincere gratitude:

My supervisors; Dr G. B. Maguire who provided financial and logistic support as well as encouragement and criticism from afar!

Assoc. Prof. A. M. M. Richardson who dealt with my dramas and dramatic field trips on a daily basis.

Fishing Research and Development Corporation for the provision of a PhD scholarship.

The Department of Aquaculture and staff for logistic support, particularly Mr G. Kent and Dr. S. Hodson.

The Department of Zoology and staff, for treating me as "one of their own"

My field assistants; Nicki Chilcott, Helen Glasick, Chris Hankin, Colin McCoull, Jason Whitehead, Pam and Fiona Taylor, Trudy and Jenny Murphy, Martina Doblin and Karen Westwood, who soon learnt that marine science is seldom glamorous.

Department of Primary Industry and Fisheries, particularly Sean Riley and Rob Green for their advice and willingness to assist.

Parks and Wildlife for allowing access to Moulting Lagoon, safeguarding experimental treatments and answering the queries of curious residents.

Mr John Aadlers and the crew at Duck Bay Oysters for providing access to their oyster lease, willing helpers and keeping me amused with colourful tales.

Marine Shellfish Hatcheries and crew for providing space and assistance.

Ms Andrea Cole, Freycient Marine Farm, and Mr George Sumner, Coal River Oysters, for allowing me access to their oyster leases for pilot trials.

Kileunda Abalone Farm, in particular to Anton Krsinich for the speedy delivery of algal cultures.

Aquatic Botany for use of culturing facilities and provision of the Graeme Lush Memorial Trust Fund. Thanks in particular to Graeme and Ian who tried in vain to convince me that algae make the world go round!

Dr. S. Edwards for persevering despite my PC illiteracy and insistence that bivalves are supreme beings.

Dr N. Andersen for use of the osmometer.

Clinical Chemistry for processing blood samples.

Dr G. Knott, for hours spent in the darkroom.

Nicki Chilcott for assistance with formatting and maps and Kerrie Swadling for editorial advice.

Although gratitude is probably not an appropriate term; to my officemates and fellow PhD students for the constant supply of "interesting" sights and sounds and their unique perspective on life!

To my friends, especially Mart, thanks for indulging my caffeine habit, listening to my whining and attempting to save what remains of my sanity.

To my parents for their unwavering faith in me.

TABLE OF CONTENTS

Chapter 1:

General Introduction.

1.1 The Veneridae

The Veneridae comprise a large, diverse and possibly polyphyletic molluscan family, with as many as 500 species (Nielsen, 1963; Boss, 1982), chiefly composed of shallow-burrowing suspension filter feeders. The venerid genus *Katelysia* includes some of the most abundant and productive shallow water bivalves along the south coast of Australia, where they are frequently the major faunal component of shallow estuarine and coastal embayments (Wells & Roberts, 1980; Wells & Threlfall, 1980; Coleman, 1982; Roberts, 1983). At present, members of this genus are distributed from Augusta (Western Australia) to Port Jackson (New South Wales) with the most northern population of *Katelysia scalarina* present at Hutt Lagoon, Western Australia (Figure 1.1). The present range of *K. scalarina* does not extend to the west coast of Australia, perhaps due to the lack of sheltered waters between Cape Leeuwin and Cape Naturaliste (S. Slack-Smith pers. comm.). However, judged by their presence in the extensive Pleistocene marine deposits on Rottnest Island, and the middle Holocene and early Pleistocene shell beds of the Perth Basin, this distribution formerly extended further north on the west coast (Nielsen, 1963). Their present distribution is a result of geological changes over the last 4-5 thousand years (Roberts, 1983).

1.2 Clam Fisheries

Commonly known as cockles or clams, venerids have long been used as a food source, although it is only in recent years that they have become established as a delicacy in Japan, Western Europe, and the United States of America (de Franssu, 1990). While traditionally they have been an inexpensive food source this is no longer the case and in some countries particular species are now considered luxury foods. Although research has been limited, venerids include some of the more valuable cockles world-wide including the commercially important genera *Venus*, *Chione*, *Mercenaria*, and *Tapes*. The production of clams and cockles has become a significant industry with world wide production of 1.8 million tonnes in 1993 exceeding that of oysters (1.1million tonnes) (Maidment, 1997).

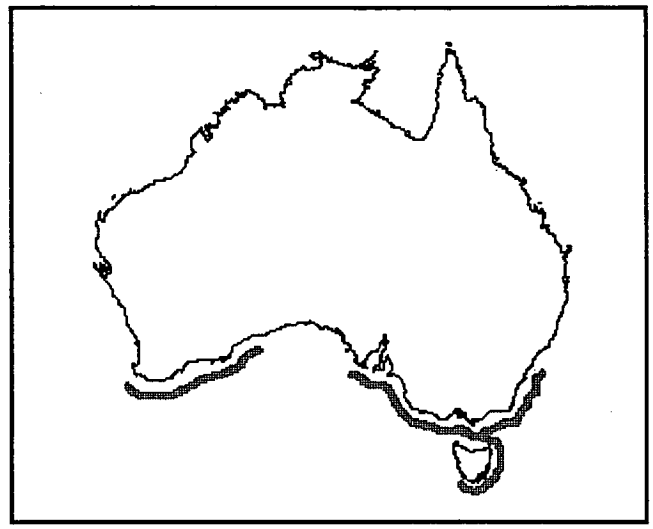

Figure 1.1: Distribution of *K. scalarina* in southern Australia

Approximately 70 species of clams world wide are considered as major regional fisheries, of these approximately 12 are also intensively cultured (Manzi & Castagna, 1989). A considerable amount of research has been conducted on the basic biology and environmental requirements of many North American commercial species such as *Mercenaria mercenaria* (quahog), *Mya arenaria* (soft shelled clam), *Spisula solidissima* (surf clam) and *Ruditapes philippinarum* (Manila clam). In Australia demand is increasing for diversification of shellfish products, with strong markets, particularly in Asia, making clams prime aquaculture candidates (Treadwell *et al.*, 1992).

In Australia, significant clam and cockle fisheries are emerging with a number of different species harvested in the southern states; Tasmania, New South Wales and South Australia. The bulk of production in Tasmania involves the stepped venerid, *Katelysia scalarina* and the New Zealand venerid, *Ruditapes largillierti.* Established in 1987 the Tasmanian clam industry is presently in its infancy, but with the establishment of Asian markets has the potential to expand rapidly. In 1987 live product wholesaled for $3.00/kg and no catch restrictions applied to the industry. However, fisheries managers have now imposed restrictions on the number of fishermen, catch quota and harvestable areas, providing a framework for preventing unsustainable expansion and degradation of fishing areas through the use of mechanical fishing methods. Currently approximately 31.1 tonnes of *Katelysia,* consisting of *K. scalarina* and *K. rhytiphora*, are harvested annually from a number of the larger bays and estuaries on east and north coast of

Tasmania (Department of Primary Industry and Fisheries, pers. comm). All the product is sold on the domestic market obtaining approximately $7.00-10.00/kg.

The primary species of clams and cockles harvested in southern New South Wales are a sublittoral bivalve, *Glycymeris flammea*, the beach pipi, *Donax deltoides* and *Tapes dorasatus* (Maguire, 1991). However, South Australia has the largest commercial harvest of clams and cockles. Two genera of clams and cockles are harvested in South Australia; the goolwa cockle or pipi, *Donax deltoides* and the mud cockle, which includes three species of the genus *Katelysia*; *K. scalarina*, *K. peroni* and *K. rhytiphora*. *Donax deltoides* is primarily harvested from high energy beaches using rakes and comprise the bulk of the South Australian clam and cockle fishery with 345 tonnes harvested in 1996/97. In contrast 73.7 tonnes of *Katelysia* were harvested in the same time period (South Australian Research and Development Institute, pers. comm.).

Clams in southern Australia are primarily harvested from natural beds. However, in recent years increasing attention has been focused on clam mariculture as a means of preventing overfishing, increasing supply to meet market demands and enhancing natural populations. In fact, clam fisheries world-wide are declining because of a number of factors, including pollution and overfishing (Manzi & Castagna, 1989). In response to declining natural populations interest in aquaculture has increased, intensive semi-controlled clam culture has been practised in North America for almost three decades (Manzi & Castagna, 1989). Similarly, it is considered that some form of aquaculture will be necessary to sustain a significant clam industry in Tasmania (Maguire, 1991). Although further expansion of the Tasmanian clam fishery in the short term is unlikely because of environmental concerns over harvesting additional areas, there may be some development opportunities in the area of enhancement of existing wild clam stocks. Successful re-seeding of hatchery reared spat to suitable on-growing sites will increase production and help ensure sustainable harvesting of clam resources.

1.3 *Katelysia* in Australia

Three species of *Katelysia* are present in southern Australia: *K. scalarina* Lamarck, 1818, *K. peroni* Lamarck, 1881 and *K. rhytiphora* Lamy, 1935. *Katelysia spp.* are usually found in sheltered sandy bays and estuaries. In southern Australia *K. scalarina* lives between the tide marks in fine to medium grain sand approximately two to four centimetres below the surface (Bellchambers & Richardson, 1995) (Plate 1.1). At high tide they are covered by

0.3 - 1.0 m of water. *K. scalarina* appears to be restricted to the intertidal zone, where it is not uniformly distributed but aggregates in groups of six or more (Nielsen, 1963). Although there may be some overlap, the zonation of *K. rhytiphora* differs from that of *K. scalarina*. *K. scalarina* is found between the tide marks while *K. rhytiphora* occurs below the low tide mark, sometimes buried in seagrass beds, with a few individuals existing within the *K. scalarina* zone (Nielsen, 1963). *K. peroni* generally occurs towards the upper limits of the *K. scalarina* zone towards the high tide mark and is generally uncommon in most locations, particularly in Tasmania.

Relatively little is known about the biology of *K. scalarina*, although a large amount of research has been conducted on other species of clams and cockles overseas. Nielsen (1963) examined the anatomy, distribution, general ecology and morphology of *K. scalarina* and *K. rhytiphora* (*K. peroni* was omitted due to insufficient samples) and discussed their association with a number of communal and parasitic animals. This work was conducted to resolve the complex synonymy of the three species and concluded that although there were no marked differences in gross anatomy of the three species they were distinct species, based on shell morphology, with a reduced chance of interbreeding. While the three species are anatomically distinct, misidentifications can occur when shell anatomy is used (Soh *et al.*, submitted). However, they can be separated conclusively using allozyme patterns and this technique has also provided evidence of occasional hybridisation of *K. scalarina* and *K. rhytiphora* (Soh *et al.*, submitted). Roberts (1981, 1983) also published observations of *K. scalarina* populations in Augusta and Albany (WA), including aspects of their anatomy, distribution and reproduction. Woodward (1985) documented recruitment, population density and predation of *K. scalarina* on a Tasmanian tidal flat as part of a wider study of the ecology of intertidal molluscan assemblages. More recently Peterson & Black (1988a, b, 1993) and Peterson *et al.*, (1994) have examined the roles of competition for space and food resources, predation and recruitment on populations of *K. scalarina* and *K. rhytiphora* in Princess Royal Harbour, Western Australia, in an attempt to determine which processes are the structuring forces in populations of intertidal suspension feeders.

Plate 1.1: *Katelysia scalarina.*

1.4 Aquaculture

When exploited stocks of shellfish decline due to natural or man made environmental changes or overfishing, hatchery programs are frequently implemented to rebuild stocks by increasing natural reproduction (Malouf & Bricelj, 1989). The success of hatchery programs depends on a number of factors: the biological characteristics of the species involved, the environment into which hatchery releases are made and the nature of the fishery (Malouf & Bricelj, 1989; Treadwell *et al.*, 1992). Populations regulated by density independent factors, such as environmental variables, will not benefit significantly from re-seeding programs if recruitment is adequate and environmental perturbations are common. Hatchery releases will have a minimal impact on these populations, as in favourable years naturally produced organisms will be abundant. In contrast, during unfavourable years hatchery reared organisms will suffer the same high mortality as the natural organisms (Malouf & Bricelj, 1989). Populations regulated primarily by density dependent factors may benefit from hatchery programs, as the impact of artificially produced animals on population size and overall recruitment can be predicted (Malouf & Bricelj, 1989). However, this is not a guarantee that hatcheries can successfully increase stocks, since a number of additional factors must be considered. An important assumption regarding hatchery programs is that the environment is

capable of sustaining hatchery shellfish after they are released (Malouf & Bricelj, 1989). This implies that reductions in the abundance of naturally produced stock are not caused by environmental unsuitability, and that the environment is capable of sustaining higher population densities. Despite this, fisheries that normally only receive high levels of recruitment may occasionally benefit from enhancement programs as such recruitment is typical of many coastal bivalves. Occasional high levels of juvenile recruitment have been observed in Tasmania for both *K. scalarina* (DPIF, unpublished data) and *R. largillierti* (A. Flintoff, pers. comm.). In addition to allowing fisheries enhancement, subject to appropriate genetic conservation policies (Soh *et al.*, submitted), the production of hatchery reared spat affords the opportunity for aquaculture of clams in predator exclusion enclosures (Toba *et al.*, 1992). Both *K. scalarina* and *R. largillierti* have been considered to have potential for aquaculture (Treadwell *et al.*, 1992)

1.5 The Estuarine Habitat

An estuary is a semi-enclosed body of coastal water that has a free connection with the open sea and within which seawater is measurably diluted with freshwater derived from land drainage (Pritchard, 1967). Estuaries are highly dynamic in nature with more variable conditions than those in either fresh water or the coastal marine environment (Ketchum, 1983). Water characteristics within the estuary change continuously under the influence of tidal forces, land runoff and winds (McLusky, 1981; Wolff, 1983) and many environmental variables show continuous and irregular patterns of change. The hydrological regime in combination with rapid salinity fluctuations, high water energy and pattern of sediment suspension and deposition make the estuarine environment unstable as well as unpredictable (Wilson, 1988). Despite these characteristics estuaries are biologically dynamic and are areas of naturally high productivity due primarily to the hydrodynamic factors including freshwater input of nutrients and the system's ability to trap and re-release nutrients (McHugh, 1976; Wilson, 1988; Day *et al.*, 1989). Although species diversity in estuaries is usually low, the ecosystem provides positive advantages for benthic animals able to cope with the prevailing environmental fluctuations (Wolff, 1983; Day *et al.*, 1989) and provide the basis for some of Australia's most productive fisheries. Estuaries are a relatively sheltered habitat, rich in food from various inputs. Moreover, the shallow nature of most estuaries means that suspended food particles are readily available for benthic animals through sinking and downward transport by turbulent water movement (Wilson, 1988; Morrisey, 1996). Despite the advantages of inhabiting estuaries, especially in terms of food resources, the

variable nature of these areas means that fisheries are particularly prone to natural fluctuations in population numbers due to high mortality events, thus increasing the chances of over-exploitation.

1.6 Structuring Forces in the Estuarine Environment

Numerous studies have focused on the factors controlling the structure of marine benthic communities. The accessibility and ease of manipulation of rocky intertidal communities have led to rigorous evaluation of ecological processes in these habitats (Paine, 1966; Dayton, 1971; Connell, 1972). However, significantly fewer studies have focused on soft bottom habitats despite the fact that the ecological problems faced by organisms in soft and rocky substrate habitats are vastly different.

In the absence of both biological (Paine, 1966) and physical (Dayton, 1971) disturbance, competition for space is the primary determinant of species distribution and abundance on rocky shores (Connell, 1961a; Peterson, 1979). Similarly competition has been used as an explanation for patterns in soft sediment habitats (see review by Peterson, 1980). In contrast, several studies have also suggested that the role of competition is not important in some estuarine habitats (Olafsson, 1986; Peterson & Black, 1993). Various other factors have also been suggested as explanations for the patterns of species abundance and distribution observed in soft sediment habitats such as disturbance (Eagle, 1975; Rhoads *et al.*, 1978; Armesto & Pickett, 1985; Flach, 1992), predation (Peterson, 1979; Hulberg & Oliver, 1980; Peterson, 1982b; Sanchez-Salazar *et al.*, 1987; Wilson, 1991) and recruitment (Woodin, 1976; Woodin, 1991; Peterson & Black, 1993). These factors are reviewed briefly below.

1.7 Physical factors

1.7.1 Tides

Tides are the dominant physical feature of many shores, although they may not be the primary cause of zonation (Raffaelli & Hawkins, 1996). Tidal range may vary enormously between estuaries and even within a single estuary, with regions closest to the mouth typically displaying a much greater tidal range than landward regions where almost no tidal activity may occur. However, it is not only tidal range that may vary between and within estuaries but the periodicity of tides, as there are several variations on the common semi-diurnal tidal pattern. Differences

between predicted and actual tides may also occur due to meteorological conditions. Differences in the duration and height of tides are mainly caused by wind or variations in barometric pressure. The essential feature which tides impose upon the shore is the periodic emersion and immersion, such that critical levels of exposure may determine the upper and lower levels of species assemblages (Newell, 1970; Connell, 1972). During exposure inhabitants are faced with increases in temperature variability and increased desiccation. During submersion temperature extremes and desiccation are minimised. Wave action can also come into play by dislodging individuals or predators. Thus zonation in the intertidal zone is often attributed to tides and the degree of exposure experienced by organisms during the tidal cycle.

Little work has been conducted on the distribution and abundance of *K. scalarina* within the intertidal zone. Woodward (1985) described the vertical distribution of *K. scalarina* at Pipeclay Lagoon, Tasmania, with larger, older individuals low on the shore and smaller, younger individuals higher on the shore. Similarly, Bellchambers (1993) reports that populations of *K. scalarina* reach their greatest abundance and largest size at low tidal positions at Moulting Lagoon, Tasmania. However, no previous studies have examined the combined effects of density and tidal position on the survival and growth of *K. scalarina*.

1.7.2 Salinity

Salinity is an important factor determining the distribution of coastal and estuarine fauna (Bayne, 1975; McLusky & Elliot, 1981). The salinity of estuaries is extremely variable ranging from freshwater, in landward areas to fully marine, approximately 35‰, at the mouth. Depending on the region salinity may vary seasonally due to river input, freshwater runoff and precipitation. Salinity may also vary vertically with ebb and flood of the tide.

Salinity limits the distribution of many fauna groups (see Weinburg, 1985) as it affects the physiological processes of estuarine species (Dame, 1996), although the results of laboratory and field studies do not always agree (Bayne *et al.*, 1976). Under laboratory conditions few aquatic species attain optimal growth rates in salinities outside waters which sustain maximum population densities (Kinne, 1971; Kiørboe *et al.*, 1981; Jørgensen, 1990). However, results from laboratory conducted studies are not always an accurate indication of the natural process. Salinity may affect the structure and functional properties of animals through a change in 1) total osmotic concentration, 2) the relative proportion of solutes, 3) the coefficient of absorption and saturation of dissolved gases, or 4)

density and viscosity (Kinne, 1964). Changes in environmental salinity may disrupt the balance between input and output of cellular water and solutes (Hawkins & Bayne, 1992). To survive in such a variable environment, estuarine organisms have developed various mechanisms to cope with the osmotic stress imposed by fluctuations in salinity such as burrowing, secretion of mucus and shell closure (Shumway, 1977a). Although, Nell & Patterson (1997) studied the salinity tolerance of a closely related species *K. rhytiphora*, nothing is known about the tolerance of *K. scalarina* to fluctuations in salinity. Similarly the effects of variations in salinity on the growth and other metabolic processes are at this stage unknown. Salinity fluctuations are also one possible explanation for the periodic observation of mass mortality in natural populations of *K. scalarina*.

1.7.3 Sediment

Sediment composition is another important physical parameter that affects the spatial distribution of estuarine fauna (Dame, 1996). The grain size distribution of estuaries is primarily the result of water movement and wind action (McLachlan, 1983; Morrisey, 1996) and biological processes such as burrowing of estuarine fauna. Zones of low energy or little water movement are dominated by finer sediments while areas of high energy are composed of coarser sediment like sand. Particle size may affect beach contour and grade, which in turn may affect drainage, exposure and porosity (McLachlan, 1983). All these factors may determine the distribution and abundance of estuarine fauna which are intimately linked with the sediment which they inhabit. Generally, deposit feeding bivalves are prevalent in finer sediments and filter feeding bivalves are common in coarser sediments (Rhoads & Young, 1970). Although spatial dynamics are largely a result of local hydrodynamics and food availability some are due to a species' ability to cope with unstable sediments, which in turn may be caused by the inhabitants themselves. Benthic suspension and deposit feeders often display inverse patterns of spatial distribution. Rhoads & Young (1970) state that deposit feeders intensively rework the surface sediment and produce the following changes: 1) an uncompacted sediment of faecal pellets, reworked material and semi consolidated mud and 2) sediments high in water content. Sediment alteration of this nature may limit the distribution of suspension feeding estuarine fauna by 1) clogging filtering structures, 2) resuspending newly settled larvae or 3) inhibiting the settlement of suspension feeding larvae.

As *K. scalarina* is a sediment dwelling bivalve there is a need to determine whether variations in sediment characteristics influences the results of growth trials.

1.8 Biological Factors

1.8.1 Competition

In the absence of large scale disturbance soft sediment habitats are often densely populated (Woodin, 1997). As each individual requires access to the sediment surface and may frequently utilise the same food resources, competition should be a predominant force in determining the structure of these communities (Woodin, 1997). However, there is some conflict regarding the importance of pre-emption of space as a structuring force as there is no real equivalent in soft sediment habitats for the kinds of spatial competition that is prevalent amongst rocky shore species (Peterson, 1979). In contrast to rocky shores, soft sediment invertebrates are mobile and inhabit a three dimensional habitat, therefore the effects of competition may be diffused by migration, preventing opportunities for overgrowing and crushing (Lee, 1996). While more diffuse and often not the predominant structuring force, competitive interactions leading to exclusion do occur in soft sediments (Peterson, 1979; Wilson, 1991).

Levin (1981) suggests that interference competition between individuals of the spionid polychaete *Polydora paucibranchiata* results in even spacing of individuals. Similarly, adults and juveniles of the same species may be partly responsible for local distribution by preemption of space. For example, larger individuals of the amphipod *Corophium volutator* (Raffaelli & Milne, 1987) and the polychaete *Ceratonereis eythrocephala* (Kent & Day, 1983) exclude conspecific juveniles in the absence of shore birds and flatfish. Under normal predator densities co-existence of large and small animals is promoted by preferential selection of large prey items by the predators. Similar adult-recruit interactions within and between species are common in soft sediments, although in most cases the actual mechanisms remain to be established (Raffaelli & Hawkins, 1996).

Interspecific competition has been reported between *Nereis diversicolor* and *Corophium volutator* (Olafsson & Persson, 1986) and for other burrowing and tube building polychaetes (Woodin, 1974). Similarly, evidence of interspecific competition has also been reported between bivalves. Levinton (1977) reports that the bivalve *Nucula proxma* avoided areas where *Yolidia limatula* were abundant. Peterson & Andre (1980) report a similar situation in which *Sanguinolaria nuttallii* avoids areas of high *Tresus nuttallii* and *Saxidomus nuttallii* densities. Although preemption of resources is less evident in soft sediments (Dayton, 1984) and is rarely the sole factor determining distribution and abundance, an example of this type of competition is the mudsnail *Hydrobia*

(Fenchel, 1975a, b). Interspecific competition between *Hydrobia ulvae* and *H. ventrosa* for particulate food caused the two species to partition the sediment resource according to body size. Although there is some debate as to whether character displacement actually occurs in *Hydrobia* spp., it is evident that competition for food resources does occur.

Numerous authors have reported that competition is more likely to be for food rather than space (Buss & Jackson, 1981; Peterson, 1982a; Peterson & Black, 1988a; Dobbinson *et al.*, 1989; Olafsson *et al.*, 1994) since food supply typically regulates benthic secondary production by affecting individual growth and fecundity of adult invertebrates (Wilson, 1983; Olafsson *et al.*, 1994). Density dependent growth effects have been reported in several bivalve species, suggesting intraspecic competition (Peterson, 1982a; Bertness & Grosholz, 1985; Weinburg, 1985; Olafsson, 1986; Peterson & Black, 1987; Peterson & Beal, 1989; Peterson, 1992). However, the effects of food depletion on the growth and fecundity of soft sediment individuals are chiefly localised, and effects at the community scale remain unclear (Peterson & Black, 1987; Raffaelli & Hawkins, 1996). Raffaelli & Hawkins (1996) suggest that any such effects may be obscured by major changes in other factors, such as physical disturbance and variations in recruitment patterns.

Peterson & Black (1993), working on *K. scalarina* and *K. rhytiphora* in Princess Royal Harbour, report that competition was evident in only one of five time intervals. However the absence of competition in these experiments may be due to the fact that clam densities were not high enough to cause limitation of resources. Therefore there is a need to further investigate the role of intra-specific competition by increasing the density of experimental treatments to examine when or if competition for resources limits the growth and survival of *K. scalarina*. Stress induced by overcrowding may also be another possible explanation for the mass mortality events observed in natural populations of *K. scalarina*. Secondly, if *K. scalarina* is to be commercially farmed defining the diet and potential for competition with other species in particular Pacific oysters, which is a likely candidate for polyculture, is essential.

1.8.2 Sediment Disturbance Induced by Bioturbation

While exploitation competition, preemption of space, is unlikely to be a major structuring force in sediment communities (Peterson, 1979; Dayton, 1984; Wilson, 1991) changes in the sediment induced by disturbance may be more important. Natural physical disturbance has been recorded as a structuring force

in many communities and a variety of natural disturbances can cause areas of opened habitat which are then available for recruitment or resettlement from other areas (Armesto & Pickett, 1985; Hall *et al.*, 1993). The extent of a disturbance patch and the frequency with which patches appear vary markedly depending on the disturbance agent (Thistle, 1981). Disturbance is common in soft sediment habitats (Woodin, 1978) and may be due either to large scale physical factors or bioturbation by a variety of estuarine organisms. Alternatively, many estuarine fauna, such as mussels, may act as sediment stabilisers within the system, inhibiting erosion by water movement (Bertness & Grosholz, 1984). Many tube dwelling species also fall into this category, especially larger polychaetes such as the sand mason *Lanice conchilega* (Raffaelli & Hawkins, 1996). At high densities the tubes of this and similar species can greatly increase the critical erosion velocities. However, if tube density is low sediment can be destabilised. Other sediment stabilisers include algal mats, mangrove trees, mussel beds and seagrasses. All these organisms can have a significant effect on sediment characteristics by creating local hydrodynamic changes (Jones & Jago, 1993) which may promote larval settlement (Eckman, 1987), they may also provide predation refuges for small epibenthic and infaunal species.

Particle processing by intertidal organisms can be substantial and involves a range of different organisms (Raffaelli & Hawkins, 1996). Sediment destabilisation is mainly caused by deposit feeders which manipulate, sort and process sediment particles (Rhoads & Young, 1970), for example the polychaete *Arenicola marina,* which ingests particles from below the surface and eventually deposits them as faeces on the sediment surface (Rhoads, 1974). Eventually the surface becomes a loose fabric of large particles, with high water content, increasing the sediment's susceptibility to erosion. Water pumping by burrowing organisms also has the potential to alter sediment characteristics, by disturbing the redox layer (Rhoads, 1974; Baillie, 1986). The presence of small crabs which burrow several centimetres below the surface over extensive areas can also cause substrate disturbance (Hall *et al.*, 1993). The sediment reworking of lugworms (*Arenicola* spp.) in the Wadden Sea has negative effects on the juveniles of other macrofaunal species including the polychaete *Nereis diversicolor*, *Vapitella capitata* and the bivalves *Mya arenaria*, *Crastoderma edule*, *Macoma baltica* and *Angulus* (=*Tellina*) *tenuis* (Flach, 1992). Some species may also have a direct effect on the meiofaunal and plant populations through predation although the majority of species are deposit feeders. All these processes generate small scale patchiness in sediment habitats and maintain

community structure and composition in the absence of strong competitive interactions seen in rocky substrates (Dayton, 1984).

Previous authors have suggested that sediment disturbance as a result of harvesting procedures has a negative effect on the survival of *K. scalarina* (Bellchambers, 1993; Bellchambers & Richardson, 1995). Juveniles are particularly vulnerable to disturbance and if covered by large amounts of reworked sediment are unable to recover their near surface position resulting in mortality.

1.8.3 Sediment Disturbance Induced by Predators

Sediment disturbance can also be caused by epibenthic predators searching for prey. These predators range in size, and resulting sediment disturbance, from large predators such as grey whales (Oliver *et al.*, 1984; Oliver & Slattery, 1985), intermediate predators such as skates, rays and crabs (Woodin 1978; Van Blaricom 1982) to small gastropods and juvenile fish (Wilste, 1980; Hunt *et al.*, 1987). The activities of epifaunal predators have two components, the direct effect on the prey item and the indirect effect on the community structure and composition (Woodin, 1997).

Epibenthic species may physically disturb the sediment by taking bites of the sediment and filtering the contents i.e. whales, flounders, rays, and shelducks or in the case of shorebirds by trampling and excavating of individual prey items (Savidge & Taghon, 1988; Cadee, 1990; Raffaelli *et al.*, 1990). This kind of disturbance creates a discrete depression or pit that eventually fills, acquiring sediment and biological characteristics different from those of the surrounding area. In shallow sublittoral areas predators such as larger crabs (Thrush, 1986; Hall *et al.*, 1991) and stingrays (Van Blaricom, 1982) excavate large pits in pursuit of prey. These pits may become a trap for organic material, attracting organisms, such as amphipods, capable of exploiting these high quality patches, alternatively they may provide a refuge for poor competitors, such as capitellid worms. Therefore sediment disturbing predators can generate a mosaic of patches each in different sucessional stages (Grassle & Sanders, 1973). However, exclusion of flounders (Raffaelli & Milne, 1987), eiders (Raffaelli *et al.*, 1990) and crabs (Hall *et al.*, 1991) shows that any biological changes occurring within the individual pits do not extend to areas outside these depressions.

Paine (1966) suggested that predation can maintain a high diversity in benthic communities on rocky shores by keeping competitor numbers low. The contrasts between predators on soft sediment shores and rocky shores are vast. In soft sediment communities with large tidal ranges, shorebirds are often the predators with the highest feeding rates (Baird *et al.*, 1985). However, the exclusion of shorebirds from invertebrate prey does not lead to the dramatic changes in community structure and composition that are shown by predator exclusion in rocky shores (Quammen, 1984; Reise, 1985; Raffaelli & Milne, 1987). Thus keystone species do not appear to play the community-structuring role evidenced on many rocky shore communities. However, small epibenthic predators, such as *Carcinus maenas* can have a significant effect on the density of sediment dwelling invertebrates (Gee *et al.*, 1985; Jensen & Jensen, 1985; Reise, 1985) and may contribute to the co-existence of species (e.g. Woodin, 1974) by exclusion and maintaining a balance between two competing species. Woodin (1997) suggests that complex tropic interactions are common in soft sediments and may explain the spatial complexity evident in these systems. Most intertidal communities are preyed upon by a diverse range of predators rather than being controlled by a specific predator (Seed, 1993).

There are a number of studies investigating predation on *K. scalarina* (Chilcott, 1995; Taylor, 1995; Mackinnon, 1997) all of which examine the effects of a single predator guild. However there is a need to determine if the presence of predators/competitors interferes with the results of growth trials.

1.8.4 Recruitment

Several workers have suggested that recruitment influences community structure (Denley & Underwood, 1979; Underwood & Denley, 1984). In fact debate over the relative importance of recruitment limitation compared with post settlement processes continues in the literature (see Olafsson *et al.*, 1994 for review). Woodin (1991) states that a large difference in the success of recruitment can dramatically alter the structure of adult populations through inter- and intraspecific relations and physical processes. Recruitment may be limited by two mechanisms; firstly on a physical scale through hydrological factors which reduce the number of larvae reaching a site (Levin, 1984; Butman, 1987) and therefore choosing substrate on behaviour criteria (Woodin, 1985, 1986; Butman *et al.*, 1988). Secondly, post settlement mortality and emigration can determine the success of larvae (Levin, 1981; Woodin, 1986).

Field manipulation studies suggest that adult-larval interactions play an important role in population dynamics (Andre & Rosenberg, 1991; Andre *et al.*, 1993). Studies by Møller (1986), working on *Mya arenaria* and *Cerastoderma edule,* suggested that plots with high densities of *M. arenaria* had fewer settled juveniles of *M. arenaria* and *C. edule* than-control plots with low densities of *M. arenaria*. Andre & Rosenburg (1991) concluded that the settlement of bivalve larvae may be decreased up to 40% in the presence of adult *C. edule* and *M. arenaria*. However a growing body of evidence suggests that benthic filter feeders in soft sediments are unlikely to achieve a detectable decrease in recruitment by limiting the settlement of larvae alone (see Peterson, 1982a; Hunt *et al.*, 1987; Ertman & Jumars, 1988; Olafsson *et al.*, 1994 for examples).

1.9 Physical versus Biological

Differences of opinion regarding the importance of physical and biotic process as structuring forces of intertidal estuarine communities is evident in the literature. However, there is now acceptance that both can act concurrently at various temporal and spatial scales within the same population (Vincent *et al.*, 1994). It appears that the structure of soft sediment habitats cannot be attributed to a single process but is the result of several processes acting independently or together at various temporal and spatial scales (Vincent *et al.*, 1994; Dame, 1996). Dayton (1984) suggested that competition, both exploitative and interference, appears to be an unimportant force in the structure of soft bottom communities. These communities are probably shaped by the combined effects of larval-adult interactions and competition for food resources (Ambrose, 1984; Reise, 1985; Wilson, 1991) and sporadically modified by the effects of physical disturbance (Møller, 1986). Recent reports have suggested that the observed distribution and abundance of soft bottom macrobenthos is a result of the complex interaction of all these factors and may not be simply explicable to one particular dominate force (Black & Peterson, 1988a; Peterson & Black, 1993).

2.0 Study Aims

The aim of this study was to investigate the role of biological and physical forces on structuring populations of the clam, *Katelysia scalarina*, firstly, in an attempt to explain the distribution and abundance of natural populations, and secondly to determine how these factors could aid or hamper attempts to use aquaculture techniques to establish marine farming, enhance natural populations and prevent over-exploitation.

1. Many intertidal invertebrates segregate according to size across the shore, producing distributions that vary with tidal level (Bally, 1983; Wendt & McLachlan, 1985; Jaramillo, 1987; Jaramillo *et al.*, 1993). This form of zonation within intertidal populations is related to the different physical and biotic factors associated with tidal levels and exposure (Creese & Underwood, 1982; Bally, 1983; Jaramillo *et al.*, 1995; Roegner & Mann, 1995). Evidence suggests that mortality at high shore levels is principally due to the rigours of temperature, desiccation, salinity and other physical conditions, while predation and other biotic interactions account for mortality low levels on the shore (Vermeij, 1972; Woodward, 1985; Black & Peterson, 1988; Dobbinson *et al.*, 1989). A series of caged manipulations were used to determine the factors enhancing or inhibiting the vertical population structure of *K. scalarina*. A number of sediment characteristics were also examined in an attempt to explain population structure in terms of physical attributes of the sediment at different tidal positions. Other researchers have identified sediment preferences as a key variable in limiting the suitability of potential growout sites for the culture of clam species (de Valence & Peyre, 1990; Spencer *et al.*, 1991; Patterson & Nell, 1997).

2. It has been suggested that altering the natural abundance and distribution of suspension-feeding bivalves may have a significant effect on either local food depletion (Buss & Jackson, 1981; Peterson & Black, 1982a, 1988b; Dobbinson *et al.*, 1989; Peterson & Beal, 1989), mortality (Dobbinson *et al.*, 1989) or juvenile recruitment (Peterson & Black, 1993). Experimental manipulations were conducted to investigate the role of competition for limited resources, including food and space, in determining the density of natural populations of *K. scalarina*. Stocking density is also a key production limiting variable in aquaculture (Treadwell *et al.*, 1991).

3. Previous authors have stressed the importance of physical factors in determining the distribution and abundance of estuarine species (Connell 1972; Menge & Sutherland, 1987) in particular salinity (Bayne, 1975; McLusky & Elliot, 1981; Weinburg, 1985). The salinity tolerance of adult and juvenile *K. scalarina* was investigated to determine whether salinity could be a factor limiting the abundance and distribution of *K. scalarina*. Secondly, the mechanisms, both physiological and behavioural, that *K. scalarina* employs to avoid or cope with salinity stress were also examined. Other researchers have identified salinity tolerance as a key variable in limiting the suitability of potential growout sites for culture of clam species (Patterson & Nell, 1997).

4. Organisms inhabiting estuaries must cope with often large fluctuations in ambient salinity and they have a variety of mechanisms for coping with salinity stress (see Burton, 1983 for review). This component of the study investigated the cost in terms of possible limitations to growth that may be experienced by bivalves inhabiting waters outside their optimal range for growth. Distortion of the energy budgets of culture species can adversely affect the economics of farming them (see Edwards *et al.*, 1997).

5. The use of oyster farms for clam culture has been identified as offering advantages in terms of dual use of existing infrastructure and microbial monitoring data for waterways (from a human health/quality assurance perspective). Thus, the gut contents of caged *K. scalarina* placed under and away from oyster racks on an existing oyster farm were examined to determine the principal items that comprise the majority of the diet of *K. scalarina* and if the potential for food competition exists between suspension-feeding bivalves in estuarine systems.

Chapter 2:

The effect of tidal position and density on growth, meat yield and survival.

2.1 Introduction

Intraspecific competition has been recognised as having a major role in the regulation of many soft bottom bivalves (Peterson, 1982b; Bertness & Grosholz, 1985; Weinburg, 1985; Olafsson, 1986; Peterson & Black, 1987; Peterson & Beal, 1989; Vincent *et al.*, 1994). Previous studies have demonstrated patterns of density-dependent growth (Peterson & Black, 1982b; Frechette & Bourget, 1985a, b; Peterson & Black, 1987) and depletion of food, near the sediment-water interface (Frechette & Bourget, 1985b; Monismith *et al.*, 1990) by suspension feeding bivalves. However, while competition for food resources may cause reduced growth in suspension feeders, it does not usually cause higher mortality or competitive exclusion (Peterson, 1982b; Olafsson, 1986; Peterson & Black, 1987; Peterson & Beal, 1989; Jensen, 1992, 1993).

Previous researchers have suggested that while there are strong spatial variations in the shell length of suspension feeding bivalves, these variations can mainly be explained by tidal level and local population density (Vincent *et al.*, 1994). Tidal gradient can be considered a strong environmental stress gradient, as physical and physiological disturbances increase with tidal height (Menge & Sutherland, 1987). Suspension feeders living at higher elevations may also encounter water masses already partial cleared of suspended food (Peterson & Black, 1987, 1988, 1991). Submersion period determines the foraging time available to intertidal suspension feeders and in turn their supply of food. A decrease in submersion time may also have adverse physiological effects, as bivalves may be exposed to harmful salinities and temperatures (Newell, 1979; Vincent *et al.*, 1994; Roegner & Mann, 1995). Higher growth rates at lower shore levels have been observed in numerous populations (Bertness & Grosholz, 1985; Peterson & Black, 1987, 1988; Jensen, 1992; Vincent *et al.*, 1994).

The aim of this component of the study was to examine the growth, survival and condition index of *K. scalarina* at different tidal levels and densities. Knowledge of the factors which govern the vertical and horizontal population structure of *K. scalarina* is important to understand estuarine species and the

processes which dominate these systems. A sound understanding of these processes is also essential to maximise the potential of any future aquaculture developments.

2.2 Methods

2.2.1 Study Site

Experimental manipulations of *K. scalarina* were conducted in the intertidal zone of Moulting Lagoon, which is a permanently open lagoon situated on the east coast of Tasmania, near Coles Bay on the Freycinet Peninsula (42°05'S, 148°10'E) (Figure 2.1). Partly enclosed by a bayhead spit, the lagoon is generally less than one metre deep and is fed by two rivers and several smaller streams which may cease to flow during summer (Dec.-Feb.) (Blackhall, 1986). The lagoon has a total water surface area of approximately 41.50 km^2.

Moulting Lagoon has a maximum tidal range between 0.8m at the mouth and 0.3m in its upper reaches; the tidal rhythm is semi-diurnal with significant diurnal inequality. At low tide, large areas of the estuary become exposed as sand or mud flats; these are most extensive in the lower and middle estuary where the tidal range is greatest (Last, 1983). The lagoon sediments are composed of sands derived from Triassic sandstone and mudstone. The oxic layer is approximately 10cm deep at the high tide mark but is much shallower towards low tide and in places is only a few centimetres deep. Located below the oxic layer is an extensive layer of organic mud and remnant shell fragments

Katelysia scalarina is the most abundant macrobenthic species within the top 5 cm of the sediment of the tidal flat. Other dominant molluscs are the gastropods *Nassarius pauperatus*, *Salinator fragilis* and *Bembicium auratum* and the bivalves *Anapella cycladea* and *Eumarcia fumigata* which are more abundant near the high tide mark. There is little aquatic vegetation in the intertidal zone.

2.2.2 Initial sampling

Juvenile *K. scalarin*a (20.0-25.0 mm shell length) were collected from Meredith Point, Moulting Lagoon between 16th - 23 Feb 1996. Size frequency distributions of clams used in the experiments were narrow, to minimise differences between experimental treatments. As a result, it was not considered necessary to individually tag clams. In addition, insufficient numbers were available in the smaller size classes to utilise in the experiment. Clams were measured along the longest antero-posterior axis (length) and dorso-ventral

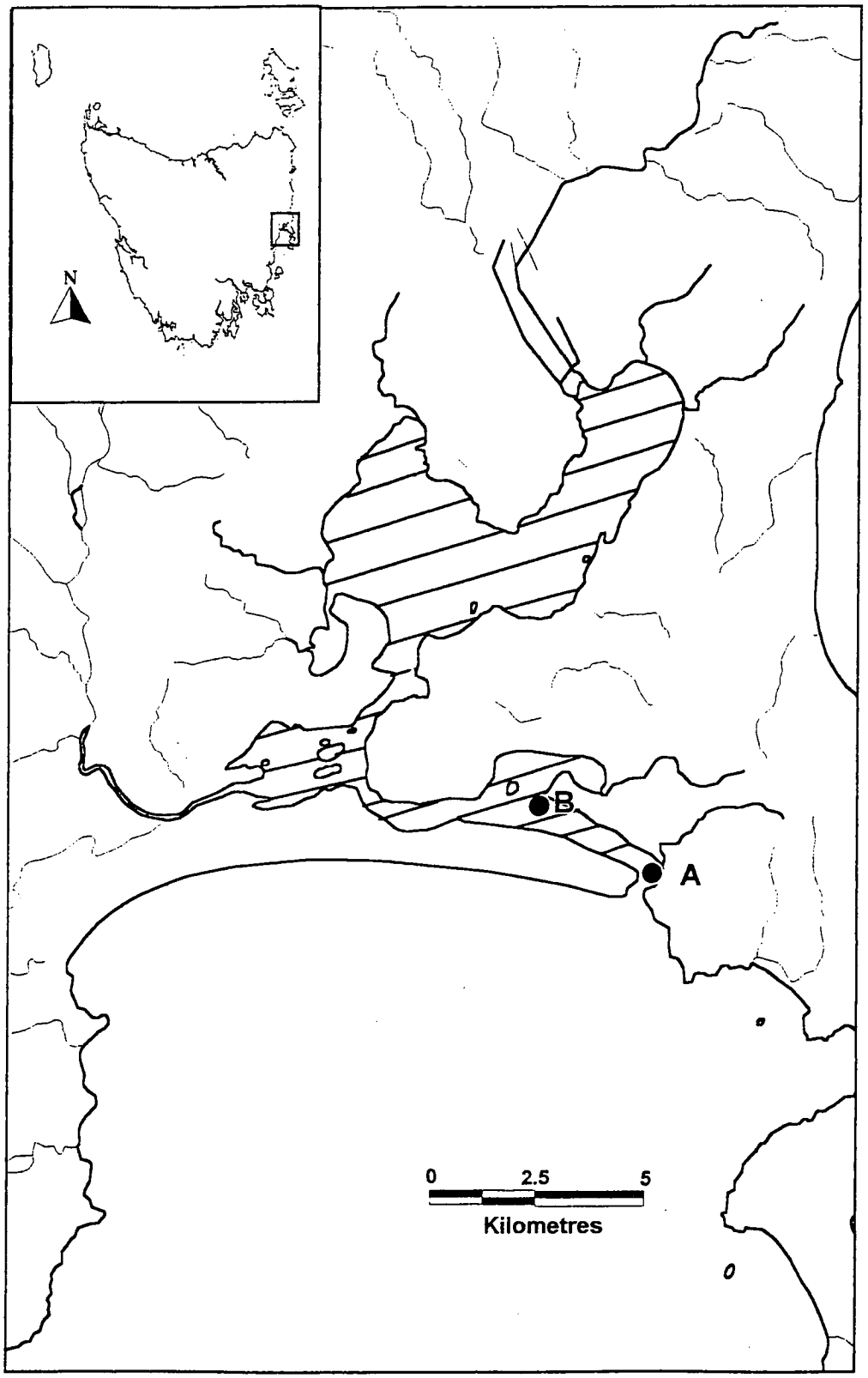

Figure 2.1: Map of Moulting Lagoon, Tasmania, Australia. Juvenile collection site is marked by B, adult collection site and experimental site is marked by A.

margin (height) to the nearest 0.1 mm with vernier callipers (Diagram 2.1) and randomly allocated to experimental combinations of density and shore position.

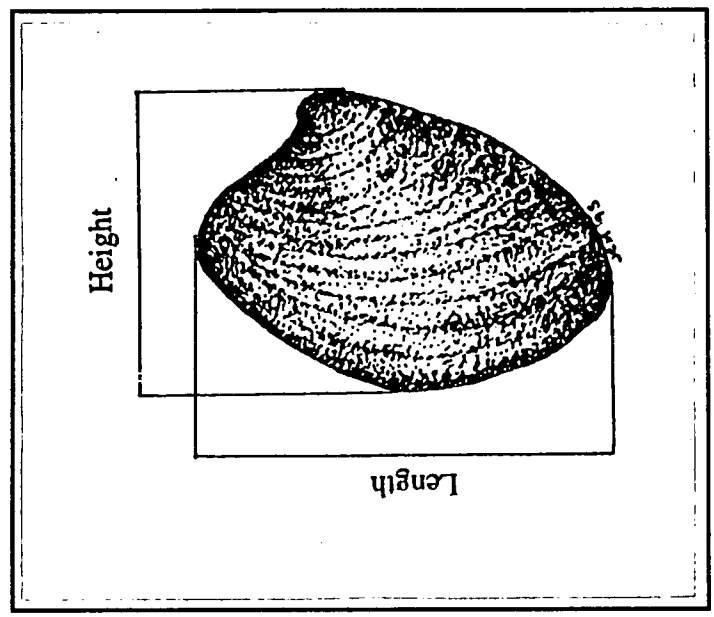

Diagram 2.1: Illustration of length and height measurements.

Experimental manipulations were conducted inside cages designed to prevent migration of *K. scalarina*, so that densities could be maintained over the experimental period. Cages were also used to prevent the access of large mobile consumers such as crabs and wading birds to the experimental densities. As all density treatments were enclosed identically, any additional effects of enclosures were held constant across treatments. Cages were constructed of 9 mm Nylex mesh (33 cm wide x 53 cm long x 14 cm deep) and fastened with plastic cable ties to wooden stakes driven into the substrate. Prior to adding the clams, sediment below each cage was removed to a depth of 10 cm and returned to the cages by sieving through a 2 mm sieve to remove all potential predators and competitors.

Experimental treatments were established in a random block design at five tidal positions, ranging from the low tide mark to the high tide mark at 30 m intervals. Theft of data loggers at an experimental site precluded the recording of average emersion times. However, depending on the prevailing weather conditions, there was approximately 3 hours difference in the aerial exposure of high and low tide treatments (pers. obs.). Three densities were used (30, 60, 120 clams/basket; 171.5-686.1 clams/m^{-2}) with four replicates of each treatment at every tidal position. Neighbouring cages were separated by 1m, neighbouring blocks were separated by 10 m (see Diagram 2.2).

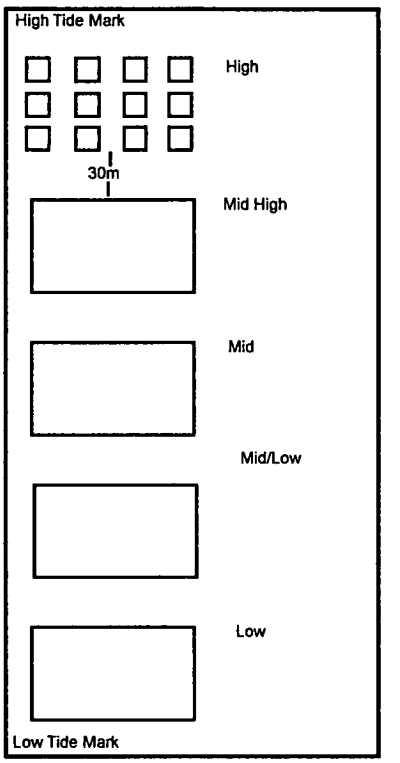

Diagram 2.2: Representation of experimental design at Moulting Lagoon, Tasmania.

2.2.3 Final Sampling

Experimental manipulations were sampled between 6 - 10th Jan 1997, approximately a year after the establishment. All cages were removed, live clams remeasured and mortalities recorded. Missing clams were a consequence of either removal by scavengers/predators or post-mortem transport. Dead shells were also classified as undamaged, drilled (by gastropods) or chipped (by crabs). Undamaged shells were the result of starvation, physiological stress or predation that leaves no evidence on the shell. Clams were remeasured to determine variations in growth according to experimental treatment. Recording mortalities and nature of mortality allowed estimates of the effect of predation and type of predation prevalent at varying densities and tidal position. The number and size of new recruits in each treatment, along with the number and species of any other macrobenthos present, were recorded to examine whether recruitment, predation intensity and type varied with local clam density and tidal position. Macrobenthos was defined as organisms retained in the cages after removal from the experimental plots and therefore included only larger macrobenthos

which may have been competing for food resources or potential predators (such as crabs, gastropods, other bivalves and small fish).

A subsample of 30 clams per cage was measured and weighed for calculation of condition index, to determine body condition. Shell and wet meat weights were recorded to the nearest 0.01g. The following equation was used to calculate meat ratios: Meat Ratio = (Meat Weight)/Total Weight x 100
Four sediment samples, approximately 3 cm in depth, were collected from both inside and outside the experimental treatments, using a 10 cm diameter sediment corer, to determine whether the presence of large aggregations of suspension feeders altered the sediment characteristics. Sediment samples were analysed for organic content and phi size. Samples were sieved through a 500 μm sieve and placed in a drying oven at 80°C for 24 h. To determine organic content, 10 g of dried sample was placed in a muffle furnace for 4 h at 450°C and percentage loss of the fraction recorded (Allen, 1989). Adjustments were made for calcium carbonate by acid digestion (Allen, 1989). The remaining proportion of each sediment sample was sorted into constituent grades through a series (<63 μm, 63 μm, 125 μm, 250 μm, 500 μm) of interlocking Endicott sieves to determine phi size (Gray, 1981). Redox and pH measurements were determined using a WTW (Wissenschaftlich - Technische - Werksttaten) Microprocesser pH meter with a redox probe. Redox and pH measurements were taken before the sediment samples were removed, in the top 2-3 cm of the sediment as this is the area inhabited by *K. scalarina* and therefore the area most likely to be affected by dense aggregations.

2.2.4 Statistical analysis

Data on growth (length and height), survival, meat ratio and number of recruits and total number and type of macrobenthos were analysed using two-way ANOVA in JMP for Macintosh (SAS Institute, 1995) with density and tidal position as factors. Non-significant results were re-analysed using one way ANOVA, averaging for block. Bartlett's test for homogeneity of variances was applied to all data to check equality of variances (Sokal & Rohlf, 1981) and residuals were tested for normality (see appendix 1 for results of statistical analysis). Survival data was transformed as arcsin (% survival x 0.01)$^{0.5}$. Sediment characteristics were tested as simple covariates to determine whether the effect of sediment characteristics depended on the treatment level.

Plate 2.1: Experimental baskets used to enclose *K. scalarina* in experimental trails.

2.3 Results

2.3.1 Survival

K. scalarina displayed high survival rates across all experimental treatments except for the high shore 120 clam treatment (Figure 2.2). The lowest mortality recorded (3.1%) was in the high shore, 30 clam treatment whereas the highest mortality recorded (41.4%) was in the high shore, 120 clam treatment. Survival data were transformed using an arcsin, square root transformation and reanalysed. Tidal height ($P = 0.0065$) and density ($P = 0.0069$) had a significant effect on the survival of *K. scalarina.* The interaction between tidal height and density was slightly significant ($P = 0.0559$). Total mortality increased as tidal height/exposure time increased.

2.3.2 Growth

There is a clear trend of decreased shell growth (in terms of length) from low to high shore treatments (Figure 2.3). Tidal position ($P = 0.0001$) and density ($P = 0.0430$) had a significant effect on the growth of *K. scalarina*, however the interaction between tidal position and density treatment was not significant ($P = 0.9674$). The largest length increase, (4.21 mm), was in the 30 clam Mid/Low

tidal treatment. The smallest length increase (2.07 mm) was in the 120 clam high tidal treatment

Results for shell height were similar to those for shell length (Figure 2.4). Both tidal height (P = 0.0000) and density (P = 0.0101) had a significant effect on the increase in shell height of *K. scalarina*, however the interaction between tidal height and density was not significant (P = 0.5151).

2.3.3 Meat Ratio

Meat ratio (% of total weight consisting of meat weight) displayed a significant response to tidal position (P = 0.0075) (Figure 2.5). The highest meat ratio (39.3%) was present in the 60 clam Mid/High treatment, while the lowest meat ratio (34.4%) was in the 120 clam Mid /Low treatment. At higher tidal heights shell growth is more depressed than meat growth and hence the meat to shell ratio improves. Neither density (P = 0.0623) nor the interaction between tidal height and density (P = 0.0623) had a significant effect on the meat ratio of *K. scalarina*.

2.3.4 Recruitment

Tidal height (P = 0.6188), density (P = 0.4584) and the interaction between tidal height and density (P = 0.7317) did not have a significant effect on recruitment of juvenile *K. scalarina* to the experimental treatments. Recruitment of juvenile *K. scalarina* was low in all the experimental treatments, with no recruits present in most treatments (Figure 2.6). The majority of recruits were found in the High and Mid/High treatments, with totals of 8 and 7 respectively. In general, higher numbers of recruits were present in the 30 and 60 clam treatments than the 120 clam treatment.

2.3.5 Macrobenthos

Numbers of macrobenthic invertebrates recovered from the treatment cages varied over the experimental treatments, with a total of 159 invertebrates collected (Figure 2.7A). In this case macrobenthos was defined as organisms retained in the cage after removal from the experimental treatments. However, neither tidal height (P = 0.7405), density (P = 0.8946) or the interaction between the two factors (P = 0.5833) had a significant effect on the total macrobenthos abundance. The highest number of macrobenthic individuals collected in one cage was 20, and the lowest was 0. Although most cages had some

macrobenthos present there was high variability between replicates at the same density and tidal position. The highest total number of macrobenthic invertebrates collected at a tidal height was 39 in the low shore treatment. Macrobenthic organisms were divided into four categories: other bivalves, gastropods, crabs and the introduced European shore crab *Carcinus maenas*. The division of the macrobenthos into these categories allowed estimates of their effects on the experimental treatments in terms of predation and competition.

The major macrobenthic category was crabs (39.62%, Figure 2.7B), the majority of which were the soldier crab, *Mictyris platycheles* and the shore crab, *Paragrapsus gaimardii*. Crab numbers were highest in the mid/low to mid/high treatments, and within this range the highest abundances of crabs were in the 60 and 120 clam treatments. Despite these differences tidal height ($P = 0.5604$), density ($P = 0.9923$) or the interaction between tidal height and density ($P = 0.9736$) did not have a significant effect on the number of crabs in the experimental treatments. *Mictyris platycheles* is a detritivore that processes large amounts of sand to extract unicellular algae (Edgar, 1997). *Paragrapsus gaimardii* is a common estuarine crab around most of Tasmania and Victoria (Edgar, 1997); although it is a small scavenging crab that consumes a range of prey, it is incapable of crushing bivalves of the size used in these experiments. Thus, despite the high numbers of these two species of crab in the experimental treatments, they posed no predation threat to *K. scalarina* and no signs of crab predation were present on any of the dead shells collected from the experimental treatments.

The next numerically dominant group was the bivalves (Figure 2.7C, 32.07%). The bivalves present were mainly *Eumarcia fumigata* and *Soletellina biradiata* which may have been competing with *K. scalarina* for food resources. Unlike the crabs, the bivalve numbers reached their highest density at the low shore treatment. Bivalves were the only group of macrobenthos which tidal height had a significant effect on ($P = 0.0113$) although density ($P = 0.2960$) and the interaction between tidal height and density ($P = 0.1647$) did not have a significant effect on bivalve numbers. However, as numbers of additional bivalves were low it is unlikely these additional recruits would have a significant impact on resources.

Gastropods also comprised a major part of the macrobenthic invertebrates collected (Figure 2.7D, 20.12%). The gastropods present were primarily *Nassarius pauperatus*, *Cominella lineolata*, and *Polinices conicus*, all of which

are carnivorous, drilling gastropods that prey on bivalve molluscs (Peterson, 1982a). *Bembicium auratum*, a small periwinkle which feeds on unicellular algae (Edgar, 1997), was also present. The total abundance of gastropods at each tidal height appeared consistent, although the distribution of gastropods within a tidal position was variable. Tidal height (P = 0.7806), density (P = 0.8110) and the interaction between tidal height and density (P = 0.1144) had no significant effect on gastropod numbers. Drilling gastropods were the only group of predators which caused mortality of *K. scalarina* in the experimental treatments, but this occurred in only one basket.

Carcinus maenas, the European shore crab, (Figure 2.7E) was placed in a separate category due to its known potential as a major bivalve predator (Gee *et al.*, 1985; McGrorty *et al.*, 1990). *C. maenas* comprised 8.17% of the total macrobenthos, but was not a major cause of mortality; in fact there was no evidence of *C. maenas* predation in any of the experimental treatments. Similarly, tidal height (P = 0.8167), density (P = 0.2940) and the interaction between tidal height and density (P = 0.2891) did not have a significant effect on the number of *C. maenas* in the experimental treatments.

2.3.6 Sediment Characteristics

None of the sediment characteristics covariates examined (particle size, organic content, redox and pH) displayed a significant effect on either the shell length or height increase of *K. scalarina*.

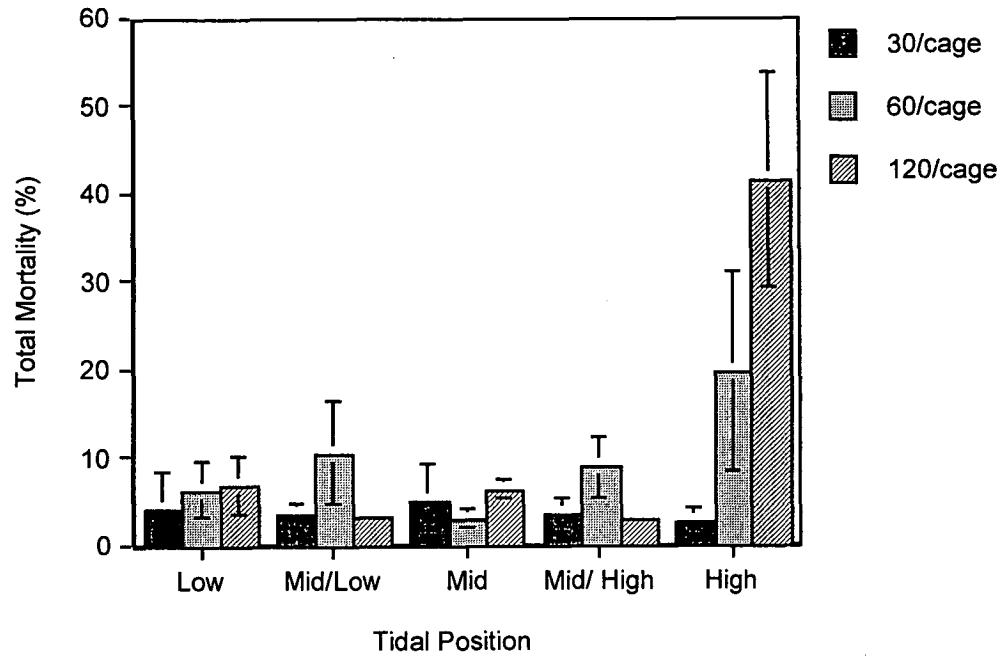

Figure 2.2: Mortality of *K. scalarina* in relation to stocking density and tidal position (mean±S.E., n=4 replicate baskets).

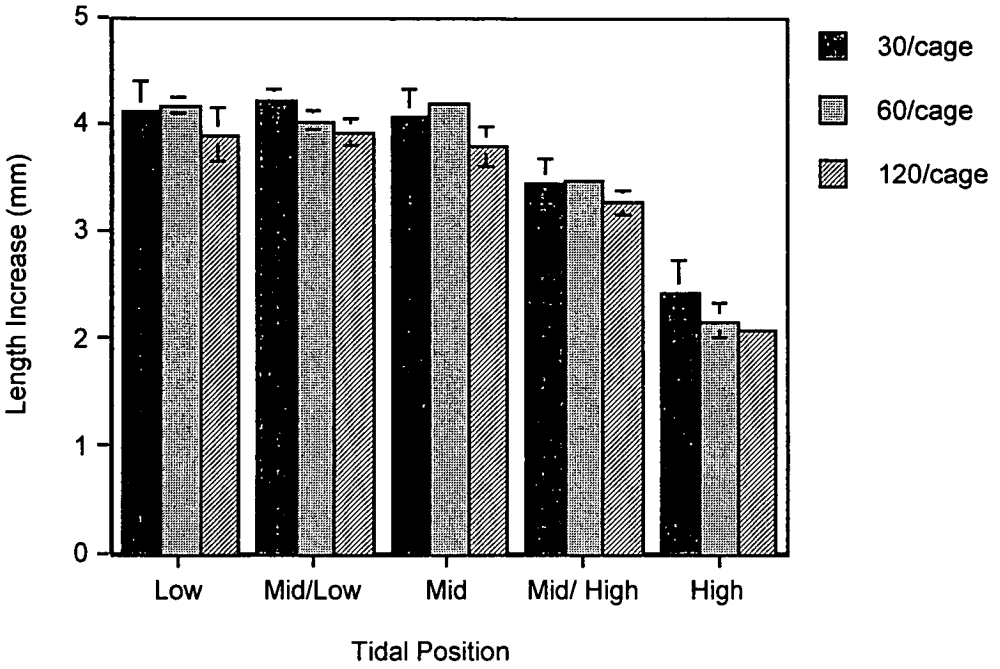

Figure 2.3: Growth of *K. scalarina* in experimental treatments measured as increased in length.(mm) over one year. (mean±S.E., n=4 replicate baskets).

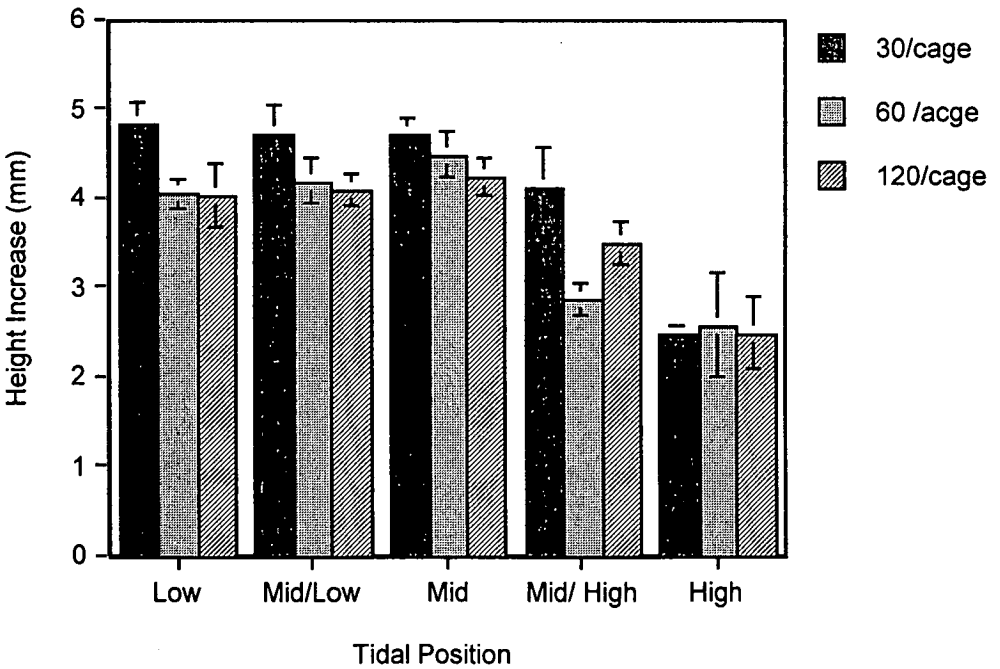

Figure 2.4: Growth of *K. scalarina* in experimental treatments measured as increases in height (mm) over one year (mean±S.E., n=4 replicate baskets).

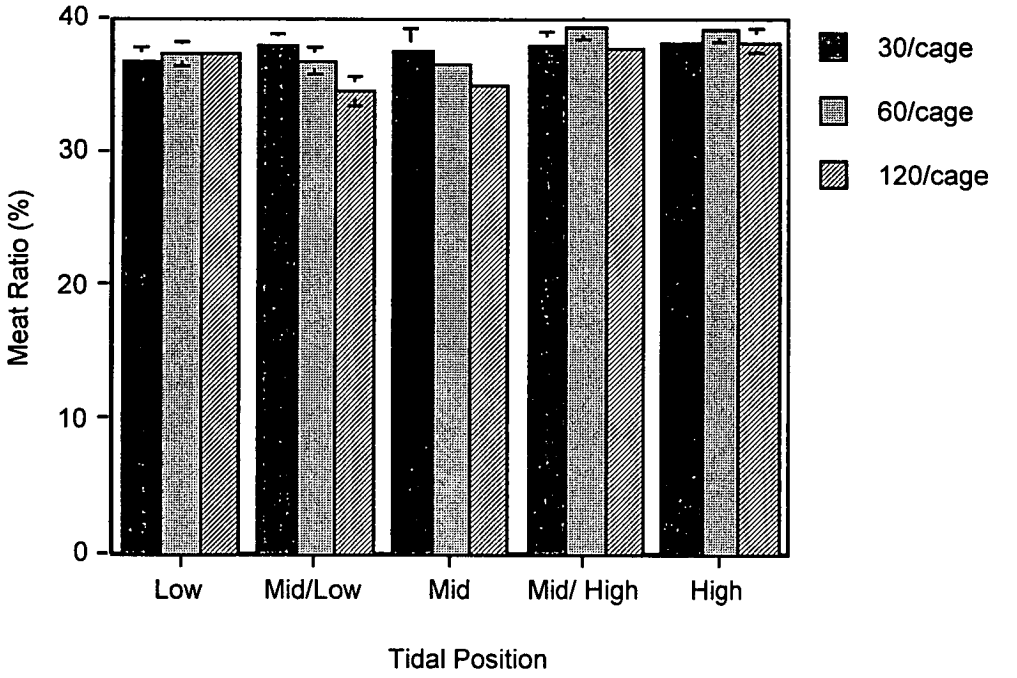

Figure 2.5: Percentage meat weight of total weight of *K. scalarina* in experimental treatments. (mean±S.E., n=30).

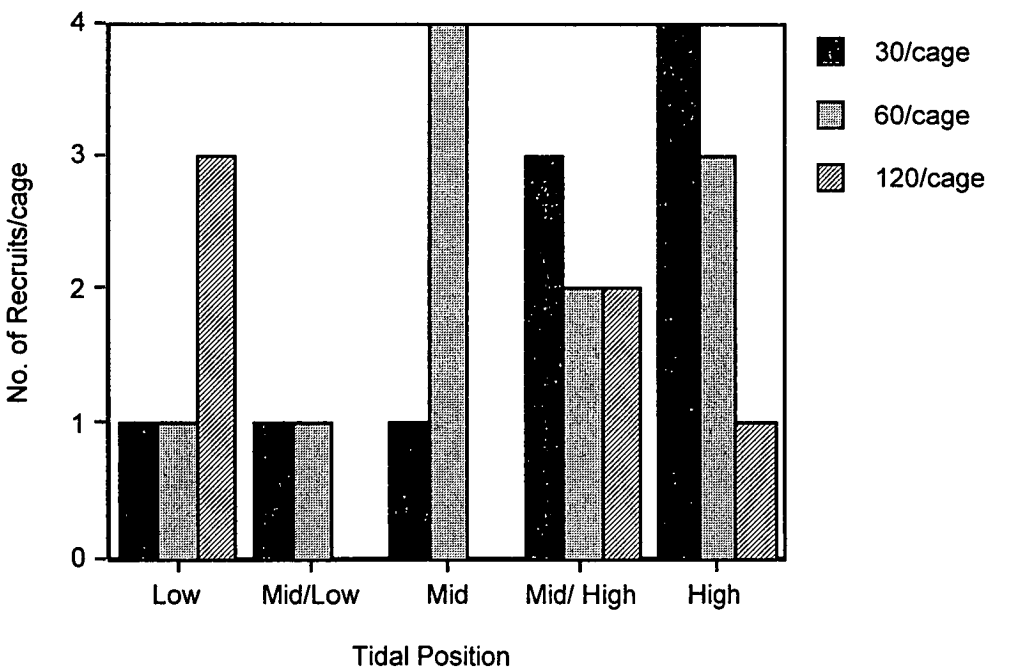

Figure 2.6: Recruitment of juvenile *K. scalarina* to experimental treatments (n=27). Each column represents the total number of recruits at the corresponding density and tidal position.

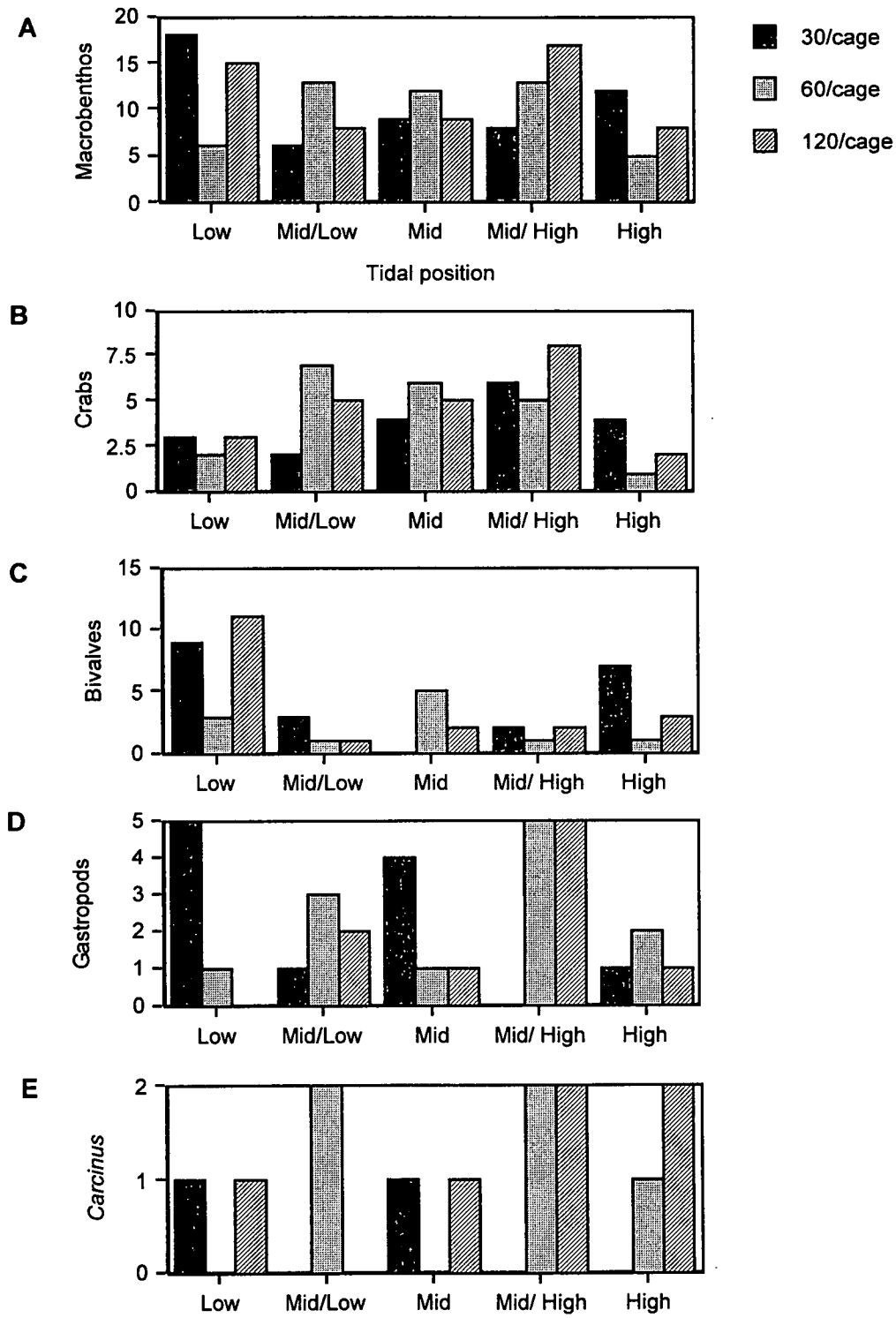

Figure 2.7: Numbers of macrobenthos/cage present in experimental treatments. A) Total numbers of macrobenthos (n=159) B) Number of crabs (n=63) C) Number of bivalves (n=51) D) Number of gastropods (n=32) E) Number of *Carcinus maenas* (n=13).

2.4 Discussion

2.4.1 Enclosure artefacts

According to Peterson (1982b) increasing density has a hierarchical effect on soft sediment bivalves; the first response is density dependent emigration, then a decrease in growth rate and reproductive effort, and finally an increase in mortality. In this study, density dependent emigration was effectively eliminated by the enclosures. In the absence of fences, experimental animals display enhanced immigration into neighbouring enclosure-free plots, a process which over time would have eroded the density treatments if manipulations had been attempted without enclosures (Peterson & Black, 1993). Bellchambers (1993) reported that while *K. scalarina* under natural conditions in almost sedentary, it is capable of significant migration to recolonise vacant spaces. Despite the necessary use of enclosures, the effect of enclosure artefacts on the results cannot be overlooked. Previous studies comparing enclosed and enclosure free plots to examine the effect of enclosing on individual growth, demonstrated a 50% reduction in growth of *Katelysia* spp. in roofed cages (Peterson & Black, 1993). In this study the presence of enclosure artefacts would not prevent a test of density or tidal position on growth because identical enclosures were used for all densities.

2.4.2 Survival

Previous authors have reported that increased density, even under conditions of extreme crowding, does not often produce mortality or competitive exclusion (Cresse & Underwood, 1982; Peterson 1982a, 1993), although few studies have investigated the combined effects of increased density and tidal position. These results from Moulting Lagoon indicate that both density and tidal position have a significant effect on survival of *K. scalarina*. *K. scalarina* displayed high survival rates across all density treatments, with the highest mortality being 41.5%, but in most treatments mortality was less than 10%. Survival was only greatly affected at the highest tidal position where density also became influential. The trend of decreased survival at higher tidal levels may be due to greater exposure causing physiological stress (Newell, 1979; Vincent *et al.*, 1994; Roegner & Mann, 1995). Similarly, Peterson & Black (1988b) working on five species of bivalves reported a decrease in survival at higher tidal positions, with mid shore survival rates of *Circe letnticularis* were 50% lower than those in subtidal treatments. *K. scalarina* is an intertidal clam and the scouring action of the channel in Moulting Lagoon meant no subtidal position was tested. In contrast Peterson & Black (1987) report that survival of the

clams, *Circe lenticularis* and *Placamen gravescens*, higher in the intertidal zone was greater than in treatments lower on the shore, largely due to predation of the clams in low shore treatments. All experimental treatments at Moulting Lagoon were enclosed in cages, and this coupled with the fact that dead shells displayed no obvious signs of predation, makes predation seems an unlikely explanation for low survival levels. A more likely explanation for the increase in mortality at higher tidal levels is increased exposure or resource depletion.

2.4.3 Growth

K. scalarina in experimental treatments displayed decreased shell growth rates with increased shore height. Clams in low shore treatments displayed growth increases double that of high shore treatments. Previous authors have reported that growth of suspension feeders decreases with tidal height (Peterson & Black, 1987, 1988b, 1991; Jensen, 1992, 1993; Vincent *et al.*, 1994; Roegner & Mann, 1995). The inverse relationship of growth and tidal height can be explained by the duration of tidal submersion (Jensen, 1992; Vincent *et al.*, 1994). Submersion time may determine the feeding period of suspension feeding and thus their food supply (Jensen, 1992). However, the prevailing tidal regime at Moulting Lagoon, and the broad nature of the tidal flat indicate that duration of tidal submersion would not vary greatly between tidal positions (approximately 1-2 h between high and low shore positions), making this explanation any unlikely one. Previous authors have suggested that submersion time alone does not explain growth reductions at higher tidal levels and a number of factors may be responsible (Peterson & Black, 1991; Jensen, 1993; Vincent *et al.*, 1994) Firstly, the physiological effects of exposure during low tide may reduce growth of suspension feeders due to high temperatures and fluctuating salinities (Hummel, 1985). Physiological stress in terms of desiccation varies between shore positions at Moulting Lagoon, despite the fact that tidal duration is similar across tidal heights. Sediments higher on the shore dry out significantly more than those lower on the shore (pers. obs.). Another explanation for decreased growth rates is the depletion of sestonic food concentrations, as the water moves up an intertidal gradient, due to suspension feeding organisms lower on the tidal flat (Hummel, 1985; Peterson & Black, 1991). Previous hydrodynamic studies have illustrated the ability of suspension feeders to deplete local supplies of benthic phytoplankton (Frechette & Bourget, 1985b; Frechette *et al.*, 1989; Monismith *et al.*, 1990). The high abundance of bivalves low on the shore at Moulting Lagoon make sestonic depletion of food resources a possible explanation for the observed patterns. Decreases in the growth rate of *K. scalarina* at high shore positions may be due to any combination of the above

factors. Density of *K. scalarina* at Moulting Lagoon also displayed a significant effect on growth. On average clams at the highest density grew 7.3% less than those at the lowest density. Numerous studies have reported reduced growth of suspension feeding bivalves (Peterson & Andre, 1980; Peterson & Black, 1988b; Olafsson, 1986; Vincent *et al.*, 1994) and it is generally associated with competition for limited resources (Peterson, 1992; Peterson & Black, 1993) such as food. Peterson & Black (1987, 1988b) designed experiments to test the importance of physical factors in the upper tidal zone, Shark Bay, Western Australia. Transplanted low shore clams (*Circe lenticularis* and *Placamen gravescens*) to high shore levels and monitored their performance. Both species grew more slowly than in their usual zone and *C. lenticularis* suffered higher mortality. However, it is unclear whether this was due to the direct effects of desiccation, reduced feeding time or aerobic respiration at higher shore levels (Raffaelli and Hawkins, 1996)

Tidal position and density displayed a significant effect on the meat ratio of *K. scalarina* at Moulting Lagoon. It has been suggested that rates of shell and soft tissue growth are not linked, and that shell growth may precede growth of soft tissues especially at high tidal levels (Hilbish, 1986; Harvey & Vincent, 1990). In contrast Beukema (1993) suggests that growth of soft tissues is dependent on tidal level. However, shell growth of *K. scalarina* at higher shore positions is apparently more suppressed than meat growth and hence the meat to shell ratio increases. Similarly, O'Meley (1995) reports that increased levels of aerial exposure also retards shell growth of Pacific oysters.

2.4.4 Recruitment

Previous studies have detected negative influences of adult density on larval settlement or juvenile recruitment (Woodin, 1976; Peterson & Andre, 1980; Peterson, 1982b; Andre & Rosenberg, 1991; Jensen, 1992; Olafsson *et al.*, 1994; Thrush *et al.*, 1996). In contrast, Peterson & Black (1993) suggest that *K. scalarina* demonstrates higher rates of recruitment where adults populations are more dense. In this study the numbers of recruits were highest in the low and mid density treatments rather than the high density treatments. Recruitment also increased with tidal height, rising from a total of 5 recruits/cage at the low shore treatments to 8/cage at high shore treatments. Jensen (1992) suggests that the abundance of *Cerastoderma edule* spat along a tidal gradient was positively correlated with submersion time. However, Woodward (1985), working on another Tasmanian lagoon, reports that there was a greater number of smaller, younger individuals at upper shore levels. Similarly, recruits at Moulting

Lagoon were more abundant at high tidal positions, however as the total number of recruits were low it is difficult to infer trends. It has been suggested that this pattern may be due to passive settlement of larvae due to depth and velocity of the overlying water mass (Jensen, 1992). In natural populations at Moulting Lagoon juveniles and small size classes are generally distributed higher on the shore than larger size classes (Bellchambers, 1993). It is possible that the cages themselves influenced the recruitment of juvenile clams and other macrobenthos, however as the total numbers of recruits were low their presence is somewhat insignificant.

2.4.5 Macrobenthos

A growing body of evidence suggests that increased densities of benthic suspension feeders in soft sediments do not cause significant reductions in recruitment of benthos (Peterson, 1982b; Hunt *et al.*, 1987; Ertman & Jumars, 1988; Peterson & Black, 1988a). Peterson & Black (1988b) demonstrated that total densities of smaller invertebrates failed to respond to changes in *Katelysia* density. This was also the case at Moulting Lagoon as the abundance of potential competitors in terms of other macro-invertebrates did not increase at low clam densities. There also appeared to be no real trend in the distribution of macro-invertebrates along the tidal gradient, with a total of 159 individuals present. However, when the macrobenthos is categorised clearer trends emerge.

The crabs *Mictyris platycheles* and *Paragrapsus gaimardii* composed 39.62% of the collected macrobenthic assemblage. Crabs display a clear preference for mid tidal treatments, with 76% of their total abundance distributed in these treatments.

Bivalves composed 32.07% of the cage macrobenthos, as total numbers were low it is unlikely that they are competing *with K. scalarina* for food. Unlike the crabs, bivalves present in the macrobenthic samples displayed a preference for the low shore treatments, this is not surprising given this is where bivalves reach there greatest abundance at Moulting Lagoon (Bellchambers, 1993).

Experimental manipulations failed to exclude all macrobenthos including potential predators such as naticid gastropods and *Carcinus maenas*. These types of predation leave distinctive signs: naticid gastropods leave characteristically drilled shells behind, whereas crab predation can be similarly inferred from crushed or chipped shells. Despite the reports of other authors that *Carcinus* is a voracious predator of bivalves (Gee *et al.*, 1985; Hunt *et al.* 1987;

McGrorty *et al.*, 1990) no signs of predation were evident on any dead shells. The only evidence of naticid predation was in one treatment cage. Therefore, predation was not a significant cause of mortality, which supports the suggestion that high shore treatments experienced higher levels of mortality due to desication or starvation through depletion of local food resources. However, as the meat ratio data does not support the theory of starvation at higher tidal levels. Woodward (1985) reports that *K. scalarina* is intolerant of desiccation and is generally restricted to lower tidal positions, therefore increased exposure appears the most likely cause of mortality.

2.5 Conclusion

Artificially increasing the density of natural populations of *K. scalarina* has been suggested as a means of both sustaining and increasing the productivity of natural populations. However, the reduction in survival and growth of *K. scalarina* exposed to high tidal positions and increased densities, coupled with the absence of predation pressure, indicates that resource limitation of some type is occurring. Survival and growth of the species is dependent on tidal position and density. Alternatively, increased mortality and depressed growth rates of high shore treatments may be due to increased exposure and physiological stress caused by variation in environmental conditions. The mechanisms limiting the growth and survival of *K. scalarina* at high shore positions are still unclear.

Chapter 3:

The effect of density on survival, growth and meat ratio.

3.1 Introduction

Competitive interactions among invertebrates in soft sediments are attributed to a variety of mechanisms: direct interference (Woodin, 1974; Peterson & Andre, 1980; Wilson, 1983; Weinburg, 1985), indirect interference by alteration of environmental conditions (Rhoads & Young, 1970; Reise, 1985), competitive exclusion (Woodin, 1976), inhibition of larval settlement (Woodin, 1976; Andre & Rosenburg, 1991) and food depletion (Levinton, 1972; Buss & Jackson, 1981; Peterson, 1982a; Olafsson, 1986; Peterson & Black, 1988a; Peterson & Beal, 1989) (see Chapter 1 for review). The existence of intra-specific competition has been recognised among suspension feeding bivalves in many soft sediment habitats (Peterson, 1982a; Bertness & Grosholz, 1985; Weinburg, 1985; Olafsson, 1986; Peterson & Black, 1987; Peterson & Beal, 1989; Vincent *et al.*, 1994, see chapter 1), however the extent to which density dependent processes influence the life history of suspension feeders is unclear (Jensen, 1993).

Manipulative field experiments have consistently demonstrated that dense assemblages of suspension feeding bivalves do not exhibit density-dependent mortality, even when competition is evidenced by reductions in growth (Peterson & Andre, 1980; Peterson, 1982a; Peterson & Black, 1987; Peterson & Beal, 1989; Peterson, 1992). Several lines of evidence suggest that food limitation is the cause of growth reductions at high densities (Peterson, 1992). Hydrodynamic studies illustrate the ability of suspension feeders to deplete local supplies of benthic phytoplankton which may result in growth inhibition of individuals (Frechette & Bourget, 1985a, b; Frechette *et al.*, 1989: Monismith *et al.*, 1990). The fact that bivalves grow less in high density patches implies that local resource limitation of some description is a common phenomenon for soft bottom suspension feeders in shallow waters (Peterson & Black, 1993). However, doubts over the prevalence of local food resource limitation among suspension feeders still persist (Levinton, 1972; Olafsson, 1986) due to the variable and unpredictable nature of phytoplankton populations.

Adult clams are presumed to interfere with colonising larvae either through direct predation (Woodin, 1976; Andre & Rosenburg, 1991) or physical disturbance and habitat alteration (Woodin, 1976; Peterson, 1982a; Andre & Rosenburg, 1991; Jensen, 1992). However, it has been suggested that recruitment of *K. scalarina* is increased around high densities of adult bivalves (Peterson & Black, 1993), even though the larvae of some bivalves may be ingested by adults and treated as food particles (Andre & Rosenburg, 1991).

Previous density manipulations have focused on detecting the negative effects of crowding, but it is possible that at high densities the penalties of competition are outweighed by positive effects via mutualistic, commensal, facilitative or promotive interactions such as predator protection and habitat stability (Peterson & Black, 1993). Some doubt still prevails regarding the effect of high densities on natural populations of bivalves (Jensen, 1993), and what role competition plays in structuring soft sediment habitats, as not all bivalve species respond in the same manner to varying density (Peterson & Black, 1987).

This component of the study examines the effects of intraspecific competition on the mortality, growth and recruitment of *K. scalarina* and the associated macrobenthic community. Experiments in this study were designed to include not only elevated abundances, but also several artificially reduced abundances to test the positive and negative consequences of density. This research is also relevant to culture of the species as stocking density can influence profitability of many forms of aquaculture (Treadwell *et al.*, 1991).

3.2 Methods

3.2.1 Initial Sampling

Experimental manipulations of *K. scalarina* were conducted in the intertidal zone of Moulting Lagoon, Coles Bay (42°05'S, 148°10'E) (see Chapter 2, Section 2.2.1 for site description). Juvenile *K. scalarina* (20.0 - 25.0 mm shell length) were collected from Meredith Point, Pelican Bay, Moulting Lagoon between 26th Feb-1st March 1996. Experimental manipulations were established in the intertidal zone of Swanwick Bay, Moulting Lagoon. The size frequency distribution of clams used in the experiments were narrow, to minimise differences between experimental treatments, and because insufficient numbers were available in the smaller size classes to utilise them

in the experiment. Clams were measured along the longest antero-posterior axis (length) and dorso-ventral margin (height) to the nearest 0.1 mm with vernier callipers and randomly allocated to a density treatment.

Density manipulations were conducted inside cages designed to prevent migration, so that densities could be maintained over the experimental period. Cages were also used to prevent the access of large mobile consumers such as crabs and wading birds to the clams. As all density treatments were enclosed identically, any additional effects of enclosures were held constant across the treatments. Cages were constructed of 9 mm^2 Nylex® mesh (33 cm wide x 53 cm long x 14 cm deep) and fastened with plastic cable ties to wooden stakes driven into the substrate. Density treatments were established by adding the appropriate number of measured *K. scalarina* to enclosures to achieve a range of densities from 57.18 - 1886.94 clams m^{-2}. Sediment below the enclosures was removed to a depth of 10 cm and returned to the cages by sieving through a 2 mm sieve to remove all potential predators and competitors.

Density treatments were established in a random block design, 90 m from the high tide mark, since this is the zone where the natural population reaches its highest abundance and greatest shell size (Bellchambers, 1993). Density experiments were located at the mid/low tidal position (see Chapter 2) and conducted concurrently with tidal height x density experiments. Density and tidal height x density experiments were separated by approximately 20 m. Nine densities were used, with four replicates of each treatment, in two fully randomised blocks. Densities ranged from 10 to 330 clams per cage with increments of 40. Neighbouring cages were separated by 1 m and blocks were separated by 5m (Diagram 3.1).

3.2.2 Final Sampling

Density manipulations were sampled between 13-17 January 1997, approximately one year after establishment. Techniques used for the final sampling of density experiments was identical to those outlined in Chapter 2, Section 2.2.3. With the exception of sediment samples with are briefly outlined below.

Four sediment samples, redox and pH measurements were collected from both inside and outside (1m) the low (50) and high (250) density treatment to

determine whether the presence of large aggregations of suspension feeders altered the sediment characteristics.

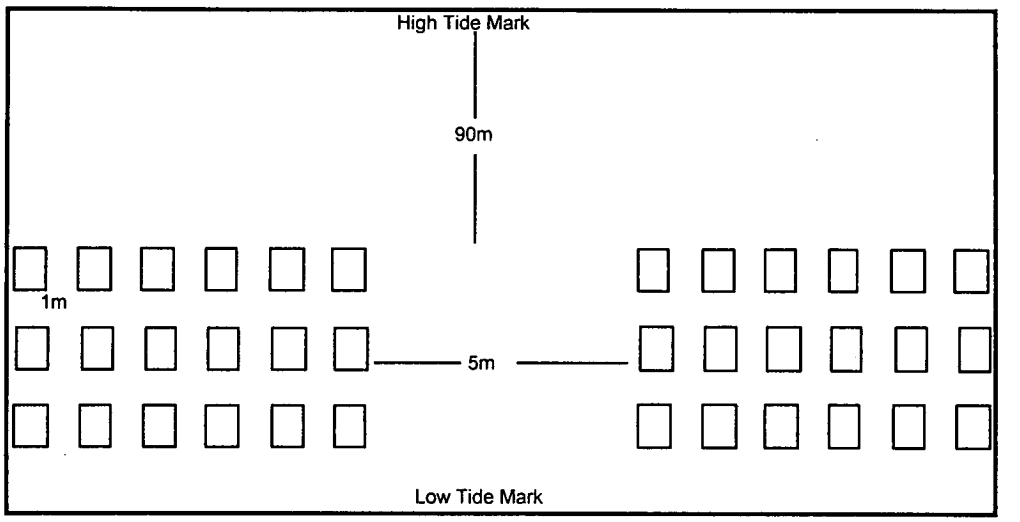

Diagram 3.1: Representation of experimental design at Moulting Lagoon, Coles Bay.

3.2.3 Statistical analysis

Data for growth (length and height), survival, meat ratio number of recruits and macrobenthos were analysed using two way ANOVA in JMP 3.0 for Macintosh (SAS Institute, 1995) with density and block as factors. Non significant results were re-analysed using one way ANOVA, averaging for block, if block and block x density interactions were non significant (see appendix 2). Bartlett's test for homogeneity of variances was applied to all data to check equality of variances (Sokal & Rohlf, 1981) and residuals were examined for departures from normality.

Soil data were analysed as a series of covariates. Sediment characteristics were tested as both simple covariates and in a factorial design to determine whether the effect of sediment characteristics depended on the treatment level. The results of statistical analysis are presented in appendix 2.

3.3 Results

3.3.1 Survival

K. scalarina displayed high survival rates across all experimental treatments regardless of density, (Figure 3.1) with the lowest survival rate being 81%, in

the 90 clam treatments in Block B. However, 100 % survival was evident only in the lowest density treatment in Block A. There was no significant effect of density on survival (P = 0.404) and the interaction between density and block was not significant (P = 0.431), but a marginally significant block effect was evident (P = 0.0402). Data were re-analysed as a one way ANOVA, averaging for block, on the basis that there was no significant block x density interaction and only a minor block effect. Again, density had no significant effect on the survival of *K. scalarina* (P = 0.492).

Clams recovered from the experimental treatments were classified into one of four categories: alive, chipped, drilled or unexplained mortality (Figure 3.2). Empty shells rarely displayed any sign of damage or death due to predation. The majority of dead clams (82.04%, Figure 3.2D) displayed no obvious signs of predation and are assumed to have died from starvation or other physiological causes. Dead shells exhibiting predator damage were either chipped by crabs (11.02%, Figure 3.2C) or drilled by naticid gastropods (6.94%, Figure 3.2B). The type or frequency of predation did not appear to vary with density.

3.3.2 Growth

Increases in length over the experimental period were small (Figure 3.3). The largest average increase in length was in the 210 clams cage^{-1} treatment in both blocks, with an increase of 4.46 mm in block A and 4.44 mm in block B. The smallest increase in length in both block A and B was in the 10 clams cage^{-1} treatment, with increases of 4.03 and 3.89 mm respectively. However, there was no significant effect of density on shell length increase (P = 0.091). Similarly, *K. scalarina* did not display large increases in height during the experimental period (Figure 3.4), the largest recorded increase was in the 50 clams cage^{-1} treatment for both block A and B, of 4.68 mm and 4.39 mm respectively. There was no significant relationship between shell height increase and density (P = 0.223).

Meat ratio (% of total weight consisting of meat weight) was very consistent across the experimental treatments. The highest meat ratio was in the 290 clams cage^{-1} treatment in block A, with a value of 40.3%. The lowest meat ratio, 34.8%, was in the 330 clams cage^{-1} treatment in block B (Figure 3.5). Density did not have a significant effect on meat ratio (P = 0.3532).

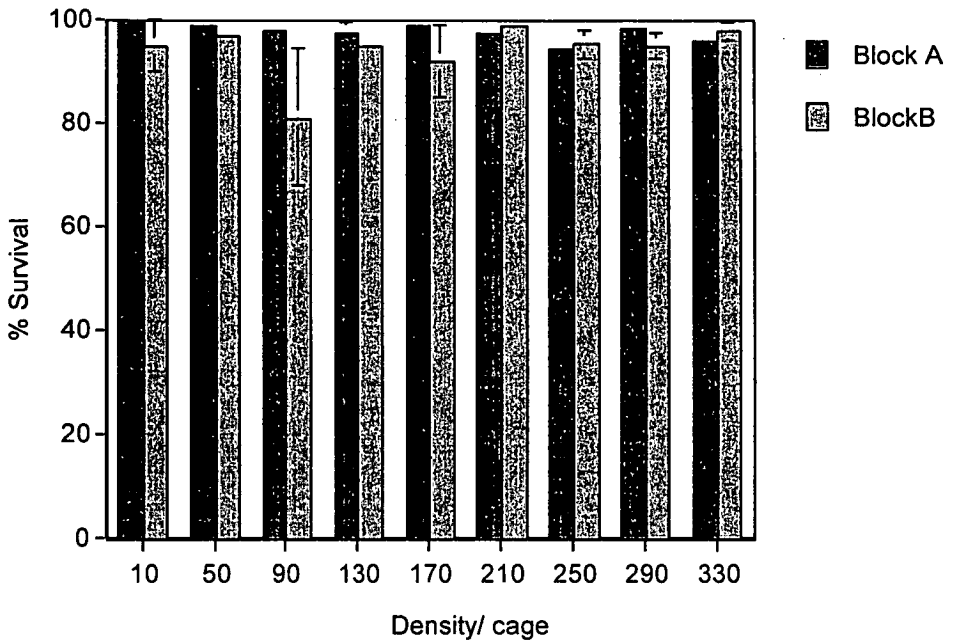

Figure 3.1: Percentage survival of *K. scalarina* in experimental density manipulations over 1 year (mean±S.E., n=4 replicate baskets)

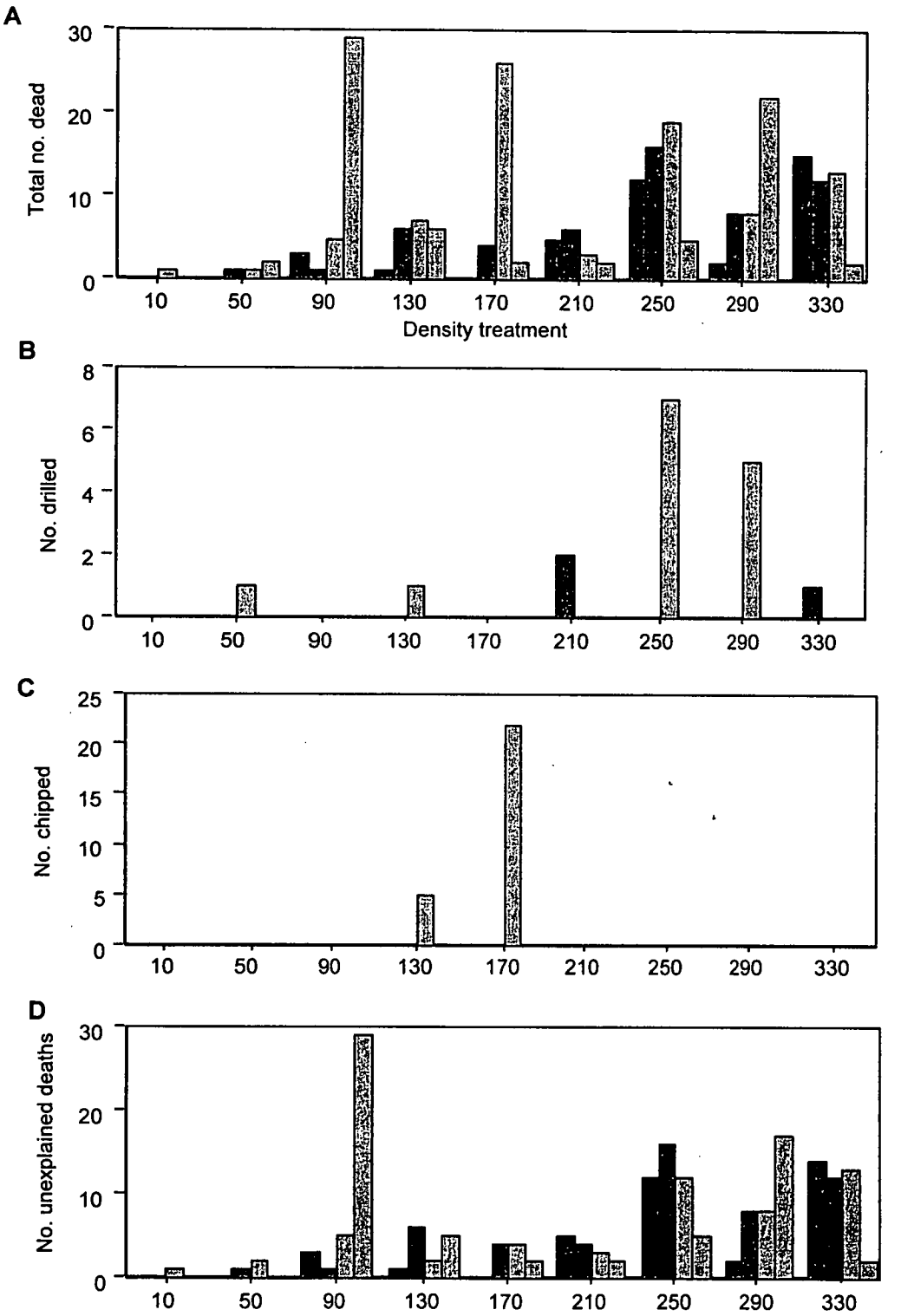

Figure 3.2: Survival of *K. scalarina* in density treatments. Each column represents one treatment basket. A) Total mortality of *K. scalarina* (n=245); B) Total number of drilled *K. scalarina* (n=17); C) Total number of chipped *K. scalarina* (n= 27); D) Total number of unexplained deaths (n=201).

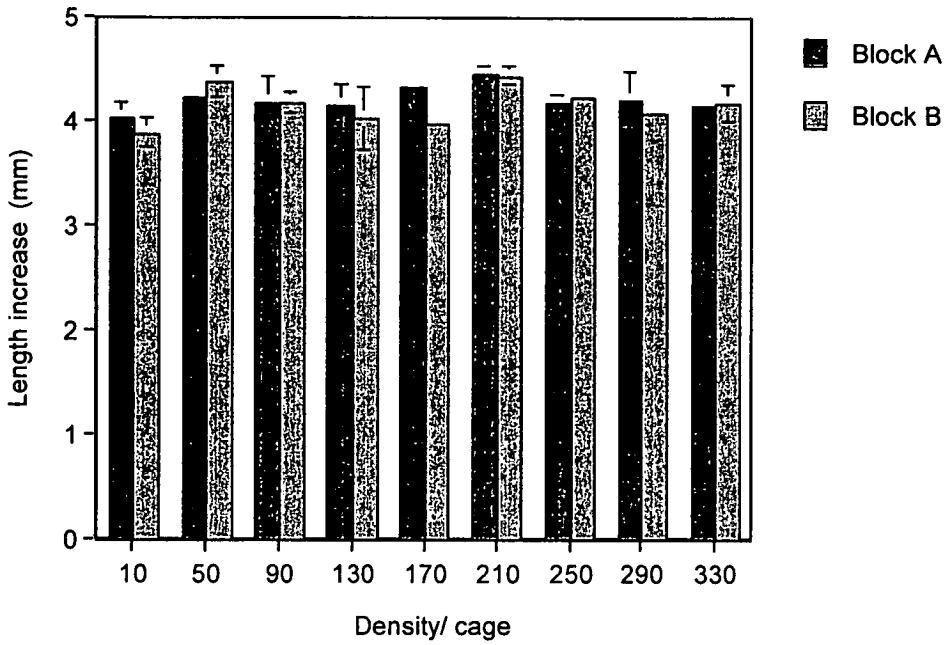

Figure 3.3: Growth of *K. scalarina* in experimental treatments measured as increase in length (mm) over one year (mean±S.E., n=4 replicate baskets)

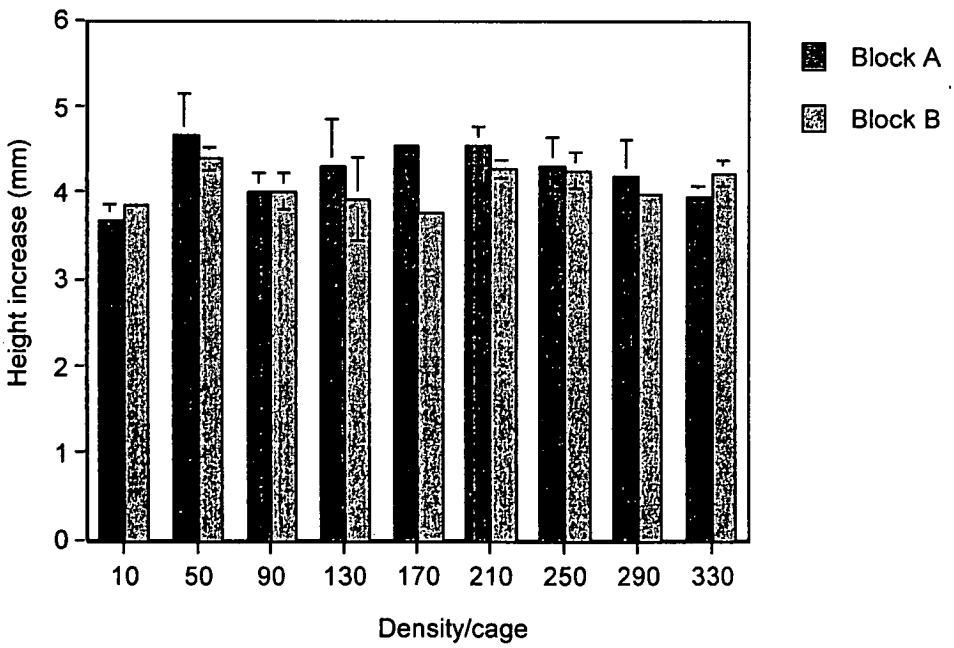

Figure 3.4: Growth of *K. scalarina* in experimental treatments measured as increase in height (mm) over one year (mean±S.E., n=4 replicate baskets)

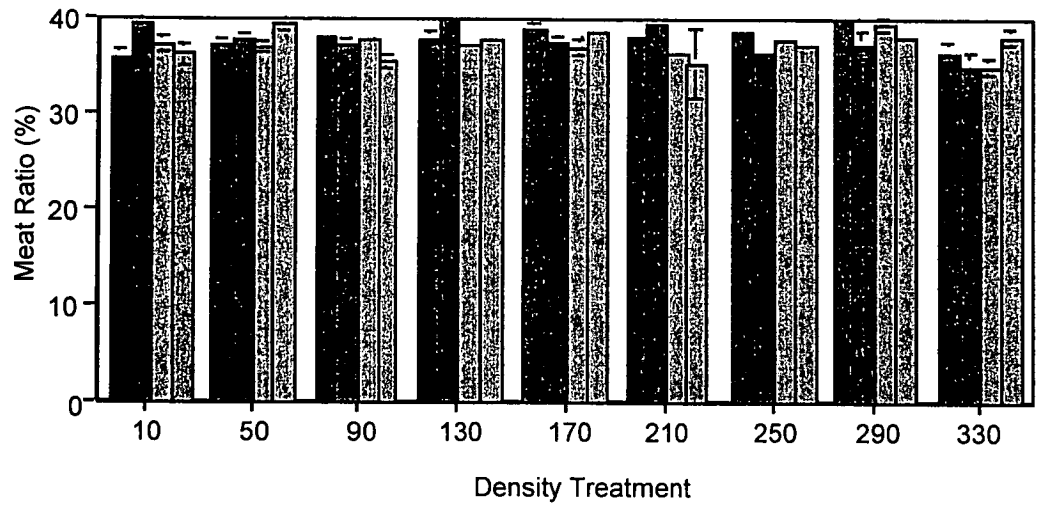

Figure 3.5: Percentage meat weight of total weight of *K. scalarina* in experimental treatments. (mean±S.E., n=30 clams)

3.3.3 Recruitment

Recruitment of juvenile *K. scalarina* was low in all the experimental treatments and most treatments had no recruits at all (Figure 3.6). The highest numbers were found in the 170 and 290 clams cage^{-1} treatments with a total of three recruits each. Recruitment did not vary significantly (P = 0.861) across the experimental treatments.

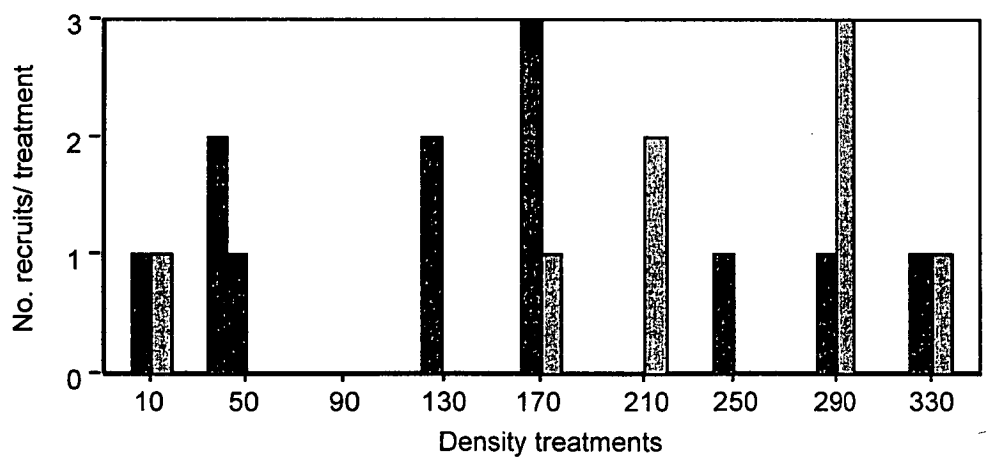

Figure 3.6: Recruitment of juvenile *K. scalarina* to experimental treatments (n=20)

3.3.4 Macrobenthos

Density had no effect on the number of macrobenthic species present (P = 0.7475). Numbers of macrobenthic invertebrates recovered from the treatment cages were relatively consistent over the experimental treatments (Figure 3.7A), with the highest number being 10 in the 170 clams cage^{-1} treatment. Overall block B displayed higher numbers of retrieved macrobenthic organisms. Macrobenthic organisms were subdivided into four categories: other bivalves, gastropods, crabs and the introduced European shore crab, *Carcinus maenas*. The division of the macrobenthos into categories allowed estimates of their effects on the experimental treatments in terms of predation potential and competition.

The most abundant taxon recorded was crabs (61.0%, Figure 3.7B), the majority of which were the soldier crab, *Mictyris platycheles* and the shore crab, *Paragrapsus gaimardii.* Despite the high numbers of these two species of crab in the experimental treatments they posed no predation threat to *K. scalarina*. Numbers of crabs did not vary significantly with density (P= 0.687).

The next numerically dominant group was gastropods (27.27%, Figure 3.7C). Gastropods present were primarily *Nassarius pauperatus*, *Cominella lineolata*, and *Polinices conicus*, all of which are carnivores, drilling gastropods that prey on bivalve molluscs (Peterson, 1982b). *Bembicium auratum*, a small periwinkle which feeds on algae was also present. The abundance of gastropods appeared to increase with clam density, however the highest frequency observed was three per treatment. Despite the number of gastropods present, numbers did not vary significantly with density (P = 183) and mortality due to gastropod predation, as evidenced by dead drilled shells, was low (6.94%, Figure 2B).

Several species of bivalves were also found in the experimental treatments (9.09%, Figure 3.7D), mainly *Eumarcia fumigata* and *Soletellina biradiata* which may have been competing with *K. scalarina* for food resources. However, as numbers of additional bivalves were low, maximum of two per cage, coupled with the fact that numbers of bivalves did not vary with density (P = 0.870) these additional recruits were unlikely to have a significant impact on the experimental treatments.

Carcinus maenas , the European shore crab, (Figure 3.7E) was placed in a separate category due to its known potential as a major bivalve predator (Gee *et al.*, 1985; McGrorty *et al.*, 1990). *C. maenas* comprised 2.60% of the total macrobenthos, but did not appear to be a major cause of mortality as it contributed to only 11.02% of the total mortality (as evidenced by chipped shells), which was mainly due to large numbers (12.94%) of *K. scalarina* consumed in one cage. However, the numbers of *C. maenas* did not vary significantly with density (P = 0.749).

3.3.5 Sediment Characteristics

Of all the sediment characteristics tested (particle size, organic content, redox, and pH) only the interaction of pH as a covariate and density within the treatment cages had a significant effect on shell height increase of *K. scalarina.* Inclusion of this covariate led to a significant result (P = 0.0423, Table 3.1) for the effect of density on shell height. Based on these adjusted treatment means, shell height gain decreased with increasing density.

Table 3.1: Covariate analysis of the effect of pH inside the treatment cages on the height of *K. scalarina.*

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	1	1	0.57317704	8.7808	0.0414
In. pH	1	1	0.00180043	0.0276	0.8762
Density*In. pH	1	1	0.56540123	8.6617	0.0423

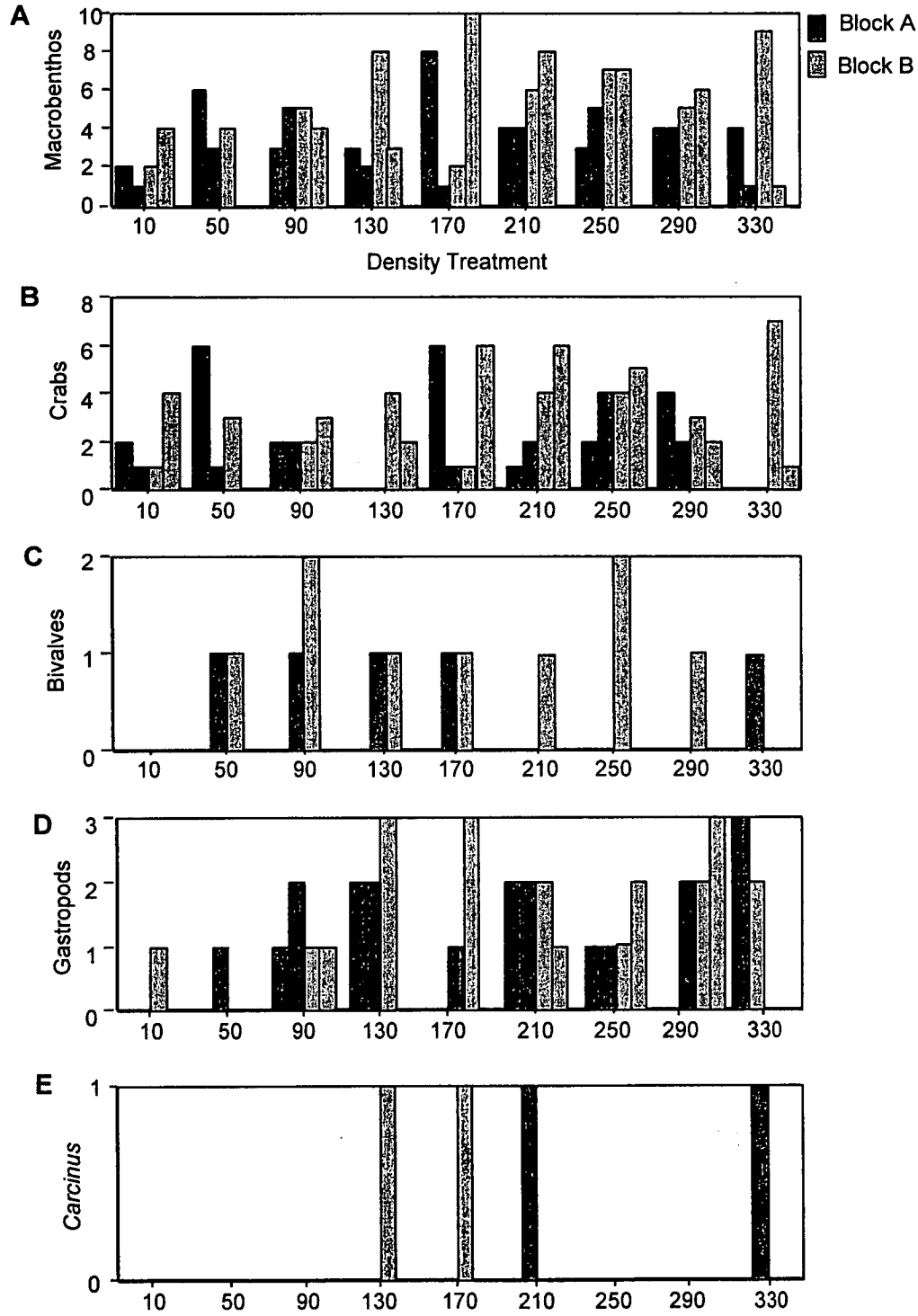

Figure 3.7: Numbers of macrobenthos present in experimental treatments. A) Total numbers of macrobenthos (n=154) B) number of crabs (n=94) C) Number gastropods (n=42) D) Number of bivalves (n=14) E) Number of *Carcinus Maenas* (n=4).

3.4 Discussion

3.4.1 Survival

Competition among suspension feeding bivalves in soft sediment environments does not often, even under extreme crowding, produce mortality or competitive exclusion (Creese & Underwood, 1982; Peterson, 1982a, 1992; Peterson & Black, 1993). In this study *K. scalarina* displayed high survival across all experimental treatments regardless of density, with the lowest survival rate being 81% in any treatment. Peterson & Black (1993) reported similar survivorship for *Katelysia* spp. in Princess Royal Harbour, over a period of eight months. Density manipulations conducted in four lagoonal systems in Western Australia and California, suggested that mortality of *Katelysia scalarina*, *K. rhytiphora*, *Protothaca staminea* and *Chione undatella* rarely responded to increases in density even when competition was evidenced by reductions in growth rate in all four systems (Peterson, 1982a; Peterson & Black, 1987, 1988a; Peterson & Beal, 1989). In this study there was no significant effect of density on survival, however there was a trend of increasing mortality at higher densities. In comparison Peterson & Black (1993) report that both survivorship and growth of *Katelysia* spp. decreased by 20 - 30 % in high density treatments, evidenced by a significant increase of dead undamaged shells. In high density treatments at Moulting Lagoon, the number of dead undamaged shells increased, indicating death by starvation or other physiological effects. Predation was a relatively insignificant cause of mortality (16.96%). However, mortality due to predation was closely linked with the presence of *Carcinus maenas* or drilling gastropods in the macrobenthic samples.

3.4.2 Growth

Growth of *K. scalarina* at Moulting Lagoon was apparently insensitive to increases in density as it remained consistent across all density treatments, even when densities were thirty times the natural adult *K. scalarina* density (50-60 m^{-2}). Peterson & Black (1993) reported that individual growth of *K. scalarina* in Princess Royal Harbour, Western Australia, only rarely varied significantly between density treatments. Previous density manipulations of suspension-feeding bivalves have shown either reductions in growth with density (Peterson, 1982a; Bertness & Grosholz, 1985; Olafsson, 1986; Peterson & Black, 1987, 1988a; Peterson & Beal, 1989; Jensen, 1992) or failure to respond to density (Peterson & Black, 1993). Density manipulations which have revealed marked declines in growth with increased density are primarily from oligotrophic coastal lagoons (Peterson, 1982a; Peterson & Black, 1987), while examples where crowding induced small

reductions in growth (Peterson & Beal, 1989), or failed to result in any detectable effect (Olafsson, 1986) were from nutrient-rich systems. Moulting Lagoon is characterised by persistently clear waters, a large tidal range, regular flushing, and is populated by relatively high densities of suspension feeding bivalves. Natural densities of *Katelysia* spp. populations in Princess Royal Harbour (~160 m^{-2}) are also much higher than Moulting Lagoon populations (~60 m^{-2}).

Moulting Lagoon does not fit clearly into either of the above categories, but the failure of clams to respond to density treatments may be due to the position of the experimental treatments within the intertidal zone. Density manipulations were conducted low on the shore, approximately 90 m from the high tide mark, as it is in this region that *K. scalarina* reaches its highest abundance and size (Bellchambers, 1993). Previous authors report no effect of density on shell growth in low shore treatments (Peterson & Black, 1987; Vincent *et al.*, 1994). It has been suggested that the absence of density dependence in low shore treatments is a consequence of longer submergence time, allowing bivalves to obtain organic matter and an undepleted food source as little or no extraction of organic matter by other suspension-feeders has yet occurred (Peterson & Black, 1987). Other explanations may include the high percentage of sand (Olafsson, 1986) and high mobility of sediment, which enhances the resuspension of the benthic diatoms and other deposited particles. Thus, suspension feeders experience an unpredictable and variable food supply during the growing season (Peterson & Black, 1988b). Therefore periods of intense competition for food resources are interspersed with periods of abundant food supply, rendering mortality or long term growth restrictions due to inadequate food resources unlikely.

Comparable studies of *K. scalarina* in Princess Royal Harbour reported growth rates of 0.031 mm $month^{-1}$ (Peterson & Black, 1993), but the lowest increase in this study (0.324 mm $month^{-1}$) was approximately ten times higher. The high growth rate in comparison to the work of Peterson & Black (1993) suggests that *K. scalarina* in high density treatments were not suppressing shell growth in order to maintain body tissue and basic metabolic processes. Conversely, the failure of *K. scalarina* to display a decrease in meat ratio indicates that clams in higher density treatments were not allocating resources to shell growth at the expense of body condition. It would appear that adequate resources were available to maintain both body tissue and shell growth.

Riley *et al.*, (1996, unpublished data) determined the age of *K. scalarina* at three locations around Tasmania and suggest that the rate of growth varies between sites. Estimates of the age of *K. scalarina* at commercial size (>32 mm) obtained using von Bertalanffy growth curves suggest that *K. scalarina* reaches commercial size in 4 years at Ansons Bay, 5 years at Little Musselroe Bay and 6 years at Cockle Creek (Riley *et al.*, 1996, unpublished data). However, the relationship between the growth rate of enclosed populations and the natural population at Moulting Lagoon remains unclear. Although enclosing density manipulation was necessary to maintain experimental densities, the fact that enclosing clams in cages may have reduced growth rates cannot be overlooked. However as some form of predator protection will be required to minimise loss of aquacultural stock the growth rate of caged clams may provide a more accurate indication of growth rates that can be obtained in an aquaculture venture.

3.4.3 Sediment characteristics

The main factor influencing organisms in soft sediment habitats is the nature of the sediment (Raffaelli & Hawkins, 1996). Examination of sediment characteristics (as covariates), such as phi size, organic content and redox potential, within and outside treatment cages did not significantly affect the growth of *K. scalarina.* However, density and its interaction with pH inside the treatment cages displayed a significant effect on the height of *K. scalarina.* These differences may be due to sedimentary modifications created by the cages (Hulberg & Oliver, 1980). Many authors have reported that although soft sediment organisms are intimately associated with and reliant on the sediment for survival, dense aggregations of infauna have the ability to significantly alter the physical and chemical structure of their habitat (Rhoads, 1974; Reise, 1985; Hall *et al.*, 1993; Woodin, 1997). Thus these result should be interpreted with caution, as while pH may be influencing the growth of *K. scalarina*, conversely the dense aggregations may be affecting the pH of sediment within the treatment cages. This conclusion is supported by the fact that pH outside the treatment cages was not a significant covariate.

3.4.4 Predation

The experimental manipulations failed to exclude all potential predators. The cage design prevented access of large mobile consumers such as fish, rays,

starfish and wading birds, however a number of small macrobenthic predators were found in the cages. Two main types of predators were found, naticid gastropods and the crab, *Carcinus maenas*. These types of predation leave distinctive signs: naticid gastropods leave characteristically drilled shells behind, whereas crab predation can be similarly inferred from crushed and chipped shells. *C. maenas* is an efficient predator of benthic invertebrates (Jensen & Jensen, 1985; Reise, 1985) and experiments have shown they can greatly decrease prey populations (Gee *et al.*, 1985; McGrorty *et al.*, 1990). Experiments were not specifically designed to test predation, therefore predation was limited to cages which predators were able to penetrate.

Predation was not a major cause of mortality at Moulting Lagoon, with only 16.96% of dead shells displaying signs of predation and, despite previous reports, only 11.02% were attributable to *C. maenas*. Peterson & Black (1993) reported that mortality of *Katelysia* spp. due to these categories of predation was also low in Princess Royal Harbour. Macrobenthic predators were present at Moulting Lagoon, however average densities were less than four crabs cage^{-1} which may be insufficient to cause significant mortality. Mackinnon (1997) reports that predation of *K. scalarina* by *C. maenas*, in feeding trials decreases dramatically once clams exceed a preferred size range, with 5.25 clams (5-15mm shell length) consumed per hour while only 0.5 clams (16-29 mm shell length) were consumed in the same time period. Clams used at Moulting Lagoon were in excess of 20 mm suggesting that the absence of predation by *C. maenas* may be due to the clams exceeding the preferred prey size range. Previous authors have suggested that high densities of suspension feeders act in a positive manner to prevent predation, as the structural complexity of dense shell assemblages may prevent the access of predators (Peterson & Black, 1993) thereby indirectly affecting survivorship.

3.4.5 Recruitment

The majority of previous studies have detected negative influences of adult density on larval settlement or juvenile recruitment (Woodin, 1976; Peterson & Andre, 1980; Peterson, 1982a; Andre & Rosenberg, 1991; Jensen, 1992; Olafsson *et al.*, 1994; Thrush *et al.*, 1996). Larvae that respond positively to the presence of adult suspension feeders may be subjected to greater risk of cannibalism immediately prior to settlement (Woodin, 1976; Andre & Rosenberg, 1991) but this risk appears to be small (Ertman & Jumars, 1988). Experimental manipulations at Moulting Lagoon displayed no significant

effect of adult density on juvenile *K. scalarina* recruitment, however as the number of recruits were extremely low it is difficult to infer trends. Previous studies have suggested that *K. scalarina* demonstrate higher rates of recruitment where adults are densest, however this trend was detectable in only one of the two experimental years (Peterson & Black, 1993). The failure of juvenile recruitment of *K. scalarina* at Moulting Lagoon to respond to adult densities may be due to the sporadic and infrequent spawning of adults in the lagoon (pers. obs.) rather than a reflection of negative cues or density induced responses.

3.4.6 Macrobenthos

Previous studies have reported a failure of benthic invertebrates to respond to manipulations of suspension feeding bivalves (Peterson, 1982a; Hunt *et al.*, 1987) and imply that competitors do not increase at low densities. Density manipulations of *K. scalarina* at Moulting Lagoon displayed no apparent effect on the macrobenthic abundance. Macrobenthic invertebrates collected in the cages were low, with a total of 154 individuals present across all density treatments.

Mictyris platycheles and *Paragrapsus gaimardii* composed 61.04% of the collected macrobenthic assemblage. Both these species are common estuarine crabs and are abundant in Moulting Lagoon. Bivalves composed 9.09% of the cage macrobenthos, and as total numbers were so low it is unlikely that they are competing with *K. scalarina* for food. The other two groups of macrobenthic invertebrates, drilling gastropods and *C. maenas*, represent potential predators of *K. scalarina*, however predation was a minor cause of total mortality.

Peterson & Black (1988a) also demonstrated that total densities of smaller invertebrates failed to respond to changes in *Katelysia* spp. density. A growing body of evidence suggests that increased densities of benthic suspension feeders in soft sediments do not cause significant reductions in recruitment of benthos (Peterson, 1982a; Hunt *et al.*, 1987; Ertman & Jumars, 1988 ; Peterson & Black, 1988a). Peterson (1979) suggests this is because competition is relatively ineffective in structuring communities of benthic infauna in soft substrate.

3.5 Conclusion

The failure of *K. scalarina* populations at Moulting Lagoon to display density dependent responses supports previous suggestions that competition plays a relatively insignificant role among suspension-feeding invertebrates in soft sediments (Peterson & Black, 1993). The absence of density dependent competition may be due to abundant resources, both food and space, to support a higher population density than currently exists. However, this raises the question of what prevents natural populations from achieving their optimal density. Peterson & Andre (1980) suggest that the potential for competition may exist but is not realised because of infrequent physical and biological disturbances which maintain low infaunal densities. This is certainly not the case with *K. scalarina* in caged experiments but may partially explain the patterns of abundance and distribution in natural populations. Alternatively, populations in these systems may be limited by some other factor such as high predation of pre-recruits or erratic recruitment patterns. In terms of aquaculture and fisheries enhancement the absence of density dependent responses is a positive factor indicating that current populations of *K. scalarina* have the potential to support much larger number of individuals without any detrimental effect in terms of survival or growth.

Chapter 4:

The effect of salinity on the osmotic and ionic composition of body fluids.

4.1 Introduction

Estuarine organisms exist under the stress of frequent and large variations in ambient salinity, caused by tidal fluctuations, periods of heavy rain and freshwater run-off (Hoyaux *et al.*, 1976; Shumway, 1977a; Djangmah *et al.*, 1979; Posey, 1987; Neufeld & Wright, 1996). Salinity fluctuations can affect both the survival and behaviour of estuarine and intertidal organisms (Posey, 1987). To survive in such a variable environment, organisms must have either a high tolerance or have developed various mechanisms to withstand the osmotic stress imposed by salinity changes (Shumway, 1977a; Deaton, 1992).

Osmoregulation in bivalves has been investigated by numerous authors (Hoyaux *et al.*, 1976; Shumway, 1977a, b; Baginski & Pierce, 1978; Djangmah *et al.*, 1979; Deaton, 1981; Mahasneh & Pora, 1981; Nossier, 1986; Deaton, 1992). Despite the variability of the estuaries in which some bivalve molluscs live, they possess no specialised osmoregulatory organs and most marine molluscs have little, if any, ability for extracellular osmotic regulation (Hoyaux *et al.*, 1976; Burton, 1983). The euryhalinity of these osmoconformers is partly due to their ability to isolate the mantle cavity by shell closure when the external salinity is unfavourable (Shumway, 1977a; Berger *et al.*, 1978), thereby reducing osmotic shock to the cells. The secretion of mucus (Shumway, 1977a), burrowing (Shumway, 1977a; Deaton, 1992) and withdrawal of sensitive body parts (Kinne, 1971) are other mechanisms for coping with osmotic stress.

Under normal salinity conditions the haemolymph of marine molluscs is generally similar to seawater in ionic composition (Burton, 1983; Wada, 1984) and most species of molluscs tend to maintain a high degree of stability in their internal ionic composition (Mahasneh & Pora, 1981). Ions play a major role in many metabolic processes and stabilisation of proteins in the cell membrane. The stabilisation of optimal ionic composition is necessary for the efficient working of the cell, particularly when environmental salinity is fluctuating. Molluscs vary greatly in their ability to regulate ions in their internal media; some marine and

brackish water species have limited regulatory abilities, while some freshwater species closely regulate ions (Burton, 1983).

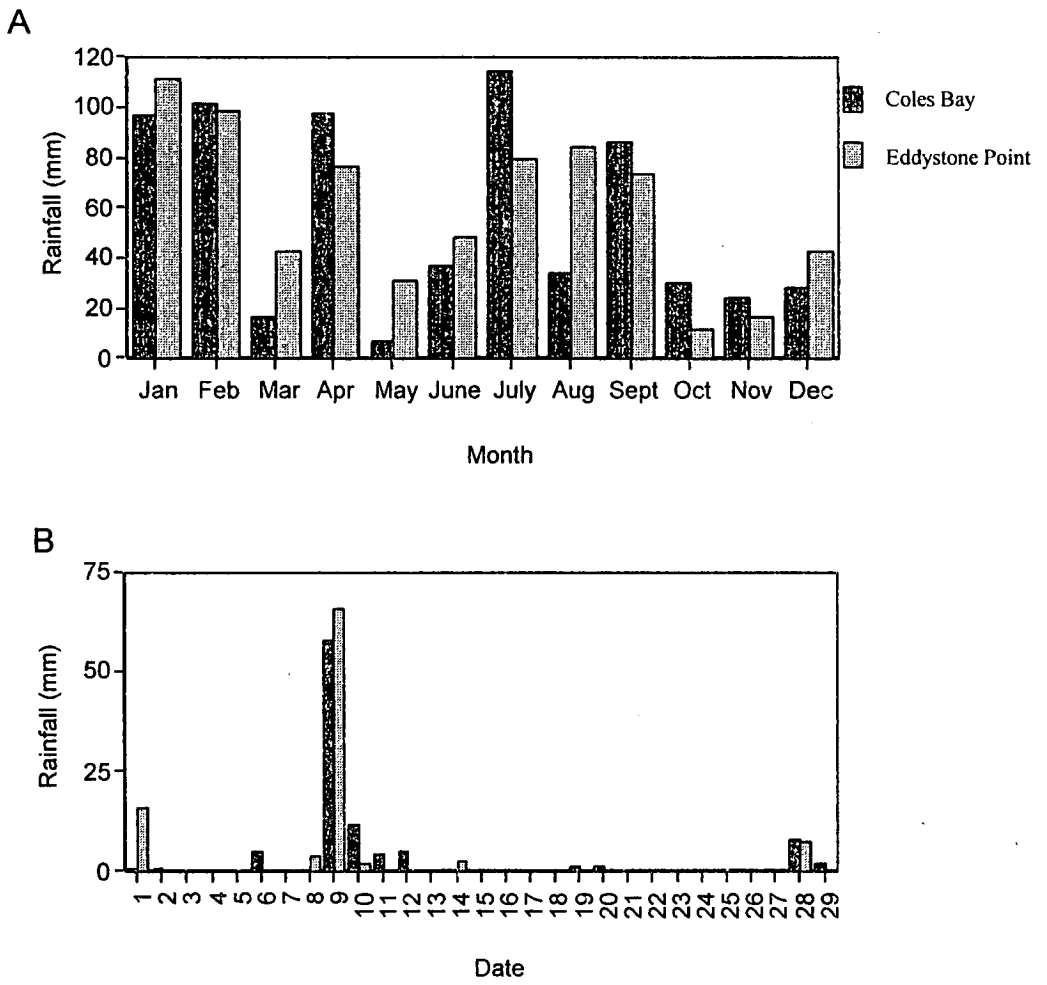

Figure 4.1: Rainfall data (mm) for the East coast of Tasmania from Coles Bay and Eddystone Point.
A) Monthly total of rainfall data for 1996 B) Daily rainfall data for February 1996. Data from the Bureau of Meteorology Tasmania.

K. scalarina comprises the dominant macrofaunal component of many bays and estuaries on Tasmania's east coast. Sheltered and partially closed to the sea by bayhead spits, these areas are prone to periods of reduced flushing and large sporadic, fresh water influxes from periods of high rainfall and freshwater runoff. The region's annual rainfall does not display any seasonality in distribution (Figure 4.1A) and tends on a monthly basis to fall in intense bursts followed by long dry spells (Figure 4.1B). These large scale salinity fluxes represent a major environmental stress for the estuarine fauna not only in terms of survival but also in relation to specific physiological processes.

Little is known of the ability of *K. scalarina* to tolerate massive freshwater influxes, however observations suggest that large scale mortality occurs (pers. obs.). The same phenomenon has been reported by numerous authors for *Crassostrea virginica* (see Shumway, 1996). Laboratory experiments were conducted to investigate the effect of hypo- and hypersaline conditions on the survival, osmoregulation and ionic concentration of *K. scalarina*. The ability of *K. scalarina* to survive large scale salinity fluxes is a critical factor in determining the position and success of any marine farming venture. Knowledge of the salinity tolerance range of *K. scalarina* may also provide an explanation for distribution and abundance of natural populations, enabling effective management of wild populations to prevent over-exploitation.

4.2 Methods

4.2.1 Salinity tolerance trials

Adult (35.0 - 40.0 mm shell length) and juvenile (20.0 - 25.0 mm shell length) *K. scalarina* were collected from Moulting Lagoon, Coles Bay (42°05'S, 148°10'E) (See Chapter 2, Section 2.2.1). Experimental treatments commenced the day after collection. Clams were transferred directly from seawater ($35^0/_{00}$) to one of the experimental salinities which ranged from 5-55$^0/_{00}$ with 5$^0/_{00}$ increments. Four aerated aquaria (3L) were used for each salinity, each containing twelve clams. Approximately 4 cm of sieved sand was placed on the bottom of each aquarium to allow clams to bury, thereby maintaining experimental conditions as close as possible to the natural habitat. Salinity treatments were established by adding distilled water or an artificial salt mixture (Coral Reef Red Sea Salt ®) to sand-filtered seawater with a salinity of $35^0/_{00}$. Water was exchanged every two days and clams were not fed during the experimental period. Temperature was maintained at 15°C throughout the experimental period. Salinities were measured daily using a WTW (Wissenschaftlich - Technische - Werksttaten) conductivity meter, calibrated using standard saline solution (Ocean Scientific International® $K_{15} = 0.99982$; Salinity =34.993$^0/_{00}$). Mortalities were removed and recorded daily until day 21, the conclusion of the experimental period. Animals were considered dead when gaping, or when the foot or siphons failed to contract in response to mechanical stimulation.

4.2.2 Osmotic Concentration of Body Fluids

Mantle fluid and haemolymph samples were collected from the salinity tolerance trials after 48 h, as this is the reported time period over which bivalve molluscs

adapt to variations in external salinity (Pierce, 1971). Samples were obtained by blotting the shell dry (to avoid mixing of internal body fluid with seawater), prising the valves open and inserting a hypodermic syringe. Approximately 50 µm of mantle fluid was obtained. Haemolymph samples were obtained by inserting a syringe into the pericardial cavity. Samples were then pooled to provide sufficient volume for analysis.

Fluid samples for osmolarity determination were centrifuged, the supernatant removed and stored frozen in capped vials until the end of the experimental period. Samples can be stored for at least a week without a change in osmolarity (Nell & Dunkley, 1984). The osmolarity of body fluids and seawater was determined using a vapour pressure osmometer (Wescor 5100B) with an accuracy of ± 0.1 mOsm.

4.2.3 Ionic Concentration of Body Fluids

Haemolymph for ionic analysis was collected in accordance with the methods for osmotic concentration outlined above. The quantities of Na^+, Cl^-, Mg^{2+}, Ca^{2+} and K^+ in the haemolymph were analysed using a Kodak Ektachem 250 Analyser.

4.3 Results

4.3.1 Salinity tolerance trials

Adult *K. scalarina* displayed a salinity tolerance range of 25-50‰ (Figure 4.2A). Salinities outside this range were lethal by day 21 of the experimental period. There was no obvious difference in the survival of clams within the salinity tolerance range, however 100% survival on day 21 was evident only in the 35-40‰ treatments. In contrast, juvenile *K. scalarina* displayed a wide salinity tolerance with substantial mortality occurring only in the 50-55‰ treatments(Figure 4.2B). However 100% survival was evident only in the 30-35‰ treatments.

Probit analysis was used to calculate LT 50s from the survival data, using PROBIT module in BIOSTAT I (Pimentel & Smith, 1990). LT 50s indicate that adult *K. scalarina* survive approximately 10 days at low salinities, (<15‰) (Table 4.1). At salinities greater than seawater (35‰) survival times were longer, with adult and juvenile *K. scalarina* in high salinities (55‰) surviving 14 and 15 days respectively. Due to the low survival of juveniles in salinities below 50‰, LD 50s could not be calculated.

Table 4.1: LT 50's of adult and juvenile *K. scalarina* in acute toxicity trials. SD = standard deviation, NA = not applicable

Salinity (‰)	Adult LT50 (days)	SD	Juvenile LT50 (days)	SD
5	9.7	1.2	NA	NA
10	10.2	1.3	NA	NA
15	9.5	1.6	NA	NA
20	23.3	6.0	NA	NA
55	13.9	2.5	14.9	2.9

4.3.2 Osmotic concentration of body fluids

The osmotic concentration of adult mantle fluid and haemolymph increased in high salinity treatments (Figure 4.3A). At high salinities the body fluids were isosmotic or slightly hypo-osmotic to the external medium (45-55‰). However, at low salinities the concentrations of haemolymph and mantle fluid were hyperosmotic to seawater (5-20‰). Differences in the osmotic concentration are evident at low salinities (5-30‰), with the haemolymph maintaining osmotic concentrations similar to normal seawater. Although there was no significant difference between the osmotic concentration of haemolymph and the mantle fluid, except at low salinities, only haemolymph was used in the subsequent experiments, since if small changes were to be detected haemolymph would provide a more accurate indication than mantle fluid, which has a buffering effect between the external medium and the haemolymph (Gilles, 1972; Hoyaux *et al.*, 1976).

There was no significant difference between the osmotic concentration of adult and juvenile haemolymph (Figure 4.3B). At salinities above 25‰ the haemolymph of adults and juveniles was isosmotic. At low salinities the haemolymph was hyperosmotic to seawater.

4.3.3 Ionic Concentration

Na^+, (Figure 4.4A) and Cl^- (Figure 4.4B) concentrations in the haemolymph displayed similar patterns. The concentration of the ions increased over the salinity range. At low salinities the haemolymph was hyperionic to the external medium (5-25‰), and at high salinities slightly hypoionic (40-55‰).

At low salinities Mg^{2+} (Figure 4.4C) remained more concentrated in the haemolymph than in the external medium (15-25‰). This situation was reversed in hyper-saline conditions where Mg^{2+} was more concentrated in the external medium, while at normal salinities the ion concentrations were equal.

Ca^{2+} (Figure 4.4D) differed in that it was the only ion to display an inverserelationship with salinity. As salinity increased Ca^{2+} concentration displayed a corresponding decrease. Ca^{2+} was initially high at low salinities (5-25‰), decreased markedly at salinities approaching normal seawater and increased again at high salinities.

In contrast to the other ions, K^{+} (Figure 4.4E) varied over a narrow range and was always hyperionic to the external medium.

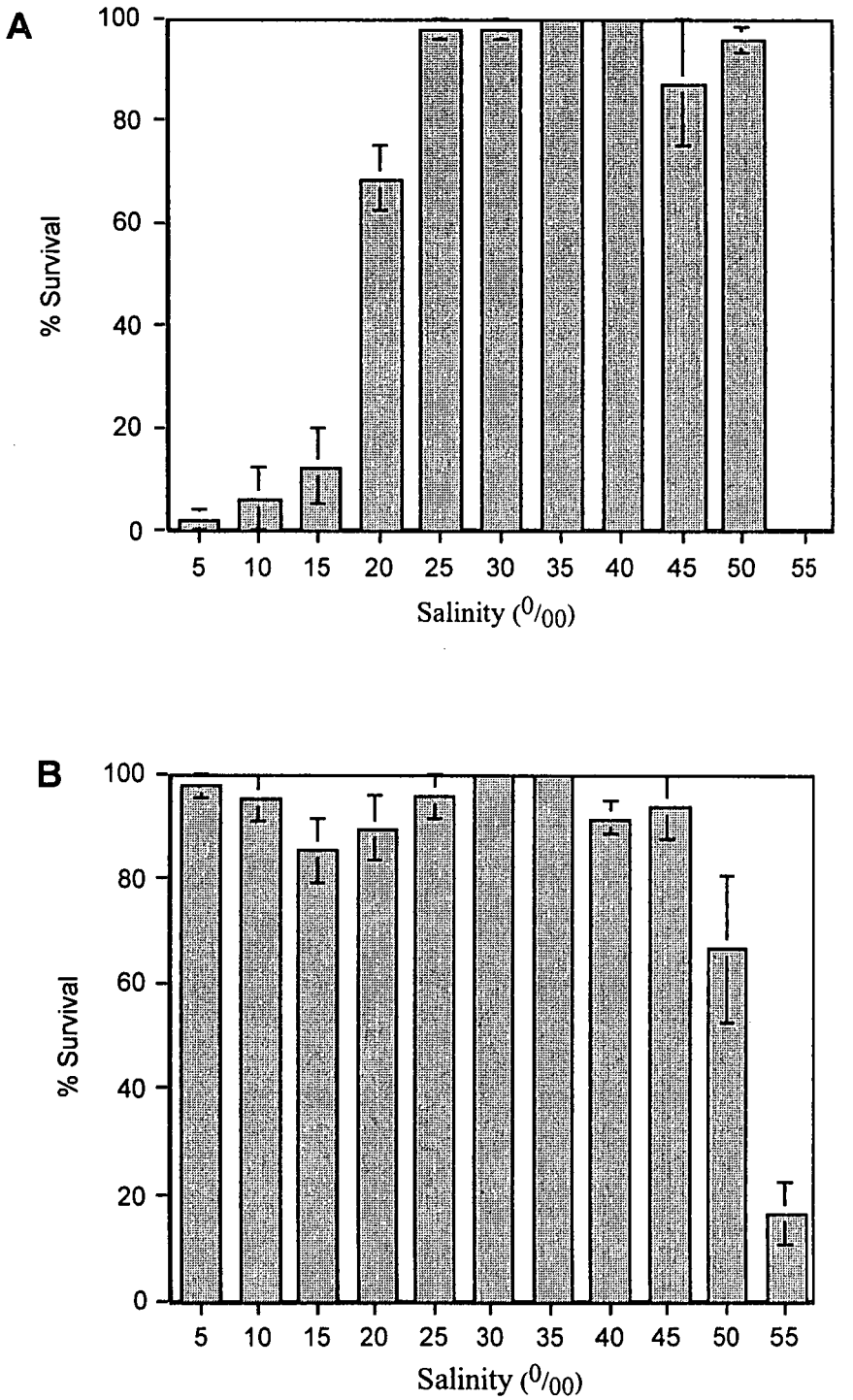

Figure 4.2: Percentage survival of *K. scalarina* at varying salinities on day 21 of the experimental period. A) adults B) juveniles.

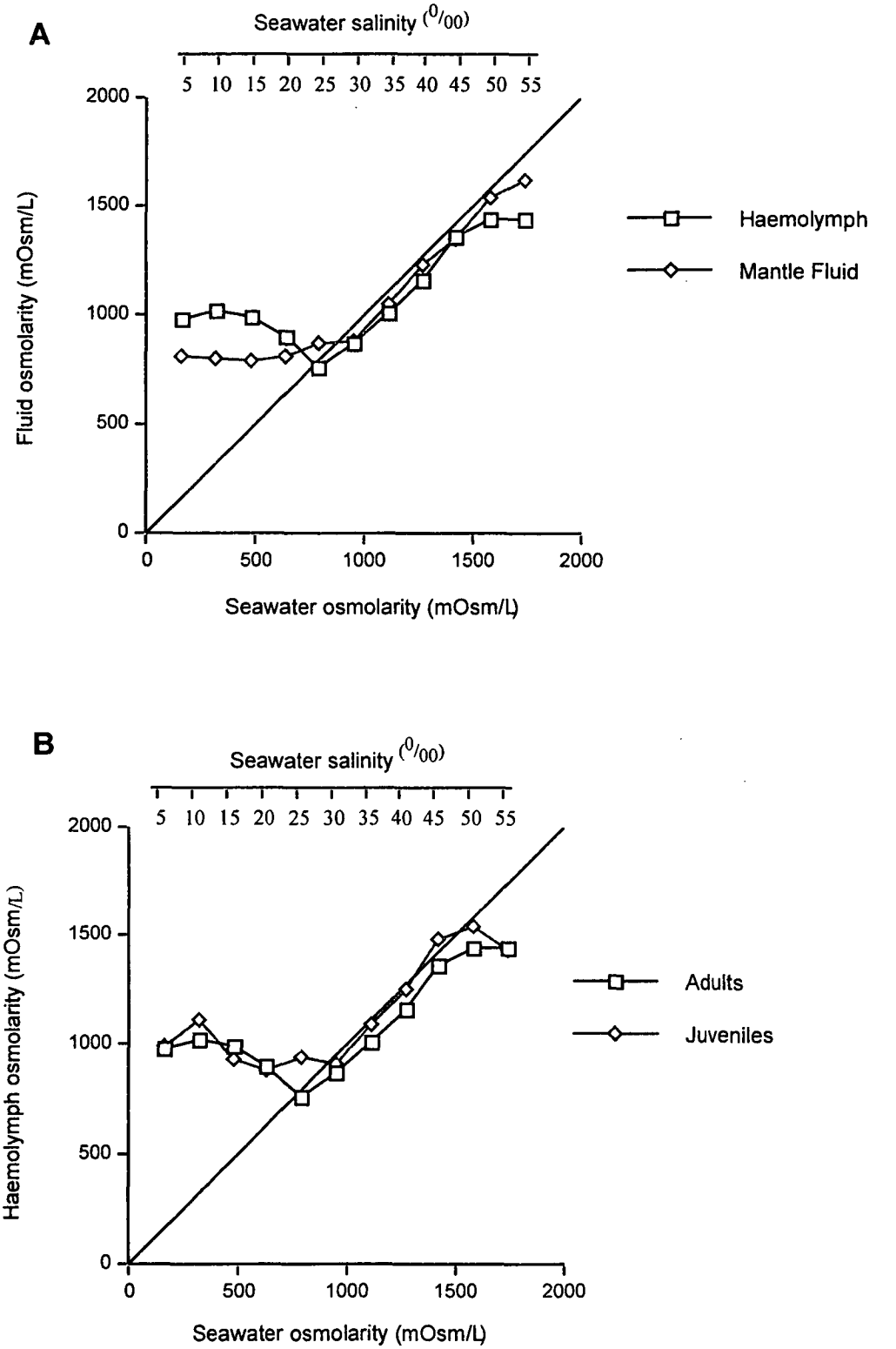

Figure 4.3: Response of the osmotic concentration of the body fluids of *K. scalarina* to changing external concentrations. A) Adult haemolymph and mantle fluid B) Adult and juvenile haemolymph. Diagonal line represents the isosmotic line.

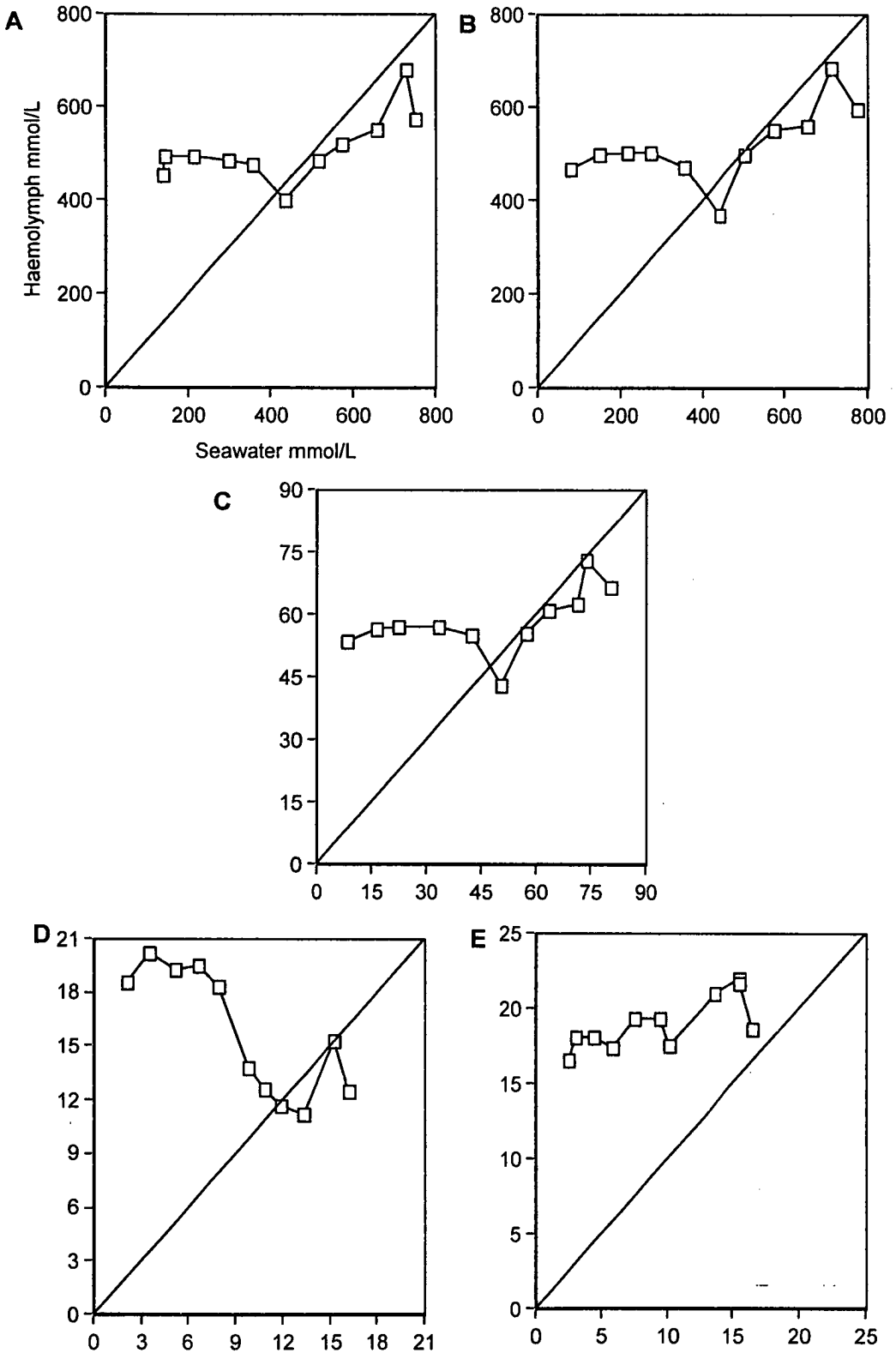

Figure 4.4: Response of the ionic concentration of adult *K. scalarina* haemolymph to changing external salinity.
A) Na^+ B) Cl^- C) Mg^{2+} D) Ca^{2+} E) K^+

4.4 Discussion

4.4.1 Salinity Tolerance

Salinity tolerance in bivalves is species dependent and habitat related (Pierce, 1970; Bedford & Anderson, 1972; Vernberg, 1969). Amongst mussels of the genus *Modiolus*, estuarine species have the widest salinity tolerance range and oceanic species the narrowest (Pierce, 1970); *Modiolus modiolus* from an open rocky coast had a salinity tolerance range of 22-44‰ after 21 days, while *Modiolus demissus demissus* from a salt marsh had a tolerance range of 8-48‰ after 21 days (Pierce, 1970). Such variation in salinity tolerance ranges can be crucial to the survival of a species, particularly in regions that experience sporadic salinity fluctuations. In this study, adult *K. scalarina* displayed a salinity tolerance range of 25-50‰, and treatments outside this range resulted in significant mortality. This compares well with the reported salinity tolerance range of *K. rhytiphora* (6.5±0.1 mm), a closely related species, of 20-45‰ (Nell & Patterson, 1997). Therefore, adult *K. scalarina* have the wide salinity tolerance range typical of many estuarine bivalves, but are generally intolerant of low salinity regimes. Juvenile *K. scalarina* displayed a wide salinity tolerance range, with significant mortality occurring only in 50-55‰. Previous authors have suggested that different stages of a clam's life cycle may display different tolerances and optima (see Malouf & Bricelj, 1989). However, reported accounts are primarily a comparison of embryos, larvae and adults (Kennedy *et al.*, 1974), rather than adults and juveniles as in this study. The differences observed here may in part reflect habitat differences of the two stages. Juveniles are frequently found higher on the shore than adults and are therefore exposed to greater variation in environmental conditions (Bellchambers, 1993) which may allow them to tolerate greater variations in salinity. The different survival time of adults and juveniles may also be due to differences in their ability to isolate the body tissues or withstand the buildup of excretory products. However, the exact mechanism which allows juveniles to survive extremes in salinity for longer periods remains unknown.

Adult *K. scalarina* exposed to low salinities (5-15‰) survived for approximately 10 days, while low salinities appeared to have little effect on the mortality of juveniles. Nossier (1986) showed that adult *Cerastoderma edule* and *C. glaucum* exposed to a comparably reduced salinity (from 32 to 5‰) survived for six days, while *K. rhytiphora* spat survived at low salinities (10-15‰) for only 3 days (Nell & Patterson, 1997). However, the effect of hypersaline conditions on most molluscs is not well known (Singnoret-Brailovsky *et al.*, 1996). Both adult and

juvenile *K. scalarina* exposed to high salinities (55‰) survived for 14 and 15 days respectively. Nell & Gibbs (1986) reported survival times for five bivalve species, exposed to both hyper- and hyposaline conditions, ranging from 2 to 11 days depending on the species' natural habitat. *Pecten fumatus*, a deep water scallop, survived 2 days in salinities outside its tolerance range, while *Anadara trapezia*, a cockle that inhabits seagrass beds, survived 11 days. In comparison, *K. rhytiphora* exposed to high salinity (50‰) showed greater than 50% mortality on day 5, with a gradual increase until day 9 (Nell & Patterson, 1997).

4.4.2 Osmotic Concentration of Body Fluids

The use of osmotic pressure measurements in the description of the relationship between an animal and its environment depends at the outset on the choice of an appropriate body fluid for measurement (Pierce, 1970). Previous authors have stated that both the mantle and pericardial fluids may be used to determine the salinity tolerance range of bivalves (Pierce, 1970; Wada, 1984; Nell & Gibbs, 1986) as their osmolarities are very similar. This study indicated no obvious difference in the osmotic concentration of haemolymph and mantle fluid of *K. scalarina* at high salinities, but at low salinities differences were evident. Under hyposaline conditions the haemolymph is hyperosmotic to mantle fluid and reflects the osmotic concentration of normal seawater. Clams were transferred directly from 35‰ to the experimental treatments, therefore differences in the two fluids may be due to shell valve closure. As reported by other authors, the mantle fluid appears to buffer the internal body fluids from unfavourable salinities (Gilles, 1972; Hoyaux *et al.*, 1976).

Despite the differences in their salinity tolerance ranges, the haemolymph of adults and juveniles displayed no significant difference in osmolarity. Both adults and juveniles were hyperosmotic to the external medium in salinities less than 25‰, due to shell closure, isosmotic between 25-45‰ and hypo-osmotic at 55‰. In comparison, *Saccostrea commercialis* held at salinities outside their tolerance range display a similar osmolarity pattern, hyperosmotic at 5‰ and hypo-osmotic and dying at 55‰ (Nell & Dunkley, 1984).

The initial response of many bivalves to a salinity which is potentially lethal is shell valve closure (Deaton, 1992). This delays equilibration with the environment, not only of their internal body fluids, but of the water in their mantle cavity which acts as a buffer to the external environment (Gilles, 1972; Hoyaux *et al.*, 1976). The advantage is obvious in estuarine molluscs, which may never equilibrate with the fluxes in salinity they are periodically exposed to (Shumway,

1977a, b; Djangmah *et al.*, 1979). Several steady state experiments have suggested that ventilation in *Mytilus edulis* and *Crassostrea gigas* stops and shell valves close at approximately 50% seawater (Shumway, 1977a), while the critical level for *Modiolus demissus* is much lower (35%). In some studies on bivalves, the valves have been propped open to allow free exchange of water (Shumway, 1977a, b). However, Davenport (1979a) has shown for *Mytilus* sp. that exchanges with dilute seawater may still be slow in the absence of artificial irrigation, due to closure of the exhalant siphons and slowing of heart and ciliary beats.

Nell & Gibbs (1986) conducted salinity tolerance trials on five species of bivalves, ranging from an oceanic scallop (*Pecten fumatus*) to an intertidal cockle (*Anadara trapezia*), and their results suggest that the bivalves were unable to remain totally inactive and keep the valves tightly closed. The animals were as active as necessary to provide minimal water exchange, thereby lowering their tissue fluid osmolarity and water balance levels until they became critical and the animals died. However bivalves, especially those from intertidal habitats, need not exchange water over short periods of time to maintain oxygen levels in their tissue fluids . Instead they can close the shell valves and revert to anaerobic metabolism (De Zwaan, 1983; Bayne *et al.*, 1976; Gilles, 1972). However, water exchange is required to reduce the concentration of excretory products such as ammonia in the tissue fluids. This buildup of excretory products may explain the mortality of *K. scalarina* at salinities less than 25‰ when the valves remained closed for an extended period.

4.4.3 Ionic Concentration of Body Fluids

The haemolymph of most marine molluscs is close to seawater in osmotic pressure and ionic composition (Burton, 1983). In euryhaline species this is true over a wide range of salinities provided a steady state exists, although behavioural mechanisms may delay the attainment of the condition. Shumway (1977b) suggested that in bivalves the regulation of cell volume by solute extrusion is a long term emergency phenomenon that periodic valve closure renders unnecessary under conditions of fluctuating salinity.

There is no evidence of ionic regulation of Na^+ or Cl^- in *K. scalarina*. Ion concentrations displayed the typical marine bivalve trend, and the concentrations of Na^+ and Cl^- in the haemolymph and seawater were almost equal above 30‰. At salinities below 30‰ the concentrations of Na^+ and Cl^- were hyperionic to the external medium as are the concentrations at 55‰, which can be attributed to

shell closure. The same pattern for Na^+ has been reported for *Mytilus edulis* and *Crassostrea gigas* (Shumway, 1977a).

Shumway (1977a) states that Ca^{2+} and Mg^{2+} exhibit a more dampened response to fluctuating salinity than Na^+. Conway (1960) has shown that small ions pass at a faster rate than large ions through cell membranes. Thus, in a system of passive equilibrium, as exhibited by *Mytilus edulis* and *Crassostrea gigas*, the larger Ca^{2+} and Mg^{2+} ions would not diffuse at the same rate as the smaller ions. However in this study, Mg^{2+} in the haemolymph was hyperionic to the external medium at low salinities, but remains at a similar concentration to normal seawater indicating shell closure. The haemolymph was isoionic at normal salinities and hypoionic at high salinities. *Mytilus galloprovincialis* displays a similar pattern of haemolymph Mg^{2+} (Mahasneh & Pora, 1981). It has been suggested that Mg^{2+} plays a protective role similar to Ca^{2+}, in that it acts as a buffering agent (Burton, 1983). Mg^{2+} can be synergistic with Ca^{2+} in antagonising K^+ (Burton, 1983).

Ca^{2+} appears to behave in a more regulated way than the other ions, and was the only ion that displayed an inverse relationship with salinity. Mahasneh & Pora (1981) reported a similar pattern of calcium regulation in *Mytilus galloprovincialis* and suggested the trend may be due to the role of calcium in composition of cell membrane structure. Shell closure may be another explanation for the patterns in Ca^{2+} regulation observed. The initial response of many bivalves to sudden change in ambient osmolarity is adduction of the valves (Deaton, 1992). If prolonged, decreased ventilation will result in the activation of facultative anaerobic pathways and the accumulation of organic acids in the tissues (De Zwaan, 1983). In the clam *Scrobicularia plana,* these acidic products are buffered by the mobilisation of Ca^{2+} as $CaCO_3$ from the shell (Akberali *et al.*, 1977). In bivalves hypercalcaemia is often correlated with survival in very dilute media (Deaton & Greenberg, 1991). The hyperionic regulation of Ca^{2+} in low salinities supports the hypothesis that to avoid unfavourable salinities *K. scalarina* relies on the behavioural mechanism of shell valve closure.

The concentration of K^+ in the haemolymph of *K. scalarina* was constantly hyperosmotic to the external medium regardless of the salinity treatment. K^+ concentration varied much less than the other ions examined, perhaps suggesting regulation. Gilles (1972) reports that *Mytilus edulis* and *Glycymeris glycymeris*, when placed in low salinities, regulate K^+ to maintain the ion concentration of the haemolymph in normal seawater. However, it is not known whether the

maintenance of K^+ concentration at a given level is important in the osmoregulation process (Shumway, 1977a). The effects of marginally raised levels of K^+ may have less physiological significance than the processes that are responsible for them, such as the absorbance of food (Burton, 1983). However this explanation is unlikely in the present case as the experimental animals were not fed during the trials.

Burton (1983) suggests that the survival of euryhaline species in different salinities shows the minor importance of absolute concentrations in the haemolymph. It may be more important that the relative concentrations of the different ions remain constant. Previous studies have suggested a correlation between the ionic concentration of Ca^{2+} and K^+ (Burton, 1983), with the two ions having an antagonistic effect on each other. The haemolymph of *K. scalarina* shows that an increase in one ion corresponds to a decrease in the other, but whether this is due to an antagonistic effect is unclear.

K. scalarina is essentially an osmo- and ionic conformer and generally intolerant of low salinity regimes. In the absence of any physiological means of regulating ionic and osmotic concentration of the haemolymph *K. scalarina* appears to rely on its ability to isolate the body tissues by closing the shell valves. By this means *K. scalarina* can survive unfavourable salinities for extended periods. The sporadic and extended salinity fluxes experienced by the sheltered estuaries and lagoons of Tasmania's east coast and the inability of *K. scalarina* to withstand low salinity regimes for extended periods suggests that the critical factors may be the duration and periodicity of salinity fluxes.

Chapter 5:

The effect of salinity on the concentration of free amino acids.

5.1 Introduction

Few species of bivalves are capable of extracellular osmotic regulation over their entire non lethal salinity range (Pierce, 1971; Gilles, 1972; Shumway, 1977a; Livingstone *et al.*, 1979), therefore it is the individual cells that withstand fluctuations in external salinity (Livingstone *et al.*, 1979; Pierce & Amende, 1981; Matsushima *et al.*, 1987). Constant altering of cell volume is likely to be disruptive and metabolically costly (Hawkins & Hilbish, 1992; Sadok *et al.*, 1997). Therefore, the initial response of many organisms to a sudden change in ambient salinity is shell valve closure (Shumway, 1977a; Davenport, 1979a; 1981). Similarly, *K. scalarina* is an osmotic and ionic conformer which utilises the behavioural response of shell closure to reduce the effects of changes in environmental salinity on haemolymph osmolarity (see Chapter 4). Nonetheless, many bivalves can survive a wide range of salinities by actively regulating cell volume. Cell volume is regulated by controlling the concentration of intracellular nitrogenous osmolytes, in particular free amino acids (FAA) (Pierce & Greenberg, 1972; Baginski & Pierce, 1978; Henry *et al.*, 1980; Sadok *et al.*, 1997) via alteration of nitrogen metabolism and excretion (Lange, 1972; Pierce & Greenberg, 1972; Schoffeniels & Gilles, 1972; Hawkins & Hilbish, 1992).

Bivalve molluscs as well as many other kinds of aquatic animals possess considerable amounts of non essential free amino acids (FAA) in their tissues. Large quantities of alanine, glycine, glutamate, proline, aspartate and taurine compose the majority of the FAA pool, although the quantities of individual compounds vary significantly between species (Du Paul & Webb, 1970; Henry *et al.*, 1980). The concentration of free amino acids in the tissues and extracellular fluids of molluscs varies with diet, season, temperature, reproductive and developmental stage, and environmental stresses related to desiccation, anaerobiosis, osmotic pressure, pollution and parasitism (see Bishop *et al.*, 1983 for review). The total size of the FAA pool varies directly with environmental salinity, therefore FAA are believed to function as intracellular osmotic regulators maintaining the isosmotic balance between intracellular and extracellular fluids (Livingstone *et al.*, 1979; Henry *et al.*, 1980; Pierce *et al.*, 1992).

FAA regulation during salinity stress has been reported for a number of other bivalve species e.g. *Mytilus edulis* (Shumway *et al.*, 1977c; Livingstone *et al.*,

1979; Smaal *et al.*, 1991; Sadok *et al.*, 1997) *Rangia cuneata* (Henry *et al.*, 1980), *Modiolus demissus* (Bartberger & Pierce, 1976; Baginski & Pierce, 1978), *Geukensia demissa* (Deaton, 1994) and *Crassostrea virginica* (Pierce *et al.*, 1992). Physiological adjustments, to salinity changes, made via tissue and haemolymph FAA levels are regulated by enzymatic adaptations such as the salinity-induced change of amino peptidase-1 activity in the cytoplasmic vacuoles and granules of the digestive and intestinal cells (Young *et al.*, 1979); these changes are directly related to experimental salinity (Deaton *et al.*, 1984).

5.1.1 Mechanism of Free Amino Acid Regulation

The mechanism of FAA regulation is simple (see Figure 5.1), in low salinity media, cells initially swell due to the establishment of an osmotic gradient between the internal body fluids and the external medium. Cell swelling is controlled by a simultaneous release of FAA and osmotically obliged water, from the intracellular pool, into the haemolymph (Livingstone *et al.*, 1979; Strange & Crowe, 1979; Henry & Magnum, 1980; Pierce & Amende, 1981). Conversely, cell shrinkage in high salinity medium is controlled by cytoplasmic increases in FAA (Gainey, 1978; Pierce *et al.*, 1992). Several routes have been suggested for accumulation of FAA e.g. cellular uptake from the haemolymph or external medium (Jørgensen, 1983; Silva & Wright, 1992), *de novo* synthesis (Henry *et al.*, 1980; Lehman *et al.*, 1985) and increased protein metabolism (Hawkins & Hilbish, 1992). In marine molluscs the intracellular concentrations of FAA are high (50 - 400 mM) in the tissues and low (0.2 - 5 mM) in the haemolymph (Bishop *et al.*, 1983).

5.1.2 Free Amino Acid Adjustment

While, the mechanism of FAA regulation is simple, the adjustment of FAA levels is a complicated process (Bishop *et al.*, 1983). Taurine and the quaternary amines are metabolically inert and are retained in the cells at high salinities (Pierce & Greenberg, 1972; Amende & Pierce, 1980; Pierce & Amende, 1981). However, glycine, alanine, proline, aspartate and glutamate display a rapid metabolic turnover (Bishop *et al.*, 1983). It appears that at high salinities, these amino acids are accumulated in the tissues by membrane related processes (Pierce & Greenberg, 1972; Shumway *et al.*, 1977c; Livingstone *et al.*, 1979; Shumway & Youngson, 1979; Strange & Crowe, 1979; Amende & Pierce 1980; Pierce & Amende, 1981) in combination with decreased catabolism and increased biosynthesis (Gilles, 1969; Baginski & Pierce, 1975, 1977; Gilles, 1979; Henry *et al.*, 1980; Zurburg & de Zwaan, 1981). Aspartate, glutamate, glycine, alanine and serine are produced from the metabolism of gluconeogenic compounds including glycogen, whereas proline is probably derived from arginine and ornithine but not

from glutamate (Bishop *et al.*,1983). All can be released from amino acids during peptide and protein turnover and may accumulate if catabolism is slowed.

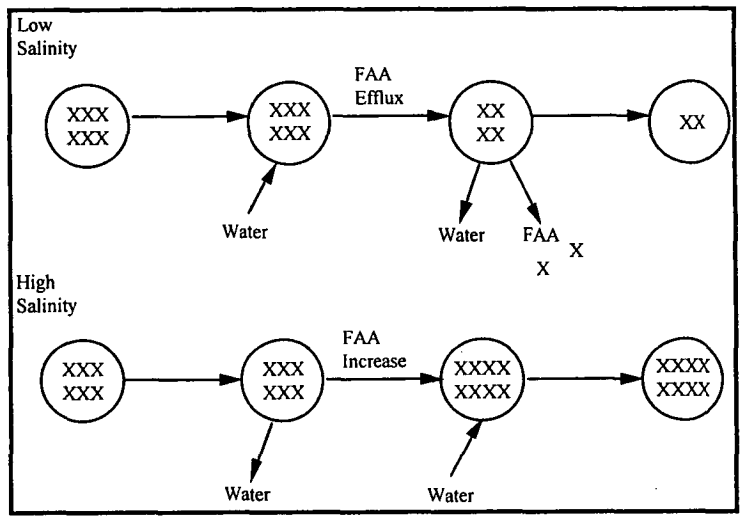

Figure 5.1: A diagrammatic representation of cell volume regulation by intracellular FAA (After Pierce & Amende, 1981).
Circles represent the cells, crosses represent intracellular solutes, in particular FAA.

The aim of this component of the research was to determine, in the absence of osmo and ionic regulation, how *K. scalarina* copes with the osmotic variation in body fluids associated with the wide salinity tolerance range (25-50‰, see Chapter 4) of the species. Secondly, to determine if accumulation of particular amino acids indicated that shell closure was being utilised by *K. scalarina* within the salinity tolerance range. Variations in the concentrations of several amino acids, in particular alanine and glutamic acid, aspartic acid and glycine, are indicative of anaerobic metabolism which occurs during prolonged periods of shell closure.

5.2 Methods

5.2.1 Experimental Procedure

Adult *K. scalarina* (35.0 - 40.0 mm shell length) were collected from Moulting Lagoon, Coles Bay (see Chapter 2, Section 2.2.1), experimental treatments commenced the day after collection. Clams were transferred from seawater of 35‰ and placed in one of the experimental salinities which ranged from 20-55‰ with 5‰ increments, representing six salinities within the salinity tolerance range and two outside the range (see Chapter 4). Two aerated aquaria (3 L) were used for each salinity, each containing ten clams. Salinities were established and

maintained in the accordance with the salinity tolerance trials (see Chapter 4, Section 4.2.1).

Samples of adult haemolymph for amino acid analysis were collected after 48 hours, to correspond with the salinity tolerance trials and secondly as this is the time period in which adjustments to the FAA pool occur (Ivanovinci *et al.*, 1981; deVooys, 1991). Three samples of haemolymph were collected from each salinity treatment. The haemolymph of approximately six clams was pooled to allow a sufficient volume for analysis. Samples were centrifuged, the supernatant removed and stored in eppendorf tubes at -80°C until amino acid analysis was conducted.

5.2.2 Amino Acid Analysis

Thawed serum samples (20 µl) were deproteinised with ethanol (400 µl), centrifuged (8000 rpm X 10 min) and the supernatant transferred to an eppendorf tube. The supernatant was dried under a stream of nitrogen on a heated dri-block (40°C, 15 min) and the solid residue dissolved in 400 µl of buffer (0.1M Na HCO_3 & 0.1M boric acid at pH 8.5 "Aminotag") and centrifuged (8000 rpm x 10min). The supernatant was removed and added to 400 µl of 1.5 mM derivatising agent (9-fluorenylmethyl chloroformate, FMOC) in acetone. After 10 minutes reaction time the mixture was extracted with pentane/ethyl acetate (80:20, 1.2 mL) and the lower layer transferred to a sample vial. FMOC derivatised amino acids were assayed using a Varian Amino Acid Analyser comprising of a HPLC ternary gradient pump (model 9010), an autosampler (model 9100), a fluorometer (model 9070, set at 270 emission, 340 detection) and UV-Vis diode array (model 9065) detectors. An Alltech Alltima C18 5µ (250 x 4.56 mm), column maintained at 32°C was used with a Waters TCM heating unit.

The standard buffers were:

A) 0.015M sodium citrate, 0.010M tetramethyl ammonium chloride (pH 2.85) with H_3PO_4.

B) 90%A at pH 4.5, and 10% methanol.

C) Acetonitrile.

The injection volume of each sample was 20 µl and a run lasted 33 minutes, with a 10 minute equilibration time between runs. The gradient profile, maintained at a constant flow rate of (1.4 mL/min), involved linear transformations between a series of compositions (Table 5.1).

Table 5.1: Compositions used for amino acid analysis of *K. scalarina* haemolymph.

Time (mins)	%A	%B	%C
0	73	0	27
0.5	68	0	32
11.5	58	0	42
13	58	0	42
13.5	31	30	39
14	0	62	38
18	0	61	39
28	0	28	72
29	0	25	75
30	0	25	75

The standard used was a mixture of Pierce type amino acid standard (SIGMA Co.) with added taurine (SIGMA Co.) and asparagine (Boehringer-Mannheim) such that the total amine concentration approximated concentrations in the serum samples. Processing of the standard followed the same derivatisation and extraction procedures as the serum samples. Duplicates of each replicate were analysed and the average used for quantification. Quantification was conducted using the fluorescence trace on an IBM compatible computer with Varian software for integration.

Final amino acid results were analysed using a one way ANOVA in SYSTAT. Homogeneity of variances was checked using Barrett's test of homogeneity of variances. Results of statistical analysis are in appendix 3.

5.3 Results

Salinity of the external medium had a significant effect on the concentration of FAA in the haemolymph of adult *K. scalarina* ($P=0.0001$), with FAA concentration displaying an inverse relationship with salinity (Figure 5.2). There was a distinct decrease in the concentration of FAA over the tolerated salinity range, from 2.22 mmol/L in 25‰ to 1.255 mmol/L in 50‰. Salinities outside the tolerated salinity range do not fit the general pattern of decreasing FAA with increasing salinity. This may be due to the breakdown of metabolic processes in salinities which are eventually lethal.

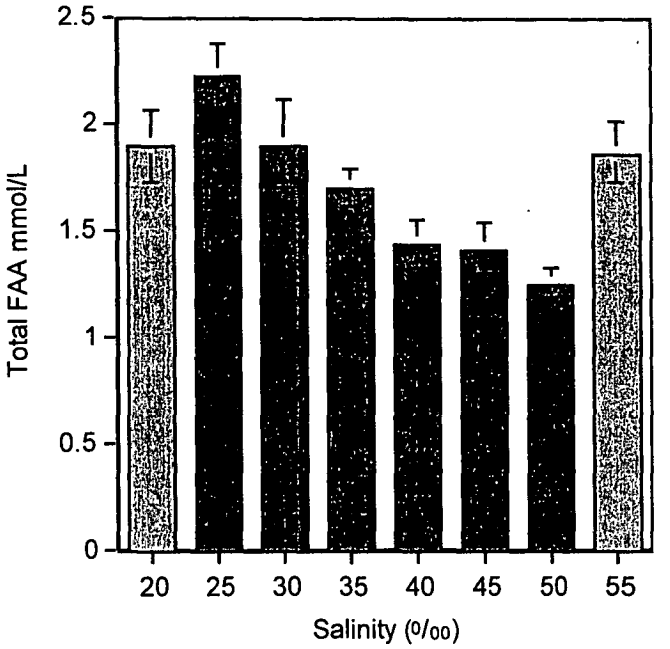

Figure 5.2: Total FAA in the haemolymph of *K. scalarina* (mean±S.E., n=3 replicate samples).

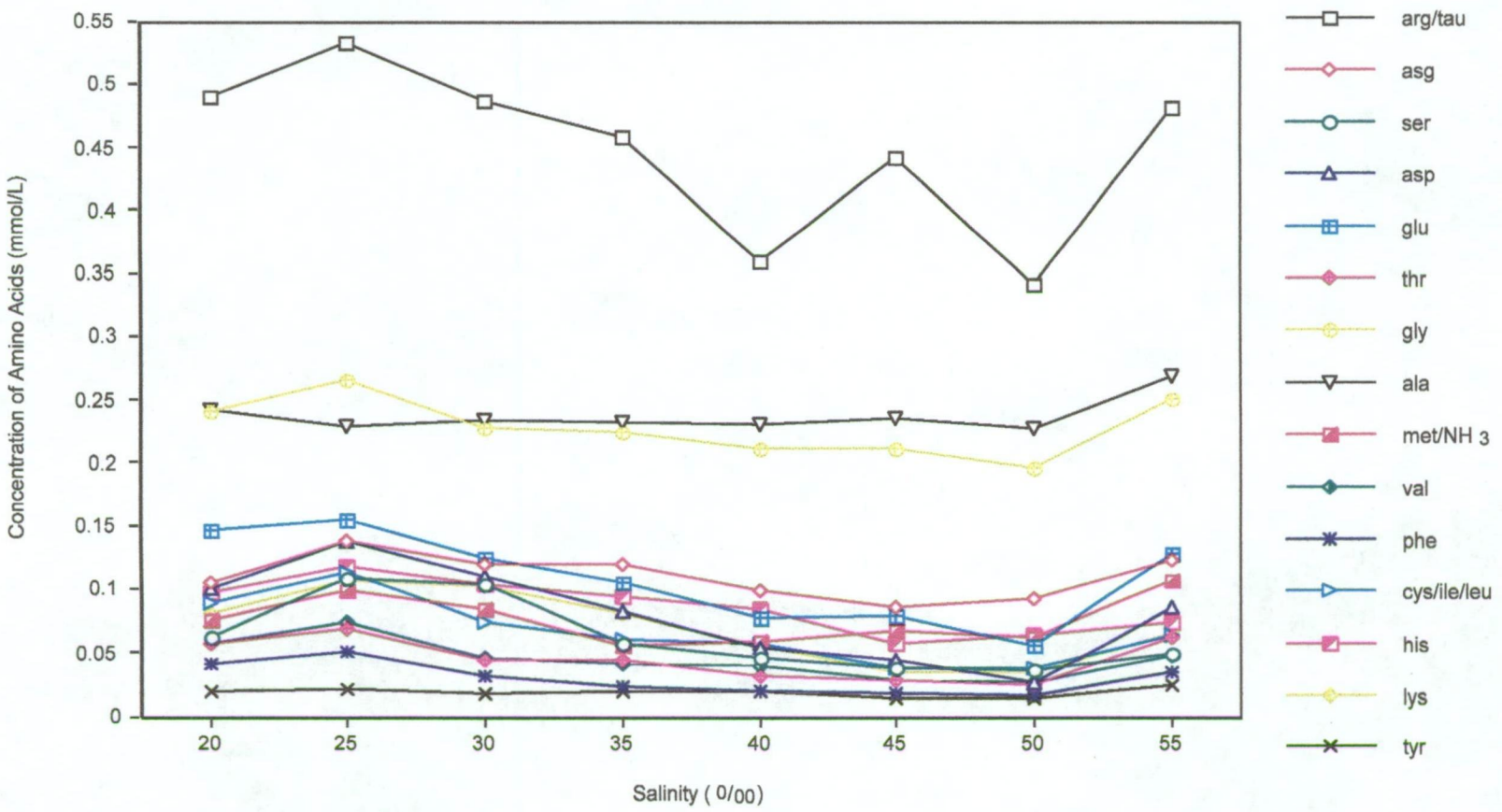

Figure 5.3: Concentration of individual amino acids in the haemolymph of adult *K. scalarina*.

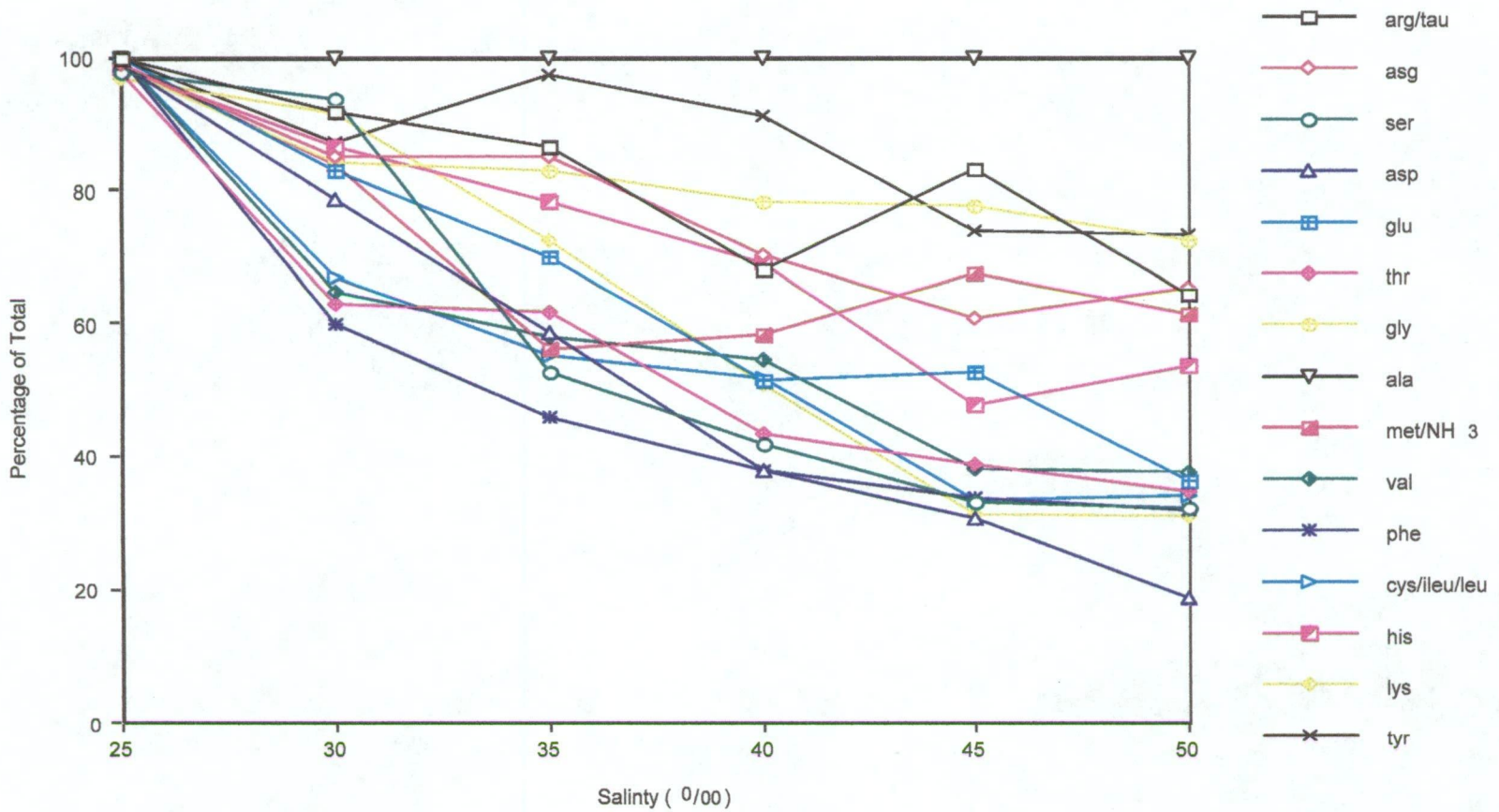

Figure 5.4: Individual amino acids expressed as a percentage of the highest individual value recorded.

Arginine and taurine composed the majority of the haemolymph FAA pool of adult *K. scalarina* (Figure 5.3). These two amino acids are grouped together as they could not always be separated, however, taurine comprised the majority (>90%) of this group in samples that resolved. Arginine and taurine reflected the general pattern of decreasing concentration with increasing salinity, ranging from 0.532 mmol/L at 25‰ to 0.343 mmol/L at 50‰. The second most concentrated FAA were glycine and alanine. Alanine appears to remain at a relatively constant level throughout the salinity range. While, glycine displayed an inverse relationship with salinity, ranging from 0.266 mmol/L in 25‰ to 0.195 mmol/L in 50‰. The remainder of FAA in the haemolymph were composed of twelve identifiable FAA or groups all of which displayed a slight decrease in concentration over the salinity range with the exception of aspartate, glutamate, lysine and serine. In contrast, aspartate (0.138 mmol/L - 0.026 mmol/L), glutamate (0.154 mmol/L - 0.055 mmol/L), lysine (0.106 mmol/L - 0.034 mmol/L) and serine (0.107 mmol/L- 0.0356 mmol/L) displayed a substantial decrease in concentration over the salinity range. Tyrosine, phenylalanine, threonine and valine remained at low but relatively constant levels despite variations in the external salinity. Proline did not resolve from the derivatising agent and so is not reported. Methionine and ammonia could not be separated and are therefore reported together. Similarly, cysteine, isoleucine and leucine are also reported as a group.

Variations in individual amino acids are more evident when the concentration at each salinity treatment is expressed as a percentage of the highest concentration for that particular amino acid (Figure 5.4). Serine, aspartate, glutamate, threonine, valine, phenylalanine, cysteine/isoleucine/leucine and lysine all display a decrease of greater than 50% over the experimental range. This represents a substantial decrease despite the fact that these amino acids did not comprise a significant component of the FAA pool in terms of concentration.

5.4 Discussion

K. scalarina does not have the physiological ability to maintain a large osmotic gradient between internal body fluids and the external medium. When exposed to salinities which vary from that of seawater, intracellular amino acids and other nitrogenous osmolytes are released into or removed from the haemolymph to maintain cell volume. The use of intracellular FAA and other NPS as the solute in cell volume regulation, in bivalves, is well documented (Pierce, 1971; Schoffeniels & Gilles, 1972; Bayne *et al.*, 1976; Hoyaux *et al.*, 1976; Baginski & Pierce 1975, 1977). Previous authors have used a range of molluscan tissues and fluids for amino acid analysis e.g. ventricles (Baginski & Pierce, 1977, 1978),

gills (Baginski & Pierce, 1977, 1978; Pierce *et al.*, 1992), adductor muscle (Shumway, 1977c; Henry *et al.*, 1980; Deaton *et al.*, 1985; Pierce *et al.*, 1992), mantle (Baginski & Pierce 1978; Deaton *et al.*, 1985) and haemolymph (Bartberger & Pierce, 1976; Sadok *et al.*, 1997). Each body tissue or fluid has its own distinct trends in amino acid accumulation and release (Bishop *et al.*, 1983), which makes comparisons between the trends observed in this study and those of previous studies difficult.

Salinity had a significant effect on the concentration of FAA in the haemolymph of adult *K. scalarina*. The concentration of FAA displayed an inverse relationship with salinity, decreasing from 2.22 mmol/L in low salinities (25‰) to 1.255 mmol/L in high salinities (50‰) after 48 hours exposure. The extracellular FAA pool of *K. scalarina* displays a step-wise increase in FAA in salinities less than 35‰, due to the influx of FAA from the tissues. Conversely, in salinities greater than 35‰, the haemolymph of *K. scalarina* displays a decrease in FAA concentration due to an efflux of FAA from haemolymph into the intracellular fluid. Similarly, Bartberger & Pierce (1976) state that a decrease in external salinity is reflected by an increase in haemolymph amino acid concentrations which is in turn reflected by a decrease in the tissue FAA pool. Enlargement of the NPS pool (ninhydrin positive substances), which includes all amino acids, has been attributed to the degradation of endogenous proteins (Hawkins & Hilbish, 1992), the transmission of the products of protein catabolism to those of NPS that accumulate during hyper osmotic shock (Deaton *et al.*, 1985), and/ or to an active transport, against an amino acid gradient from the haemolymph to the intracellular fluid (Zurburg & de Zwaan, 1981). In comparison, Matsushima *et al.* (1987) states that the intracellular FAA pool of the bivalve *Corbicula japonica* increases during the initial stage of hyperosmotic stress (short term), plateaus and then continues to increase over a long period (long term).

The pattern and time course of accumulation is different for the respective amino acids (Baginski & Pierce, 1975, 1977). Many studies of bivalves have shown that there is both an immediate increase in the efflux of amino acids from tissues (Pierce & Amende, 1981; Deaton, 1990) and a longer term decrease in the content of amino acids (Livingstone *et al.*, 1979; Amende & Pierce, 1980) when animals are exposed to a decrease in ambient salinity. Previous authors have argued that there is no general pattern in role of amino acids in the regulation of intracellular osmolarity, however, the non essential amino aids together with taurine played a greater part than the essential ones (Hoyaux *et al.*, 1976; Livingstone *et al.*, 1979).

The majority of the FAA pool of *K. scalarina* was composed of taurine and arginine, these two amino acids comprised approximately a quarter of the total FAA pool. Taurine and arginine are grouped together as in the majority of samples these two amino acids could not be separated, although taurine composed the majority of this group in samples that did resolve. As taurine and arginine and reported together it is difficult to comment with any certainty on the individual trends of arginine and taurine in the haemolymph of *K. scalarina.*

Previous studies indicate that taurine is frequently present in significantly higher concentrations than arginine (see Baginski & Pierce, 1977; Shumway *et al.*, 1977c; Livingstone *et al.*, 1979 for examples). The results of Shumway *et al.* (1977) from the adductor muscle of 8 species of bivalve molluscs indicate that taurine comprised between 10.78% - 50.09% of the FAA pool while arginine composed between 1.49 - 7.30%. In most of the species studied taurine levels were approximately ten times higher than arginine. Therefore, although arginine and taurine could not be separated in this study results from other studies suggest that taurine composes the majority of the FAA pool and it is likely that this is also the case for *K. scalarina.*

While comprising the majority of FAA in the haemolymph of *K. scalarina,* taurine and arginine also displayed the largest decrease in concentration with increasing salinity, from 0.532 mmol/L (25‰) to 0.343 mmol/L (50‰). Taurine is thought to have an important role in osmoregulation (Lange, 1963) and declines in intracellular fluids with an abrupt decrease in salinity (Livingstone *et al.*, 1979). Taurine is typically synthesised from cysteine, cysteic acid and cysteine sulfinic acid (Bishop *et al.,* 1983) however, despite its wide occurrence, taurine biosynthesis in invertebrates is poorly understood (Pierce *et al.*, 1992). Baginski & Pierce (1977) working on *Modulis demissus demissus* suggest that taurine composes the majority of FAA pool and is used for long term salinity acclimation. Long term taurine acclimation probably relieves the requirement for utilisation of other essential amino acids for intracellular regulation (Lange, 1963). There are several other bivalves in which taurine dominates the FAA pool e.g. *Mytilus edulis* (Lange, 1963) *Modiolus squamosus* (Pierce, 1971) and *Mercenaria mercenaria* (Du Paul & Webb, 1974). Patterns of taurine accumulation in the haemolymph of adult *K. scalarina* reflect previous studies and indicate that taurine may be the major amino acid used in maintaining intracellular volume under salinity stress.

While taurine displayed the largest decrease in terms of mmol/L and comprised the majority of the FAA pool when decreases in FAA concentration are expressed

as a percentage of the total concentration of each amino acid, several other amino acids display much larger decreases in overall concentration than taurine. Aspartate (80%), glutamate (65%), serine (65%), threonine (60%), valine (60%), phenylalanine (65%), cysteine/isoleucine/leucine (65%) and lysine (70%) all displayed decreases substantial decreases in the percentage total amino acid in comparison to taurine (35%). However, as these amino acids comprised only a small proportion of the total FAA pool decreases in their overall concentration may not be significant in coping with osmotic stress.

An intracellular increase in arginine, a nitrogen bearing precursor to urea, is consistent with a detoxification process under conditions which prevent the normal excretion of ammonia i.e. shell closure due to salinity stress (Ivanovinci *et al.*, 1981). *K. scalarina* certainly uses the behavioural response of shell closure to minimise the effects of unfavourable salinities (pers.obs., Chapter 6). However, due to the grouping of taurine and arginine and the fact that taurine composed the majority of the samples that resolved it is difficult to comment on shell closure and the subsequent restriction of excretion in *K. scalarina*.

Alanine was the second most abundant FAA in the haemolymph of *K. scalarina*. Alanine is among the chief osmotic effectors in several other bivalves e.g. *Mya arenaria* (Virkar & Webb, 1970), *Modiolus demissus granosissimus* (Pierce, 1971), *Spisula solidissima* (Du Paul & Webb, 1974), *Polymesoda caroliniana*, and *Corbicula manilesis* (Gainey, 1978). In bivalves, alanine is usually rapidly synthesised by the mitochondria following the onset of salinity stress (Baginski & Pierce, 1977, 1978; Bishop *et al.*, 1981). Intracellular free alanine also accumulates under anaerobic conditions (Baginski & Pierce, 1978; Henry *et al.*, 1980; Ivanovinci *et al.*, 1981). Many organisms including bivalves utilise a pathway of glucose degradation that results in intracellular accumulation of alanine and succinate (Baginski & Pierce, 1978). In this study alanine concentrations remained constant across all salinity treatments. Coupled with the fact that haemolymph ammonia concentration displayed only a slight increase in low salinities, it is suggested that anaerobic metabolism due to shell closure during the experiment was minimal. Alternatively, anaerobic metabolism may have occurred but was interspersed with periods of aerobic metabolism allowing adequate flushing of nitrogenous wastes.

Anaerobic metabolism in many invertebrate species is characterised by higher levels of alanine and glutamic acid and lower level of aspartic acid and glycine. A marked decrease in glycine concentration with a concomitant increase in the ratio of taurine to glycine has been associated with stress conditions in a variety of

molluscan species. However, while taurine and arginine decreased, glycine was consistent after an initial drop from 0.266 mmol/L (25‰) to 0.227 mmol/L (30‰) and remained relatively constant. Therefore it is unlikely that *K. scalarina* responded to salinity stress by reverting to anaerobic metabolism, with the possible exception of clams in the 25‰ treatment.

While the major reductions in the FAA pool of *K. scalarina* were due to a decrease in taurine and a somewhat smaller decrease in glycine, substantial decreases in the total concentration of aspartate, glutamate, lysine and serine were also evident. Livingstone *et al.*, (1979) suggest that reduction in intracellular total NPS were primarily due to decrease in glycine and taurine but there were also significant reductions in aspartate, serine, threonine and arginine. These changes are consistent with the two major enzymes suggested as the possible major routes for ammonia release in bivalves, which utilise aspartate and serine as substrates.

In the absence of osmo or ionic regulation *K. scalarina* displays a wide salinity tolerance range due its ability to regulate volume during salinity stress by altering the size and composition of the FAA pool. The majority of the FAA pool of *K. scalarina* is composed of taurine, which has been reported as the major osmotic effector in several other bivalve species. It would appear due to the constant levels of alanine and ammonia in the haemolymph of *K. scalarina* that prolonged shell valve closure is not utilised by *K. scalarina* over the tolerated salinity range (25-50‰). Salinity stress is adequately, at least over the duration of this experiment, compensated for by the variations in the size and composition of the FAA pool.

It has been suggested that the limits of euyhalinity in osmoconformers depends on the ability of the cells to regulate volume in a varying salinity regime which in turn depends on the ability to which the size of the FAA pool can be regulated (Pierce, 1970, 1971; Henry *et al.*, 1980). Gainey & Greenburg (1977) state that the penetration of freshwater species into the marine environment and marine species into the freshwater environment seems to be more limited by their ability to regulate ion concentration than to mobilise amino acids for cellular osmoregulation. Similarly, the limited ion regulation capabilities of *K. scalarina* (see Chapter 4) may be a factor which prevents their survival in reduced salinities.

Chapter 6:

The effect of salinity on valve closure, respiration and algal clearance.

6.1 Introduction

Growth rates of marine and brackish water invertebrates, vary with age (Rodhouse, 1978; Branch, 1982; Bayne & Newell, 1983), physiological state (Bayne & Newell, 1983) and environmental conditions (Buxton *et al.*, 1981; Bayne *et al.*, 1973; Widdows & Bayne, 1971; Newell, 1979; Newell & Branch, 1980). Three of the most important environmental factors are salinity (Bayne, 1973; Shumway, 1979; Shumway, 1981), temperature (Mane, 1975; Newell & Branch, 1980; Wilson & Davis, 1984; Loo, 1992; Smaal *et al.*, 1997) and ambient oxygen concentration (Bayne, 1971; Mane, 1975; Shumway & Marsden, 1982; Wilson & Davis, 1984; Widdows *et al.*, 1989). Generally, growth of estuarine invertebrates is restricted to significantly narrower salinity ranges than survival (Almada-Villela, 1984; Nell & Holliday, 1988; Nell & Patterson, 1997).

In the absence of any extracellular physiological ability to osmoregulate, the initial response of many bivalves to a salinity which may eventually be lethal, is shell closure (Shumway, 1977a; Deaton, 1992). Bivalves from intertidal habitats do not have to exchange water over short periods to maintain oxygen levels in their tissue fluids, instead they can close the shell valves and revert to anaerobic metabolism (Gilles, 1972; Bayne, 1976; de Zwaan, 1983). This mechanism delays equilibration with the environment, not only of their internal body fluids, but of their mantle fluid which acts as a buffer between the external environment (Gilles, 1972; Hoyaux *et al.*, 1976). Shell closure minimises the need for solute adjustment, but it may cause anoxia and the accumulation of organic acids due to anaerobic metabolism (Burton, 1983). Thus, valve closure is only a temporary means of protection against adverse environmental conditions such as reduced salinities (Shumway, 1977a). Water exchange is required to reduce the concentration of excretory products such as ammonia in the tissues, as long-term valve closure results in a build up of anaerobic by-products and eventually death (Shumway, 1977a; De Zwann, 1983).

Bivalves display distinct phases of gape, each one characterised by a different pumping rate resulting in variable oxygen uptake (Collier, 1959). Even a slight contraction of the valves will result in reduced water flow which will in turn affect the rate of feeding (Riisgård, 1991) and gas exchange (Shumway, 1996).

Similarly, exposure to non lethal salinities may cause periodic closure or irregular ventilation patterns. Decreasing filtration rates in this manner may reduce the time available for feeding, thus limiting growth potential (Bayne & Newell, 1983; Jørgensen *et al.*, 1986). Therefore, salinity is a limiting factor for many marine molluscs and may, when reduced, restrict the potential for energy acquisition, thereby limiting overall energy balance and scope for growth (Stickle & Bayne, 1982; Bayne & Newell, 1983).

A number of different variables have been used to investigate the over all effect of fluctuations in salinity within the non lethal salinity range. Previous authors have investigated the effects of salinity on oxygen consumption (Brown & Meredith, 1981; Shumway, 1981; Shumway & Marsden, 1982; Stickle & Bayne, 1982), filtration rate (Schulte, 1975), food consumption (Nell & Paterson, 1997) and growth (Bayne, 1965; Almada-Villela, 1984; Nell & Paterson, 1997).

Respiration represents the energetic cost of maintaining basic metabolic processes (Pamatmat, 1983; Dame, 1997), and is an important indicator of an organisms ability to cope with stress (Bayne *et al.*, 1976; Vernberg & Vernberg, 1981). Previous authors have suggested that fluctuations in environmental salinity may alter the metabolism of aquatic invertebrates (Kinne, 1971; Bayne, 1973; Shumway, 1979, 1980; Brown & Meredith, 1981; Shumway, 1982; Shumway & Koehn, 1982) and that reductions in respiration rate may conserve metabolic reserves during periods of stress (Newell, 1973). However, the causes of salinity effects on oxygen consumption rates are not clear. Some authors suggest that altered metabolic rates in response to changing salinity is the result of stimulation or inhibition of movement (Shumway, 1979; Brown & Meredith, 1981; Stickle & Bayne, 1982), an increase or decrease in the osmotic concentration of body fluids, changes in internal ion ratios, and interference with neuromuscular, hormonal or enzymatic mechanisms (Kinne 1971; Newell, 1973).

The effects of salinity have been investigated on both isolated tissues (Galtsoff, 1964; Van Winkle, 1968) and intact animals (Mane, 1975; Brown & Meredith, 1981; Shumway, 1981; Shumway & Marsden, 1982; Stickle & Bayne, 1982), however isolated tissues do not always exhibit the same metabolic response to environmental changes as intact animals (Shumway, 1982, 1996). This may be due to the increased energetic cost of osmoregulation in isolated tissues (Vernberg & Vernberg, 1981).

Many bivalve molluscs exhibit behavioural periodicity in their valve movements, with periods of activity and quiescence (Higgins, 1980). During activity bivalves

expose themselves to the ambient environment, to ventilate their gills for respiration and feeding. During inactivity the animal isolates itself from the surrounding conditions, is unable to feed, and generally respires anaerobically. Anaerobiosis, although vital for survival especially in intertidal organisms, is an ineffective use of organic energy stores and is generally utilised during periods when food intake declines or ceases (McMahan, 1988).

Lowered salinities result in partial or complete contraction of the adductor muscle and a slowing or cessation of water current through the gills, if animals are not actively pumping, feeding cannot occur. Conversely even though pumping activity can be underway, feeding may still not occur (Shumway, 1996). Thus salinities that result in shell closure or periodic closure may limit access to food resources and in turn may limit growth.

Although *K. scalarina* is capable of surviving a salinity range of 25-50‰ (see Chapter 4) the zone of optimal growth may be a narrower band within this range. Experiments were conducted to determine if in the absence of osmo- and ionic regulation *K. scalarina* relies on its ability to isolate the visceral mass by shell closure to survive unfavourable salinity regimes. Secondly, as insufficient numbers of small *K. scalarina* could be obtained for growth experiments, respiration and algal clearance trials were conducted to determine if shell valve closure or irregular shell closure patterns limit oxygen consumption and algal clearance, which may in turn limit growth potential.

6.2 Methods

6.2.1 Shell closure

Adult (35.0 - 4.0 mm) and juvenile (20.0 - 25.0 mm) *K. scalarina* were collected from Moulting Lagoon, Coles Bay (see Chapter 2, Section 2.2.1). Experiments commenced the day after collection. Clams were transferred directly from seawater (35‰) to one of the experimental salinities. Duplicate aerated plastic aquaria (2 L) were used for each salinity (15, 25, 35, 45 & 55‰). Each aquarium contained ten clams with approximately 4 cm of 500 µm sieved sand. Salinity treatments were established by adding distilled water or an artificial salt mix (Coral Reef Red Sea Salt®) to sand filtered seawater with a salinity of 35‰.

Filtering activity was recorded at ½ hr intervals in 4 hour blocks every 12 hours for a total of 48 hours from commencement of the experiment. The number of clams, in each aquarium, with siphons protruding from between the shell valves were counted to give an indication of shell closure in different salinities.

6.2.2 Respirometry

Respiration trials were conducted at Marine Shellfish Hatcheries, Bicheno, from 19th Feb. to 6th March 1997. Adult *K. scalarina* (35.0 - 40.0 mm shell length) were collected from Moulting Lagoon, Coles Bay (see Chapter 2, Section 2.2.1). Clams were maintained prior to respirometry in tanks of flowing aerated seawater ($35^0/_{00}$). Clams were fed a mixed algal diet, (4 L), consisting of *Pavlova lutheri* and *Tetraselmis suecica* every third day. Clams used in the respiration trials were starved for 24 hours prior to and during respirometry, to ensure that oxygen consumption was not altered by digestive processes.

Salinity treatments were established by adding double filtered freshwater (1 μm nominal and activated charcoal) or an artificial salt mixture (Coral Reef Red Sea Salt®) to seawater with a salinity of $35^0/_{00}$. Seawater was drawn from an exposed section of the coastline unaffected by freshwater runoff. Salinities were measured daily using a WTW (Wissenschaftlich - Technische - Werksttaten) conductivity meter, calibrated using standard saline solution (Ocean Scientific International® $K_{15} = 0.99982$; Salinity $=34.993^0/_{00}$). Water was exchanged after every trial and clams were utilised only once.

Respiration trials commenced the day after collection, clams were placed in the respirometer chambers which were filled with seawater of the appropriate salinity. Each chamber contained approximately 17-20 clams (400 g total wet weight). Groups of clams rather than individuals were utilised in both respiration and feeding trials for a number of reasons. Firstly, the sensitivity of the equipment available was inadequate to detect the small variations obtained if experiments were conducted with individual clams. Secondly, the effect of varying experimental conditions on groups of clams provided a more accurate indication of culture conditions. Similarly, as natural populations *K. scalarina* are found in dense aggregations using groups of clams provided a more accurate indication of the response of natural populations to variations in salinity.

Pilot trials using sand in the bottom of chambers to allow the clams to bury, thus maintaining conditions as close as possible to field conditions, proved unsuccessful causing blockages in the equipment and unreliable results. All subsequent results were conducted without sand in the respirometer chambers. Salinity trials were conducted as duplicates for each of two salinities and a control ($35^0/_{00}$ with no clams). The control enabled an estimate of microbial oxygen consumption/production so that a correction factor could be applied to the data. Trials were conducted for a period of 48 hours, firstly to mimic earlier osmotic and ionic trials (see Chapter 4) and secondly as it is during this period that

stabilisation of oxygen consumption occurs for bivalves following a salinity change (Kinne, 1971; Bayne, 1973).

6.2.3 Respirometer

The respirometer was composed of five elliptical perspex chambers (2.3 L) set up with two replicate chambers for each treatment and one chamber as a control (no animals) in a continuous flow open circuit design, for a detailed description see Maguire *et al.* (submitted). Water from a reservoir entered at the base of each chamber and was regulated by a rotameter. Flow rates were maintained at 80 mL min^{-1} and were checked manually twice daily. Water leaves the respiration chambers from at the top where it is diverted by solenoids to either waste or to a flow cell containing an Orion (model 9708) oxygen electrode. Each of the five chambers is monitored for 10 minutes every hour and the remaining ten minutes in each hour is used to calibrate the oxygen electrode using fully aerated seawater. Data from the oxygen electrode are collected by a data logger (Data Electronics Australia, model DT600) every second, averaged every minute and downloaded to a computer at the end of each experiment. The datalogger also stored water temperature data from thermocouples placed throughout the system.

Final mV output was converted to oxygen levels in the chambers using a LOTUS spreadsheet, where drift between calibrations are assumed to be linear (for both flow and oxygen calibrations). The amount of oxygen used by each tank is then calculated as a percentage of the full saturation value using the calibration adjusted mV output from the oxygen electrode. This is converted to mg O_2h^{-1} using the following equations:

$$Oxygen\_consumption(mg.h^{-1}) = \_$$

$$Flow(L.h^{-1}) \times O_2\_in\_fully\_saturated\_seawater(mg.L^{-1}) \times \%\_full\_saturation\_used$$

and to calculate the oxygen content of fully saturated seawater in $mg.L^{-1}$;

$$O_2 = 10^{\left(\frac{781.29}{T(^\circ K)} - 1.70842\right)} \times \frac{b.p.(torr)}{1000} \times [0.994857 - (0.0091099 \times Cl‰)]$$

which is derived from standard tables. The first term is a Clausius-Clapeyron form of equation correcting for temperature where T is given in °Kelvin. The second term correcting for barometric pressure (in torr). The third is based on a

linear regression and corrects for salinity given in ppt Chlorine (see Maguire *et al.*, submitted, for details)

Values for tanks containing animals are corrected for the oxygen uptake of the control tank and the final values were divided by the wet weight of the animals to express results as oxygen consumption in mg $O_2kg^{-1}h^{-1}$.

Data from low salinity treatments (15 & 25‰) resulted in negative values for oxygen consumption, due to nutrient enrichment of the freshwater used to dilute the seawater, resulting in elevated microbial activity. The microbial population, as indicated by oxygen consumption in the control chamber, was stable after 24 hours. Therefore, the data presented for low salinities is from the second 24 hour period. The application of oxygen consumption/production of the control tank microbial population to experimental data as a correction factor resulted in unrealistic values and so an alternative correction factor was applied. As the clams in all treatments displayed dormant periods where oxygen consumption approached zero, this number was used to correct for any microbial addition

6.2.4 Algal Clearance

Two types of algae were used in separate trials to ensure clearance rates were not masked by particle size selection. The prymnesiophyte, *Pavlova lutheri* (4-6 μm) and the green flagellate, *Tetraselmis suecica* (12-14 μm) were used as these two species are common aquaculture species. Adult (35.0 - 40.0 cm, shell length) and juvenile (20.0 - 25.0 cm, shell length) *K. scalarina* were collected from Moulting Lagoon, Coles Bay (see Chapter 2, Section 2.2.1). Experimental treatments commenced the day after collection. Clams were transferred directly from seawater with a salinity of 35‰ to one of the experimental salinities ranging from 5-55‰ with 5‰ increments. Three aerated plastic aquaria (500ml) were used for each salinity, each containing four pre-weighed clams. A control treatment containing empty shells was used to determine the natural settling rate of the algae. Salinity treatments were established by adding distilled water or an artificial salt mixture (Coral Reef Red Sea Salt®) to sand-filtered seawater with a salinity of 35‰. Temperature was maintained at 15°C throughout the experimental period. Clams and algae were added to the experimental treatments and left for 48 hours. Water samples containing algae were taken from each of the treatments after 48 hours and preserved with gluteraldehyde for later renumeration.

Three replicates of each algal samples were counted using a haemocytometer and algal densities were extrapolated to 1 L. The mean number of algal cells present

in each sample were subtracted from the number of cells originally fed to the clams and the results expressed as cells consumed g^{-1} wet weight clam.

6.2.5 Statistical Analysis

Shell Closure

Data from all observation periods were averaged to eradicate discrepancies between observation periods and the results expressed as mean percentage shell closure. Data was arcsin $x^{0.5}$ transformed and analysed using one way ANOVA in SYSTAT for Macintosh. Means were compared using Tukeys (Sokal & Rohlf, 1981).

Respiration

Data from the respirometer was corrected using the equations given in Section 6.2.3. Corrected data was analysed using one way ANOVA in JMP for Macintosh. Homogeneity of variances was checked using Barretts test of homogenenity of variances.

6.3 Results

6.3.1 Shell Closure

Salinity had a significant effect on the shell closure of both adult ($P = 0.003$) and juvenile ($P = 0.0001$) *K. scalarina*. Shell closure of adult and juvenile *K. scalarina* was highest in salinities outside the salinity tolerance range (15 & 55‰) (Figure 6.1). Clams in 25‰ also displayed a high percentage of shell closure (adults, 88%; juveniles,76%) although this salinity is within the established salinity tolerance range. However, a lower percentage of juveniles were closed in 25‰ treatments. Overall juveniles were more active than adults and were less likely to have their valves closed during the observation periods.

6.3.2 Respiration

Adult *K. scalarina* did not maintain a constant rate of oxygen consumption throughout the experimental period (Figure 6.2). Oxygen consumption of clams in 35‰ illustrates that respiration went through a series of fluxes, or periods of activity and quiescence. Salinity significantly affected oxygen consumption of adult *K. scalarina* ($P = 0.0128$). Optimal oxygen consumption was evident in 35‰, oxygen consumption decreased progressively at salinities either side of this point (Figure 6.3). However, oxygen consumption decreased most dramatically in low salinity treatments, with respiration of clams in 15‰ substantially different from all other treatments.

6.3.3 Algal Clearance

The highest number of *P. lutheri* cells consumed by adult *K. scalarina* was in the 25-50‰ treatments (Figure 6.4A). Algal consumption decreased dramatically in salinities outside this range. The optimal number of algae consumed was in 40‰. However, there was no substantial difference in algal consumption between 25-50‰.

A similar pattern of *P. lutheri* consumption was displayed by juvenile *K. scalarina* (Figure 6.4B), with high rates between 25-50‰ However, there was a more obvious increase in algal consumption between 30-45‰. Algal consumption decreased in salinity treatments greater than 45‰ and less than 25‰. The lowest numbers of algae were consumed were in the hypo-saline treatments.

Consumption of *T. sueccia* by adult *K. scalarina* (Figure 6.5A) displayed a similar pattern to *P. lutheri.* With high consumption rates between 25-50‰ and a decrease outside this range. However, the decrease in consumption outside the optimal range was not as pronounced as was displayed with *P. lutheri*

Juvenile consumption of *T. sueccia* was highest between 25-50‰ (Figure 6.5B). Again, algal consumption of clams displayed a marked decline in salinities outside this range. The highest algal consumption was in the 25-40‰ treatments.

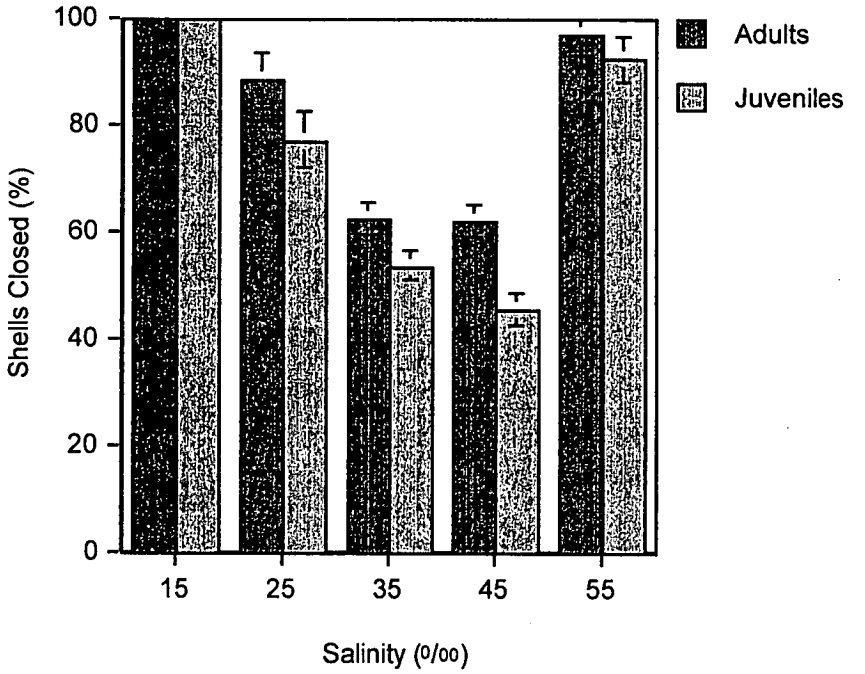

Figure 6.1: Percentage of adult and juvenile *K. scalarina* with shell valves closed during observation periods (mean ± S.E.)

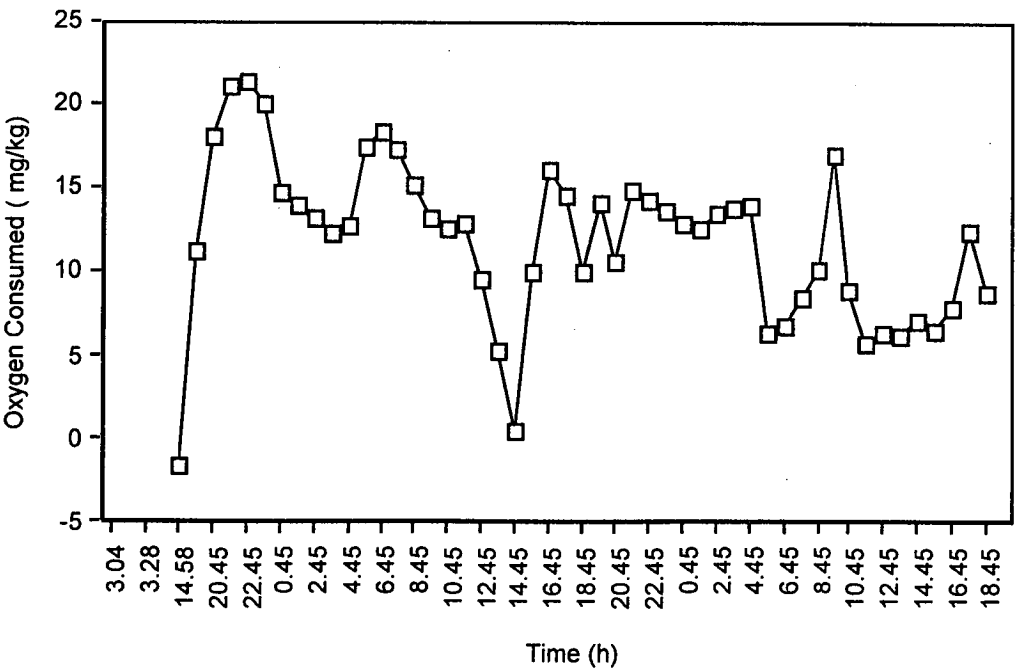

Figure 6.2: 24 h pattern of oxygen consumption of adult *K. scalarina* in 35‰ treatment.

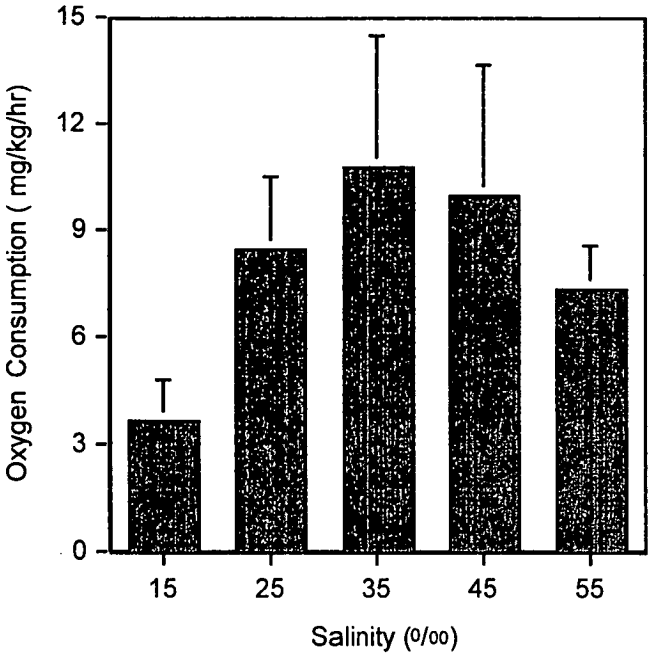

Figure 6.3: Oxygen consumption of adult *K. scalarina* (mean ± S.E.).

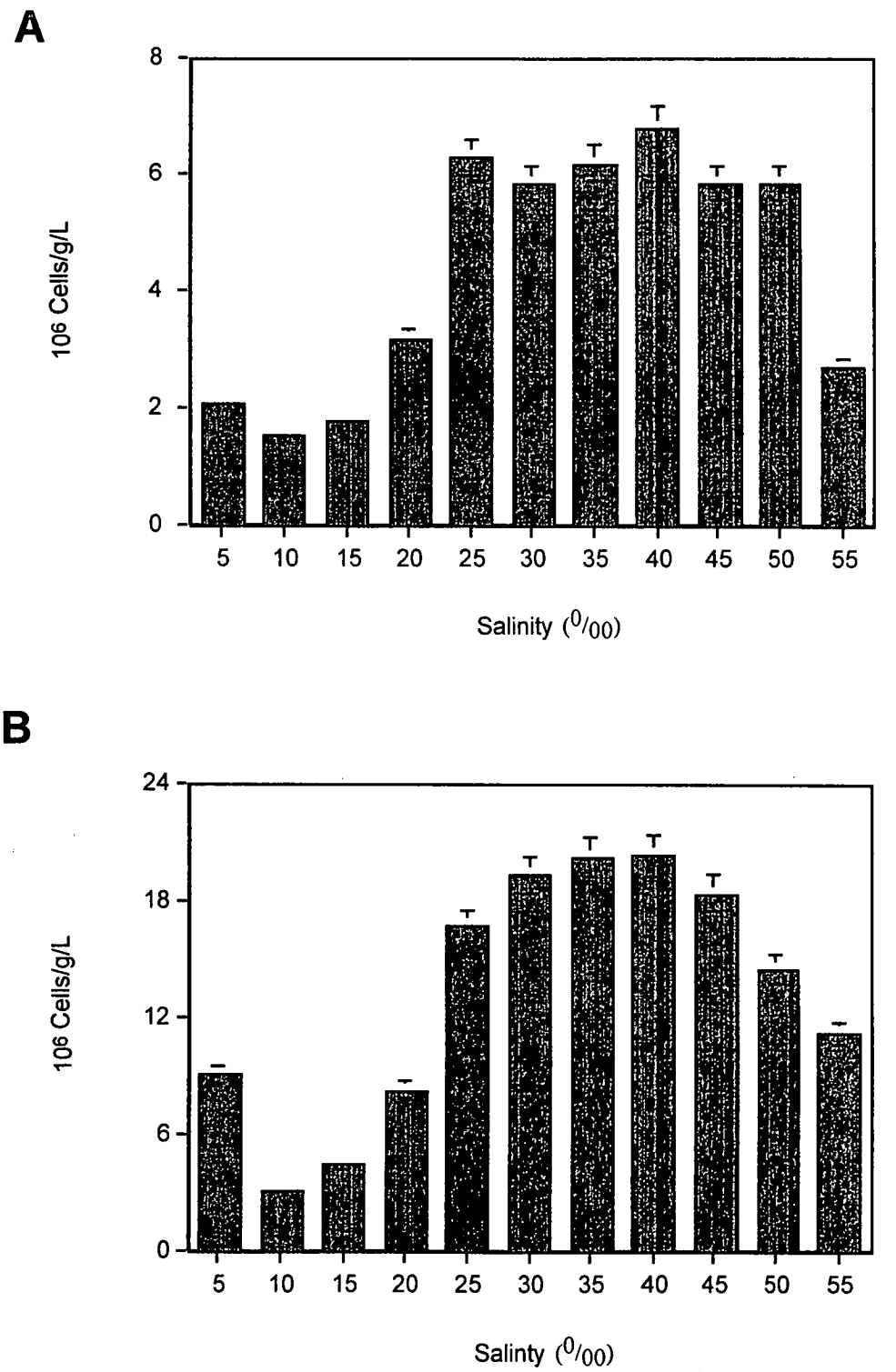

Figure 6.4: Clearance of *Pavlova lutheri* by *K. scalarina* (mean ± S.E.). A) adults B) juveniles.

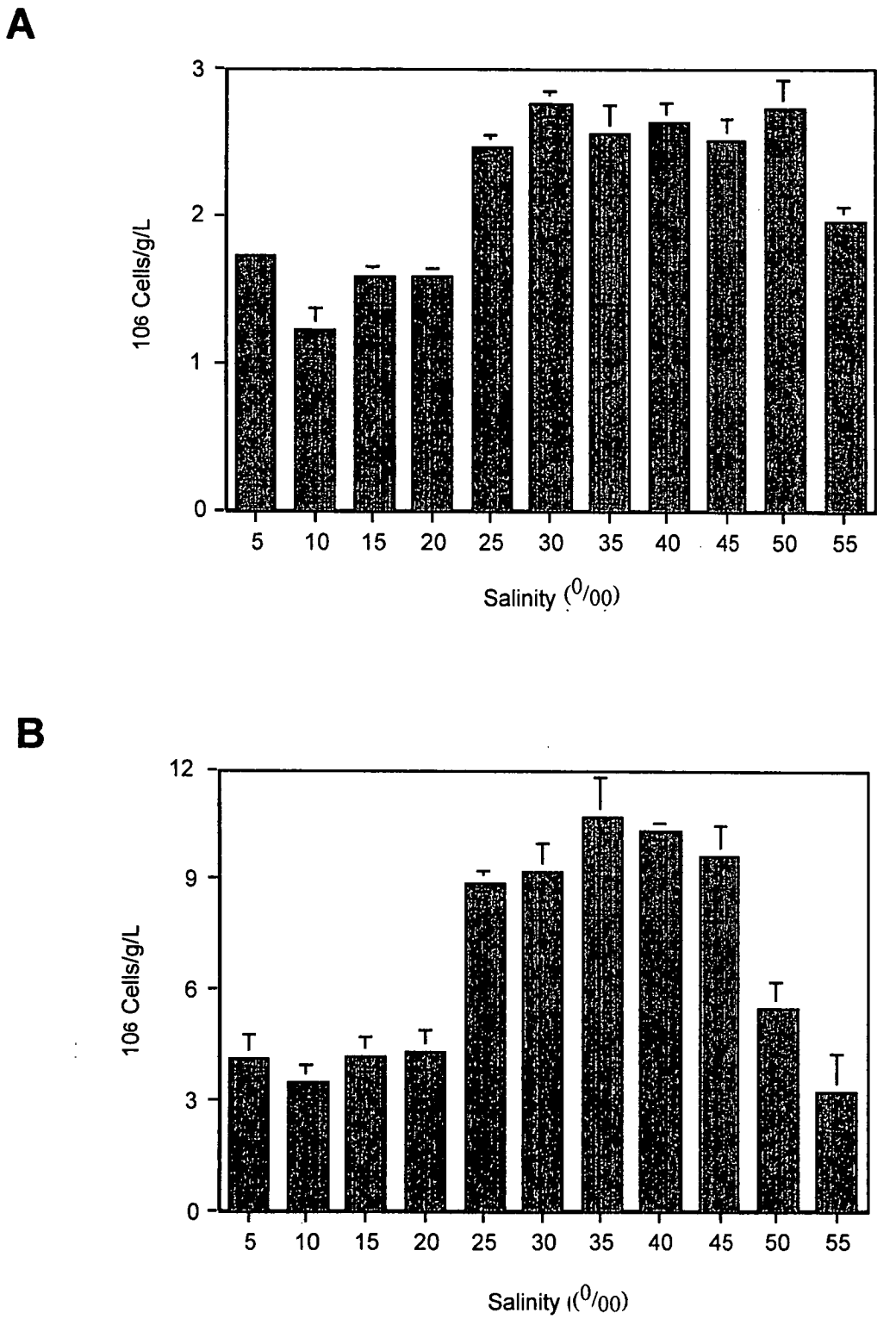

Figure 6.5: Clearance of *Tetraselmis sueccia* by *K. scalarina* (mean ± S.E.). A) adults B) juveniles.

6.4 Discussion

The immediate response of bivalve molluscs exposed to an environmental stress, especially unfavourable salinity, is shell valve closure (Shumway, 1977a; Deaton, 1992; Widdows & Donkin, 1992). *K. scalarina,* like other bivalves molluscs, exposed to salinities outside its salinity tolerance range (25-50‰) closes the shell valves and isolates its tissues from the ambient conditions. Shell closure experiments indicate that in 15‰ all clams, both adults and juveniles, remained closed over the entire experimental period (48 h). Shell closure was also high (75-100%) in 25‰, although this treatment is within the salinity tolerance range of this species. Overall juveniles displayed the lowest rates of shell closure, although salinity tolerance trials (see Chapter 4) illustrate that juvenile *K. scalarina* have a wider salinity tolerance range (5-50‰) than adults (25-50‰). The number of juveniles closed in 35-45‰ treatments varied between 40-55%, while the number of adults closed in the same treatments ranged between 30-80%. Previous authors report that for bivalves held in undisturbed conditions shell valves will remain open the majority of the time (Galstoff, 1964; Widows & Donkin, 1992). In comparison Pierce (1971) reports that *Modiolus demissus* may remain consistently closed for up to 7-10 days, depending on the extent of the salinity change.

Benthic macroinvertebrates display a range of responses to altered salinity (Vernberg & Vernberg, 1981). However, Kinne (1971) divided the effects of salinity on the rate of oxygen consumption in marine and brackish water invertebrates into four categories: 1) increase in hyper-saline media and or decrease in hypo-saline media 2) increase in hyper- and hypo-saline conditions (e.g. Mane, 1975; Shumway & Marsden, 1982) 3) decrease in hyper- and hypo-saline media (e.g. Lukanina & Gurina, 1977; Brown & Meredith, 1981; Shumway & Koehn, 1982; Stickle & Bayne, 1982) 4) both remain essentially unaffected (e.g. Galstoff, 1964; Shumway, 1979; 1981).

Salinity had a significant effect on the oxygen consumption of *K. scalarina.* Respiration trials indicate that *K. scalarina* undergoes an initial period of osmotic shock in which the shell valves are closed but resumes a stable pattern of reduced respiration in salinities outside that of normal seawater within 24 h. Previous authors have suggested that following a salinity shock respiration rate is initially reduced, followed by a phase of stabilisation (Kinne, 1971) lasting a few hours, and leading to a new steady state within 2 days (Kinne, 1971; Bayne, 1973). *K. scalarina* appears to follow this general pattern.

The highest rates of oxygen consumption for adult *K. scalarina* were in 35‰ treatments (10.78 mg/g/hr), decreasing to the lowest rate in 15‰ (3.62 mg/g/hr) which was significantly different from all other treatments. Decreases were also evident in 25 and 45‰ treatments although these salinities are within the salinity tolerance range. Other authors have reported that marine invertebrates display a range of responses to a change in the ambient salinity. Galstoff (1964) found no significant change in the respiration of *Crassostrea virginica* after 3 days in dilute sea water, however a substantially narrower salinity range (24.1-31.5‰) was studied than the ones discussed for *K. scalarina*. The rate of oxygen consumption by *Amphibola crenata*, a marine pulmonate, is unaffected by salinity, in the range 0-125% sea water (Shumway, 1981). However, Shumway & Koehn (1982) state that the oxygen consumption rate of whole oysters, *Crassostrea virginica*, increased with decreasing salinity at 10 and 20°C, similar to results reported for isolated gill tissue. Kinne (1971) states that immediate temporary elevation of oxygen consumption following salinity change may result from an increased overall alertness by the animal to counteract physiological stress. In contrast, Stickle & Bayne (1982) report that salinity and temperature had a significant effect on oxygen consumption of the gastropod *Thais lapillus*, which was highest at 30‰ and depressed at 17.5‰ and 5°C, which is in accordance with the results obtained during the duration of this experiment.

Similarly, high salinity treatments also resulted in decreased oxygen consumption. *K. scalarina* in 35‰ treatments had an oxygen uptake of 10.78 mg/g/hr while clams in 55‰ treatments had an oxygen uptake of 7.33 mg/g/hr. Brown & Meredith (1981) report that high salinities induced a marked reduction in the rate of oxygen uptake of the whelk, *Bullia digitalis*. At 45‰ mean transformed oxygen consumption was 388 µg/hr and at 51‰ it was 2411 µg/hr. The decrease in oxygen uptake in *B. digitalis*, associated with salinity changes, was partly attributed to decreased activity, however activity levels were not the only cause of the decreased oxygen consumption (Brown & Meredith, 1981). Brown (1979) has shown that immobile *B. digitalis* have a mean oxygen take of 630µg/hr in natural seawater. Therefore, such a large decrease in oxygen uptake must be due to factors other than activity state alone. In this study *K. scalarina* were held out of the substrate in smooth sided tanks and thus little movement of any kind occurred. *K. scalarina* is a sediment dwelling bivalve that, excluding burrowing behaviour, normally displays limited movement in its natural environment (Bellchambers, 1993). Thus it seems unlikely that inactivity alone explains the reduction in oxygen consumption of *K. scalarina*. Brown and Meredith (1981) state that in the absence of a respiratory current little or no oxygen uptake by the gill, of *B. digitalis* was measured during the experiments, as the animal appears to

be able to turn off or at least dramatically reduce this current indefinitely. Similarly, *K. scalarina* exposed to salinities outside its natural range may experience a reduction in the respiratory current which may account for the reduced oxygen consumption and algal clearance rates recorded. Shumway (1979), working with 11 species of gastropod molluscs, reported withdrawal into the shell as a primary response to decreased salinity, oxygen uptake ceasing thereafter.

Comparison of the algal clearance rates of adult and juvenile *K. scalarina* suggest that both groups respond to changes in ambient salinity in a similar manner. Both groups display a substantial decrease in algal clearance in salinities outside the salinity tolerance range. Although juvenile *K. scalarina* appear display a zone of optimal algal clearance between 30‰ and 45‰, patterns in adult *K. scalarina* algal clearance are less obvious. The highest rates of algal clearance are evident within the salinity tolerance range but optimal clearance varied somewhat between algal species.

In certain bivalves cessation of the respiratory current may protect the pallial organs from osmotic stress and the molluscan gill may be similar in this respect. However, complete cessation of the respiratory current or filtration rate of *K. scalarina* is unlikely. Shell valve closure experiments indicate that *K. scalarina* in hypo and hyper saline medium do not remain consistently closed but are characterised by periods of activity interspersed with periods of quiescence, this is also evident in 24 h pattern of oxygen consumption. The dramatic decrease in oxygen consumption rates and algal clearance in salinities < 25‰ is in accordance with the salinity tolerance range. While *K. scalarina* displayed a decrease in algal clearance in salinities < 25‰ there is still evidence of clearance which is either incomplete or at least intermittent. Similarly, adult *K. scalarina* clams in low salinity respiration treatments (<35‰) are still respiring, although at a reduced rate. Van Winkle (1972) suggested that in cases where shell closure is not complete, the rate of oxygen consumption may still decline, may be due to direct inhibition of the gill cilia by reduced salinity. Alternatively, filtration may be periodic as a means of ridding the mantle cavity of anaerobic by-products. If this is the case the increased salinity tolerance of juvenile *K. scalarina* (5-50‰) in comparison to adult *K. scalarina* (25-50‰, see chapter 4) may be related to the ability to rid the body cavity of anaerobic by products rather than salinity tolerance *per se*. Juvenile *K. scalarina* display decreased rates of shell closure, therefore allowing excretion of by-products preventing the accumulation of toxins that eventually lead to death.

K. scalarina utilises shell closure as a mechanism to avoid unfavourable salinities. However, shell closure is either not complete or is more in the form of irregular ventilation especially at low salinities. This is supported by algal clearance and respiration trials. Oxygen consumption trials indicate a limitation of oxygen uptake at salinities greater than or less than 35‰ even if these salinities are within the tolerance range. This may in the long term decrease the scope for growth, however evidence from adult algal clearance trials is not is clear cut. Juveniles appear to have some restriction in salinities greater than 45‰ and less than 30‰. While *K. scalarina* survives a range of salinities, optimal salinity for growth as inferred from the physiological processes investigated may be a somewhat narrower band.

Chapter 7:

Food sources of *K. scalarina*: potential for polyculture with Pacific oysters.

7.1 Introduction

7.1.1 Food sources

Despite numerous laboratory based studies on the feeding of bivalve molluscs (see Morton, 1983; Bayne & Newell, 1983 for reviews) few studies have investigated the specific food items utilised by bivalves in their natural habitat (Shumway *et al.*, 1987). It is generally assumed that filter feeders rely on phytoplankton suspended in the water column as their main food source (Shumway *et al.*, 1987; Newell & Shumway, 1993). However, in unconsolidated sediments seston and benthic phytoplankton are important food sources for benthic organisms (Langdon & Newell, 1990). Previous authors have suggested that the majority of molluscs feed on seston and that detritus is a common dietary component of bivalve molluscs (Tsikhon-Lukanina, 1982; Hummel, 1985; Thangavelu, 1988; Langdon & Newell, 1990). It is probable that bivalve molluscs do not necessarily require phytoplankton as a food source and that organic particles of suitable size and composition can be utilised (Jørgensen, 1966).

As no previous work has been conducted on the feeding of *K. scalarina* and there is some debate as to the primary feeding strategy and major dietary components of this species. For the species to be successfully farmed especially in a polyculture situation this information is essential to ensure competition for food resources is minimised. The existing knowledge of feeding strategies and mechanisms in bivalves is briefly reviewed below.

A number of different feeding mechanisms have evolved in bivalves (see Morton, 1983 for review). Although the majority of lamellibranch bivalves have been divided into two groups, the suspension feeders and the deposit feeders, there is no clear cut distinction between the two food sources (Morton, 1983; Shumway *et al.*, 1987). Suspension feeders commonly inhabit medium to coarse grained sediments in environments dominated by tidal and wave induced currents. While deposit feeding bivalves generally inhabit fine grained sediment in low energy environments. However, the two feeding modes are not necessarily exclusive and some bivalves utilise both feeding modes or switch between modes to optimise feeding under fluctuating environmental conditions (Dame, 1996). Some clam species such as *Macoma balthica* have two feeding modes: filtering suspended

food particles from the water column or filtering food particles deposited on the sediment surface (Skilleter & Peterson, 1994). Several species of bivalves can derive nutritional benefits from growing in the sediment (Hylleberg & Gallucci, 1975; Hummel, 1985; Skilleter & Peterson, 1994; Patterson & Nell, 1997). Sediment related food sources such as algae, detritus and other micro-organisms are utilised by the clam *Ruditapes philippinarum*, in particular, benthic diatoms (He & Wei, 1984). Previous authors have suggested that bivalves located in the sediment display higher growth rates than bivalves located out of the sediment, because of supplementary food sources derived from the sediment (Hylleberg & Gallucci, 1975; Patterson & Nell, 1997). Levinton (1972) suggests that the food supply is constantly fluctuating and unpredictable therefore suspension feeders must maintain an adaptive feeding strategy which maximises their food resources.

7.1.2 Suspension Feeding

The ctenidia of suspension feeding bivalves are modified for the collection of suspended or benthic food material from the water column (see Morton, 1983; Jørgensen, 1990 for reviews). There are three types of cilia present on the gill filaments that are responsible for filter feeding: the frontal, laterofrontal and lateral (Bayne *et al.*, 1976; Jørgensen, 1990; Dame, 1996). The primary role of the lateral cilia is to generate ciliary currents, maintaining a flow of water through the mantle cavity and across the gills (Bayne *et al.* 1976; Jørgensen, 1990). Particles are removed from suspension by the laterofrontal cilia. Although filtration is largely the function of the laterofrontal cilia, mucus and the patterns of water flow over the ctenidia are also important (Jørgensen, 1990). Food particles retained by the gills are bound in mucus on the gill lamellae and transported by ciliated grooves to the labial palps (Morton, 1983). Therefore, the gills are the first site of particle selection, which is based primarily on particle size, and in turn is dependent on the size of the gill ostia (Scarratt, 1994).

The labial palps are paired triangular flaps located on either side of the mouth, which regulate the amount and size of food entering the mouth and direct surplus material to rejection tracts on the mantle surface (Bayne *et al.*, 1976; Jørgensen, 1990). Generally, small particles are transported over the ridges of the palps by the frontal cilia towards the proximal oral grooves and the mouth, while large particles fall into the troughs between adjacent ridges and are rejected from the ventral edge and tip of the palps (Morton, 1983; Jørgensen, 1990). Therefore, the palps are the second site of particle selection; particles are selected according to their chemical or nutritional properties (Scarratt, 1994). Rejected particles are

expelled from the mantle cavity by muscular contractions of the valves which reverse the direction of flow and force particles back through the inhalant siphon. Particles rejected by the labial palps and particles from the mantle surface drop off the gills and are called pseudofaeces (Widdows *et al.*, 1979; Inglesias *et al.*, 1992).

Accepted particles are transported from the labial palps along the proximal oral grooves to the mouth. Food particles pass through the mouth and oesophagus to the stomach, which is a complex structure containing a system of ciliary tracts (Bayne *et al.*, 1976). The particulate matter is sorted by the stomach which directs it towards either the digestive gland or the intestine. Thus, the stomach is the third location at which particle selection may occur. However, the mechanisms for selection in the gut are not clearly understood although they are probably based on biochemical sensing (Bricelj *et al.*, 1984). The digestive gland stores nutrients and regulates their transfer to other tissues, it is also where intracellular and extracellular digestion occurs.

7.1.3 Deposit Feeding

Deposit feeders use the same pumping mechanism as filter feeders to move water through the animal. However, food particles are collected in the form of benthic phytoplankton and organic matter, from the surrounding benthic environment by extending the inhalant siphon and sucking up particles (Dame, 1996). To ensure a continuous water flow between the bivalve, which is often buried in muddy unconsolidated sediments, and the benthic-pelagic interface many deposit feeding bivalves have elongated incurrent and excurrent siphons (Dame, 1996). Due to the poor quality of food, deposit feeding bivalves have developed two approaches to feeding: bulk feeding and sorting. In bulk feeding, large volumes of sediments are processed through the digestive tract for a small amount of nutrition (Dame, 1996). Bivalve deposit feeders typically sort particles before they are ingested into the mouth and reject the majority of the particles as pseudofaeces.

7.1.4 Potential for Polyculture

Several authors have investigated the possibility of polyculture involving a range of marine species (see Wallace, 1980; Taylor *et al.*, 1992; Stirling & Okumus, 1995). Besides using the existing infrastructure of established marine farms these areas offer a number of additional food sources due to organic enrichment. Bivalves cultured on existing farms may therefore gain a more continuous food

supply resulting in enhanced growth, reproduction, and possibly decreased time to reach market size (Wallace, 1980; Patterson & Nell, 1997). However, food can also be a limiting factor for bivalve production (Beukema & Cadee, 1986). Dilution of phytoplankton concentrations by suspension feeding organisms may lower food supply for other suspension feeders and consequently create competition for food resources between bivalves, which may eventually affect their growth. Kamermans (1993) reports that both the stomach contents and growth rates of *Cerastoderma edule* are negatively influenced by the nearby presence of *Mytilus edulis* beds. Similarly, Jensen (1992, 1993) observed low growth rates of *Cerastoderma edule* within dense populations. However, Kamermans *et al.* (1992) showed that mixed populations of *C. edule* and *Macoma balthica* had no influence on one another's growth in a small scale basin experiment.

7.1.5 Polyculture Potential in Tasmania

The Pacific oyster, *Crassostrea gigas*, was first introduced into Tasmanian waters in the late 1940's and early 1950's. By January 1993 there were 91 oyster leases occupying 1353 ha, which were estimated in 1995 to be worth approximately $15 million annually to the Tasmanian economy (Wilson *et al.*, 1996). Currently a total of 1351 ha is leased for oyster farming, of which approximately a third is developed, with another third suitable for development (Department of Primary Industry and Fisheries, 1996). Traditionally oyster farming has consisted of placing oysters in mesh baskets strung between wooden racks in the intertidal zone, so that the oysters are suspended in the water column and faeces and pseudofaeces sink to the sediment below. Approximately 1351 ha are leased for oysters farming in Tasmania, with 1051 ha involved in intertidal culture techniques. Thus the use of oyster farms for clam culture has been identified as offering advantages in terms of use of existing infrastructure and microbial monitoring data for waterways, from a human health/quality assurance perspective (Maguire, 1991). In addition, *K. scalarina* occurs on several of the existing Pacific oyster leases in Moulting Lagoon and Duck Bay.

A series of trials were conducted at Duck Bay, Smithton to determine the feasibility of growing *K. scalarina* under oyster racks on existing marine farms. Experiments were situated under, and away from, existing oyster racks for several reasons. Firstly, to investigate whether *K. scalarina* and *C. gigas* compete for food resources. Such competition is likely to render the potentially attractive idea of growing clams on otherwise unused space on oyster leases unacceptable to

farmers concerned about carrying capacity of waterways used for farming oysters. Secondly, to determine whether *K. scalarina* obtained any positive benefits from being located beneath oyster racks in terms of additional food resources from oyster faecal and pseudofaceal biodeposition. The stocking of clam able to utilise benthic resources on existing oyster leases has the potential to reduce the localised environmental impact of oyster farms (Maguire, 1992).

7.2 Methods

7.2.1 Site Description

Experiments were established at Duck Bay Shellfish's Pacific oyster lease on the 30/08/95 at Duck Bay, Smithton. Duck Bay is a large continuously open bay on the North West coast of Tasmania (54°81'S 145°38'E) (Figure 7.1). Buffered from the strong northerly seas by Perkins Island and the westerly projection of the Tasmanian mainland, Duck Bay experiences less wave action and a faster tidal current than many of the North West coast bays (Last, 1983). The bay is predominately marine but receives freshwater in the southern end from the Duck River. Duck Bay is dominated by extensive subtidal and tidal flats dissected by braided channels. The tidal cycle is semi-diurnal with a period of approximately 3 hours between high and low tide. The coast is of low relief mainly fringed with salt marshes and mixtures of mud, sand and shingle.

Duck Bay is approximately 12 km^2 in area, however marine farming is only permitted in West Duck Bay, from Pelican Point to East Perkins Island, an area of approximately 7.5 km^2. There are three oyster leases in Duck Bay with a total area of 45 ha, approximately 32-35 ha are currently under cultivation.

7.2.2 Experimental Design

Adult *K. scalarina* (30.0 - 40.0 mm shell length) were collected from Moulting Lagoon, Coles Bay (see Figure 2.1, Chapter 2) as insufficient numbers were available in Duck Bay. Experiments were conducted on Duck Bay Oysters, Pacific oyster leases at Smithton. Clams were placed inside cages to prevent migration over the experimental period. Cages were also designed to prevent access of large mobile consumers, such as crabs and wading birds, to the clams. All treatments were enclosed identically, therefore any additional effects of enclosures were held constant across the treatments. Cages were constructed of 9 mm Nylex® mesh (33 cm wide x 53 cm long x 14 cm) and fastened with plastic

cable ties to wooden stakes driven into the substrate. A total of 256 cages were placed in eight blocks in four patches, so that each patch had an "under oyster rack" and an "away from oyster rack" treatment.
Oysters were located in baskets directly above the "under rack" treatments and remained in this position for the duration of the experimental period (Plate 7.1). Each block had eight cages containing 25 clams (Diagram 7.1). Experiments were sampled for gut content determination every two months with one basket removed from each block at every sampling period, making a total of eight cages sampled per period (four from each treatment).

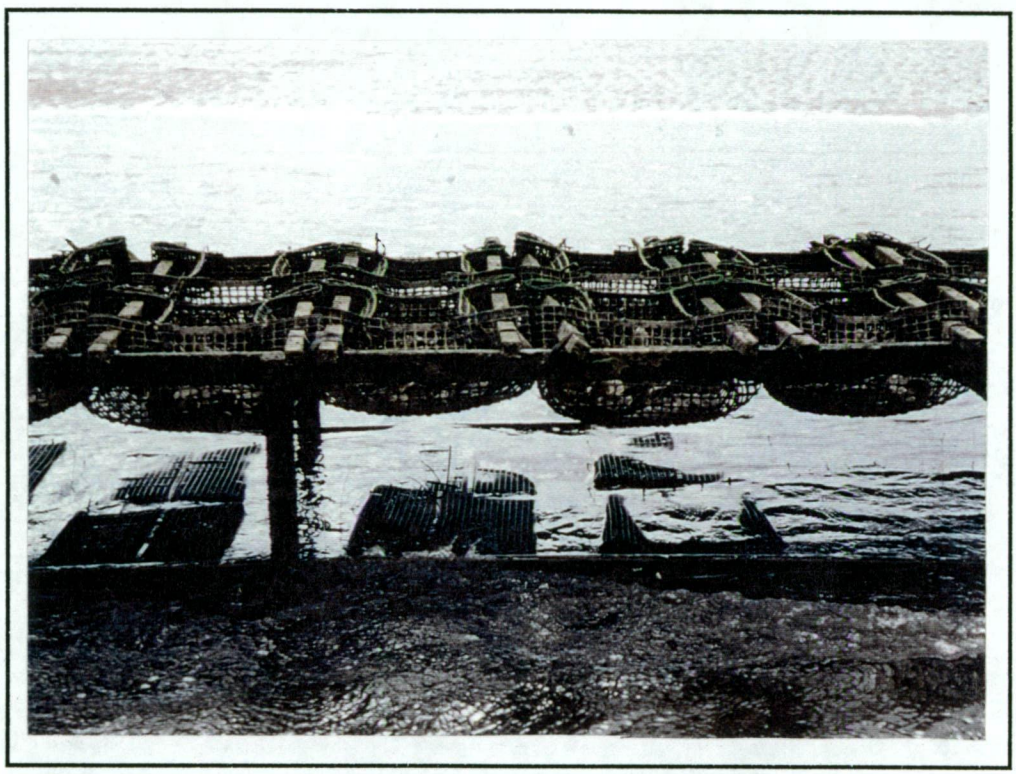

Plate 7.1: Cages containing *K. scalarina* in under rack treatments.

Two 2 L bottles of seawater were collected from the surface layers of the water column between the oyster rack and the away treatment at each sampling period for identification and estimation of the relative abundance of plankton assemblages. Two 2 L samples were preserved using Lugols fixative or gluteraldehyde and stored in darkened 2 L bottles for later identification and quantification.

Samples were generally collected on the outgoing tide, 1-2 h before low tide. After collection, clams were scrubbed to remove any epiphytes and injected with 10% formalin to prevent digestion of the gut contents until examined. To inject

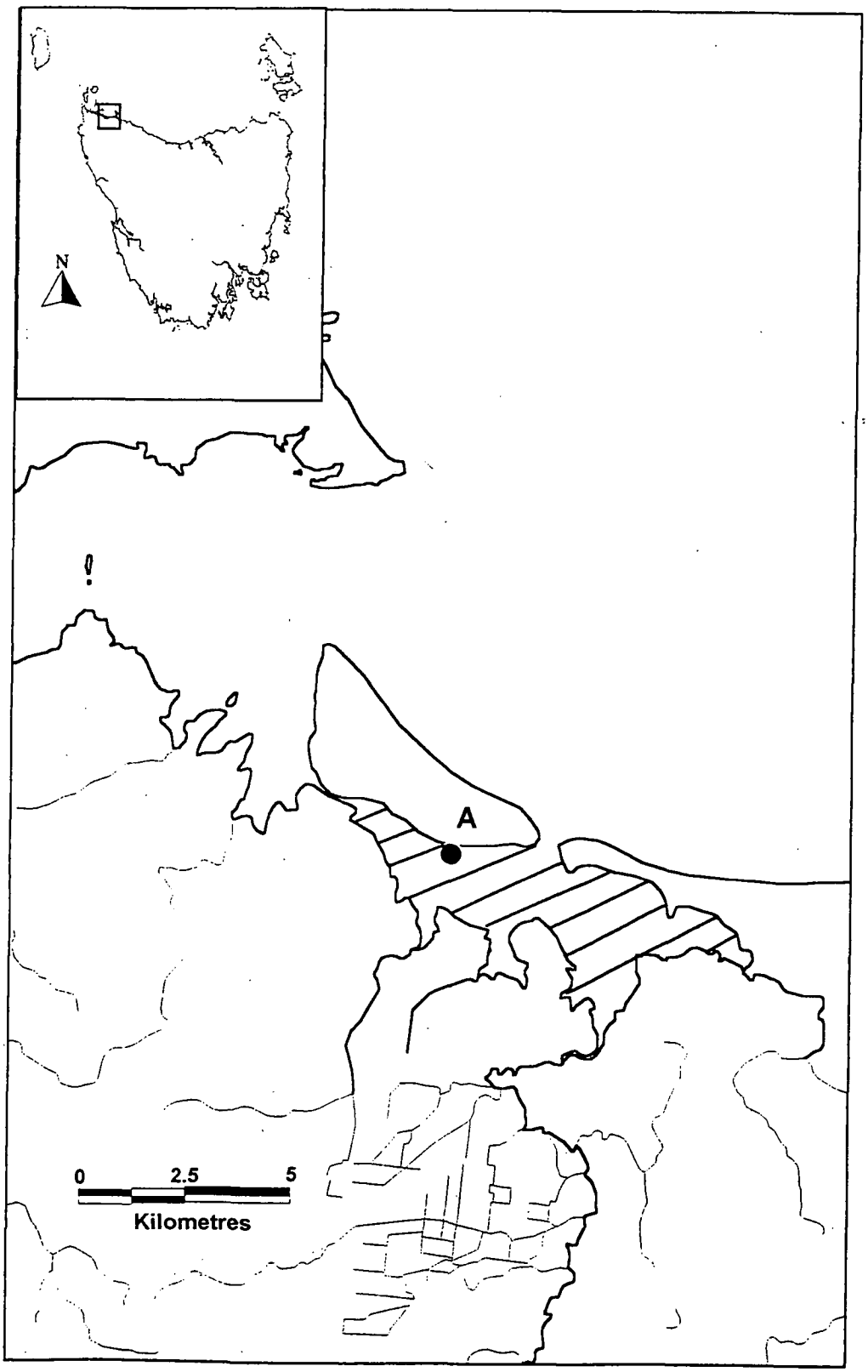

Figure 7.1: Map of Duck Bay, Smithton, Tasmania, Australia. Location of experimental treatments indicated by A.

the clams the valves were gently prised apart with a scalpel and formalin was injected with a hypodermic syringe. Clams were then placed in labelled plastic bottles containing 10% formalin and stored for later dissection. As the experimental site had to be assessed by boat, approximately 1-2 h lapsed between the collection of water and clam samples and preservation.

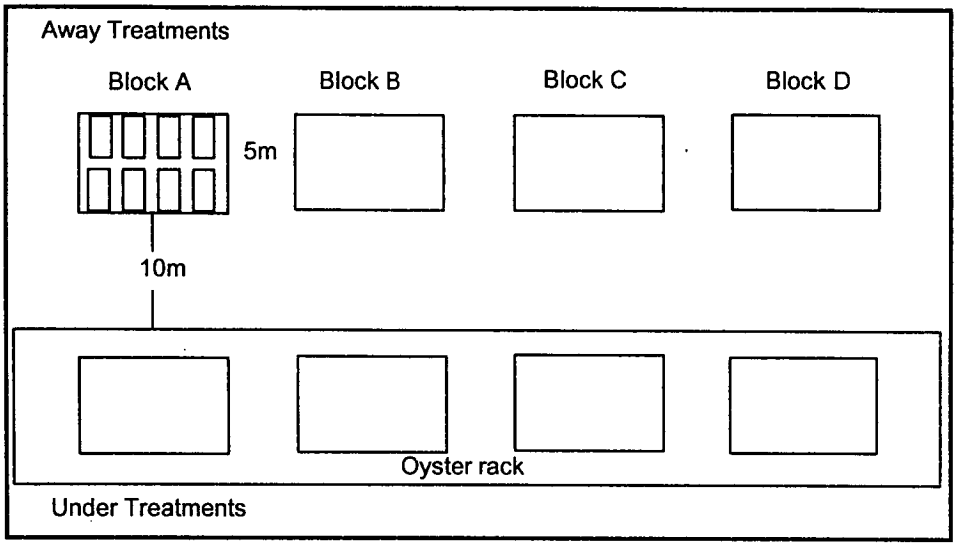

Diagram 7.1: Representation of experimental design at Duck Bay, Smithton.

7.2.3 Laboratory Techniques

To identify and count algal species present in the water column, samples preserved in Lugols were settled in 1 L measuring cylinders for at least a week (Richardson, 1991). The supernatant was siphoned off and the remaining 100 ml centrifuged at 1000 rpm for 10 minutes. The supernatant was again siphoned off and the remaining pellet re-suspended. A concentrated sub sample was then placed in a Sedgwick Rafter cell and examined on a Carl Zeiss Axiovert 25 inverted microscope. Fifty divisions of the counting cell were counted (approximately 300 algal cells) and the algae were identified to genus and where possible species level.

A sub sample of ten clam guts from each treatment were used for enumeration and identification of gut contents. Guts (mid gut and stomach) were carefully dissected from the preserved clams, any excess tissue removed and rinsed in distilled water. Gut contents were collected by slicing through the stomach with a scalpel blade and removing the contents with the edge of the scalpel blade. The gut contents were wet mounted on a microscope slide with distilled water and the

phytoplankton present were counted and identified using a Carl Zeiss Axioskop phase contrast microscope at x400 magnification.

SEM was used to obtain photos of the dominate algal types present in water and gut samples and to allow easier identification. Algae were identified by comparing preserved and photographed specimens with the descriptions and illustrations of Round *et al.* (1990). Algal identifications were checked and confirmed by consultation with algal taxonomists. Some difficulty was experienced obtaining adequate samples for SEM examination, due to the large amount of flocculate and detrital material in the water samples. This matter obscured the view on the SEM and blocked the pores of diatoms making obtaining photos for accurate identification difficult. Conventional 'cleaning' techniques would remove the excess detrital matter but also destroy many algal types present in the samples. All samples were concentrated as described for numeration and identification with the light microscope and then prepared in a number of different ways in order to achieve adequate photos.

Environmental SEM

Filtered

- samples were passed through a double filtration system of 14 mm and 7 mm Millipore filters and examined in wet mode on the environmental SEM

Air dried

- samples were rinsed in distilled water onto a 0.2 μm Nucleopore filter
-

SEM

Dehydrated and Preserved

- samples were put through a decreasing salinity series; 100, 70, 50, 30,0% (distilled water) followed by an increasing acetone series; 30, 50, 70, 100% with each sample centrifuged between steps. Samples in 100% acetone were then critical point dried and sputter coated (see Appendix 5.1).

Air dried

- samples were rinsed in distilled water onto a 0.2 μm Nucleopore filter

Algal species identified from the water samples and gut contents of *K. scalarina* were tabulated (see appendix 5.2a, b) however data presented in this chapter are expressed as weighted abundance percentages. Weighted abundance percentages were calculated by dividing the total species abundance for each genera by the

total phytoplankton abundance for that sample period and multiplying this number by 100. The use of weighted abundance percentages eradicated any identification errors which may have occurred at the species level, where small differences can lead to misidentification.

7.2.4 Algal Disappearance

Adult *K. scalarina* (30.0 - 40.0 mm shell length) were collected from Moulting Lagoon, Coles Bay (see Figure 2.1, Chapter 2). Clams were maintained in the laboratory at 15°C in aerated seawater ($35^0/_{00}$) prior to trials. Clams were starved for one week prior to the trials to ensure that the guts of the clams in the experimental treatments had no residual algal cells present. Four different algal species were utilised *Pavlova lutheri* (pymnesiophtye), *Tetraselmis suecica* (green flagellate) *Nitzschia longipes* (diatom) and *Navicula jeffreyi* (diatom).

Experimental treatments were established by placing four clams in plastic containers (500 ml) with seawater ($35^0/_{00}$), to which one of the four experimental algae was added. Each treatment was replicated and a control containing clams without algae was used. Clams were allowed to feed for 2 h and then removed from the water for a further 2 h. A two hour digestion period was used to mimic field preservation techniques. The clams were then injected with 10% formalin to prevent digestion. Clam guts were examined in the same manner as used for field samples to determine if the experimental algal species could still be observed in the gut contents.

7.3 Results

7.3.1 Water Samples

Excluding dinoflagellates, 66 phytoplankton species from 29 genera were identified from the water column at Duck Bay, Smithton (Appendix 5.2a). The majority of the phytoplankton species identified were benthic diatoms, with 22 of the 29 genera identified being pennate diatoms, indicating that benthic pennate diatoms are a major component of the phytoplankton assemblage at Duck Bay. Several species of dinoflagellate were also present but could not be identified. The majority of phytoplankton species in the water column at Duck Bay were epiphytic (living and growing on other aquatic plants) or epipelic (growing and living on inorganic objects) in origin. Some diatoms were present in water samples over the entire study period. These included *Amphora, Coscinodiscus,*

Cocconneis, Grammatophora oceanica, Navicula, Nitzschia, Pleurosigma, and *Synedra*. Despite the consistent appearance of these diatoms in every sampling period and the wide array of phytoplankton genera identified, the phytoplankton assemblage was primarily composed of four genera; *Amphora* (6.45%), *Cocconeis* (35.64%), *Navicula* (25.03%) and *Nitzschia* (9.68%).

7.3.2 Gut Contents

The abundance of phytoplankton species in the gut contents of *K. scalarina* was generally consistent between under and away treatments within sampling periods (Figure 7.2). However, for samples taken on the 13/11/96, there was a substantial difference between the gut contents of *K. scalarina* in under and away treatments on an abundance but not taxonomic composition basis. Phytoplankton abundance was highest in the first sampling period with a total of 463 algae identified from clams in away treatments and 397 cells from clams in under treatments. A sudden decrease in contents is evident in the subsequent sample (away 71, under 100) which displayed an increase in subsequent sample periods but did not reach the level of the initial summer sample.

Excluding dinoflagellates, 53 species of phytoplankton from 28 genera were identified in the gut contents of *K. scalarina* from Duck Bay, Smithton (Appendix 5.2b). Of the 28 genera 22 were pennate diatoms of benthic origin. The majority of the gut contents of *K. scalarina* were represented by three genera: *Cocconeis* (30.79%), *Navicula* (14.07%) and *Nitzschia* (26.03%). These three genera were also the major component of the water column phytoplankton assemblage in Duck Bay. Phytoplankton present in the gut of *K. scalarina* generally reflected the composition of the surrounding water column (Figure 7.3), although some genera appear to be more abundant in the gut contents of *K. scalarina* than their abundance in the water column would suggest e.g. *Nitzschia* and *Achnanthes*. Other species are less abundant in the gut than the water assemblage e.g. *Navicula* and *Entomeneis*. Some species are present in the water but not in the gut contents e.g. dinoflagellates indicating that *K. scalarina* may be displaying food selection, with *Achnanthes* and *Nitzschia* being preferred or favoured.

While the abundance of phytoplankton in the gut contents between sample periods varied the species composition was consistent between under and away treatments across all five sample periods (Figure 7.4-7.8). The majority of each sample was composed of *Cocconesis, Navicula,* and *Nitzschia,* but the percentage composition of the dominant species fluctuated slightly, largely accounting for the variation in

abundance of phytoplankton between sample periods. In general *Cocconesis* was the dominant genus present in the gut samples, followed by *Nitzschia* and *Navicula*, with the exception of sample 2 where *Nitzschia* was the dominate species followed by *Cocconeis*. The order of dominance generally reflected the proportion of the three major diatom genera present in the water samples. The presence or abundance of the other minor dietary phytoplankton varied between treatments but again remained consistent between under and away treatments and in general reflected the presence or abundance of phytoplankton in the water.

7.3.3 Algal Disappearance

All of the algal species used in the algal disappearance trials were present in the gut contents of *K. scalarina*. In fact the algal species used in the trials were the only recognisable items in the guts of the clams examined. Clams in control trials had empty guts with no recognisable items present indicating that the algal species observed in the guts of experimental clams were consumed during the trial and had been adequately preserved.

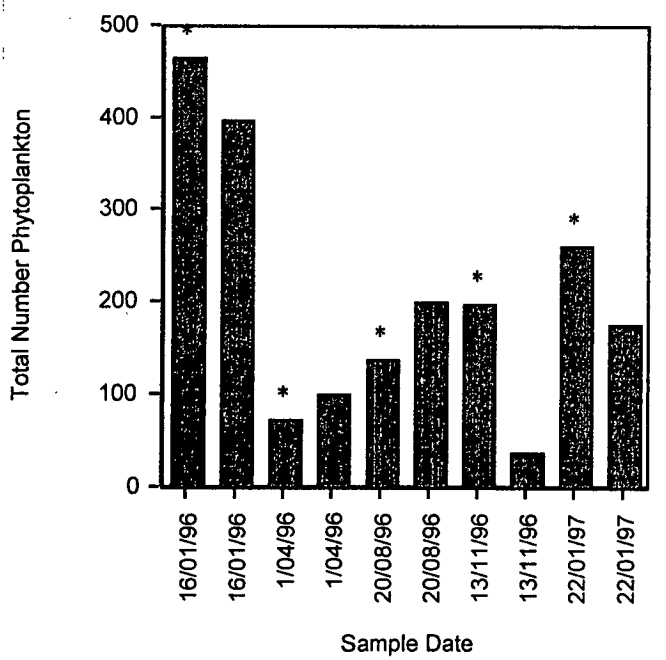

Figure 7.2: Phytoplankton abundance in the gut contents of *K. scalarina* at each sampling period. * denotes treatments away from oyster racks (n=40 clams/sample period).

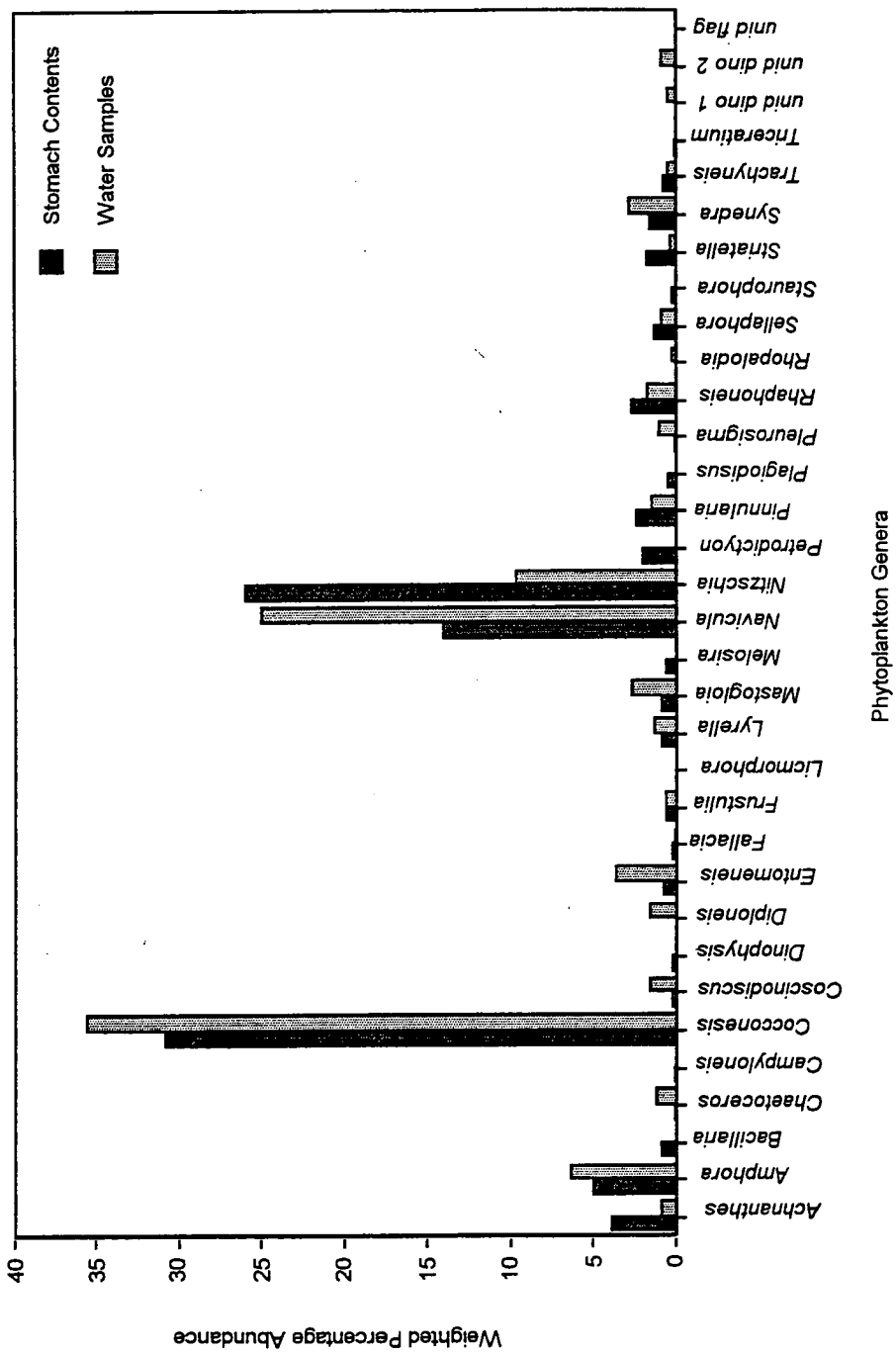

Figure 7.3: Comparison of algal composition of gut contents of *K. scalarina* and water algal assemblage.

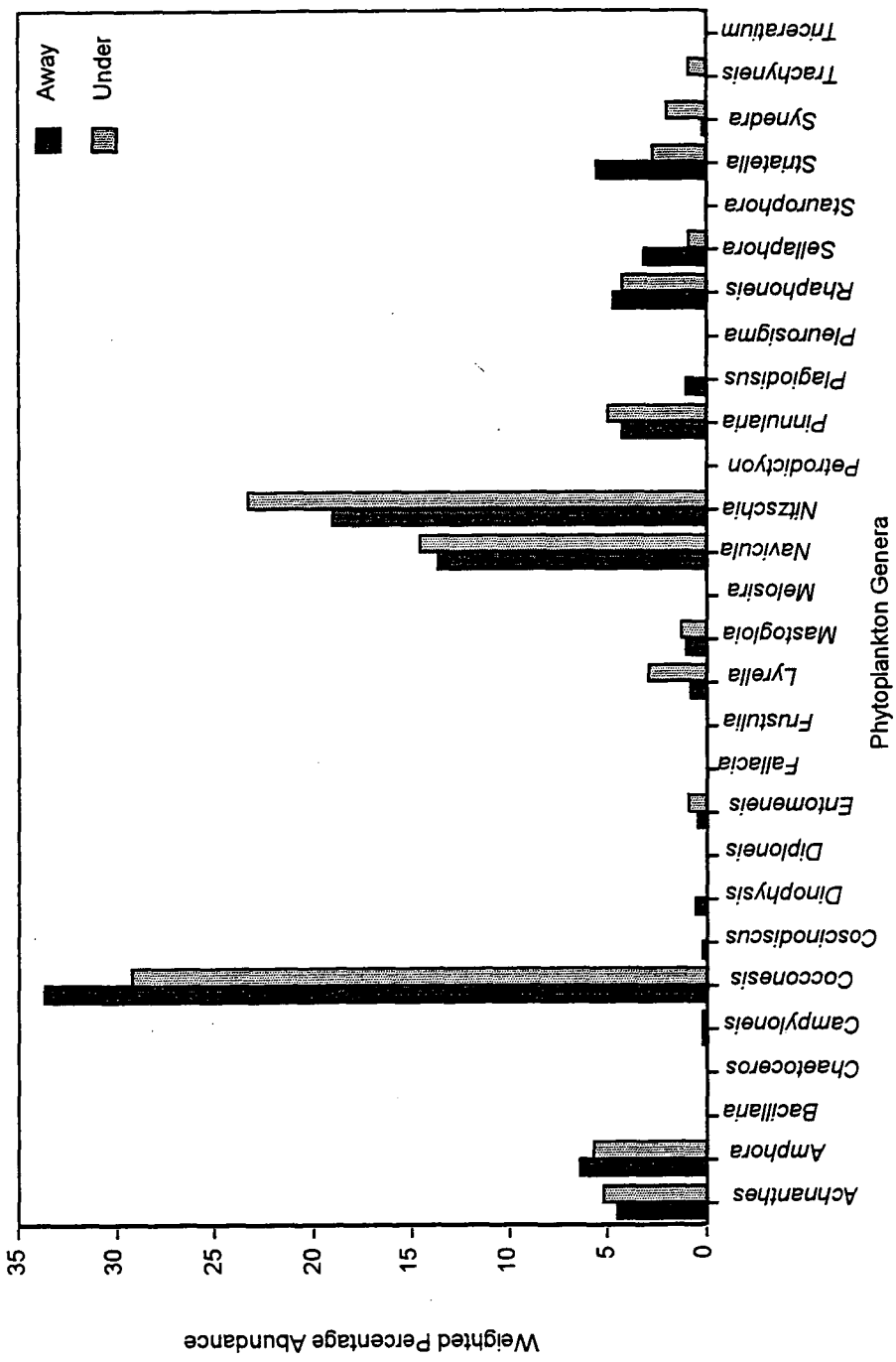

Figure 7.4: Phytoplankton identified in the gut contents of *K. scalarina* on the 16/01/96.

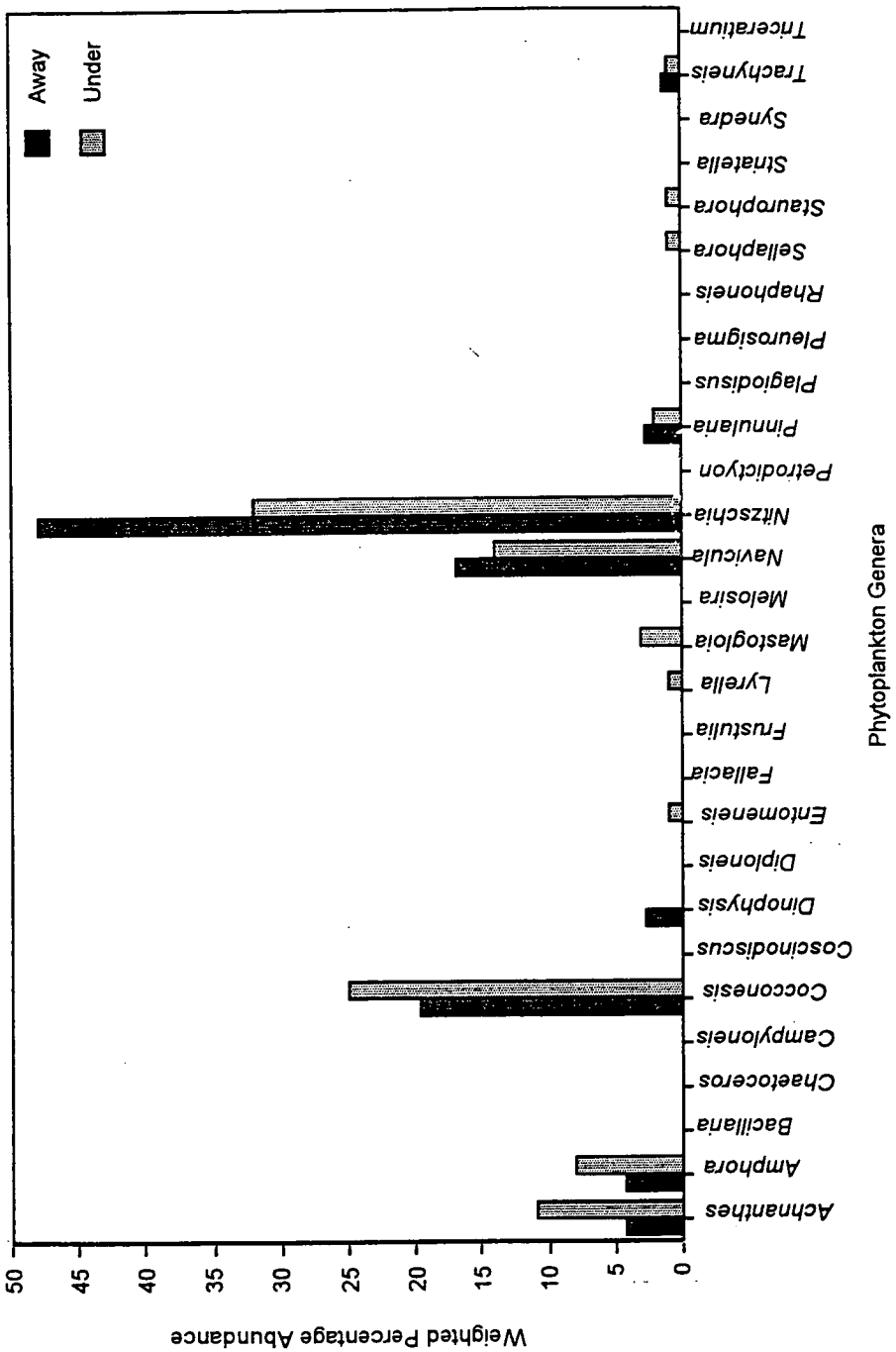

Figure 7.5: Phytoplankton identified in the gut contents of *K. scalarina* on the 1/04/96.

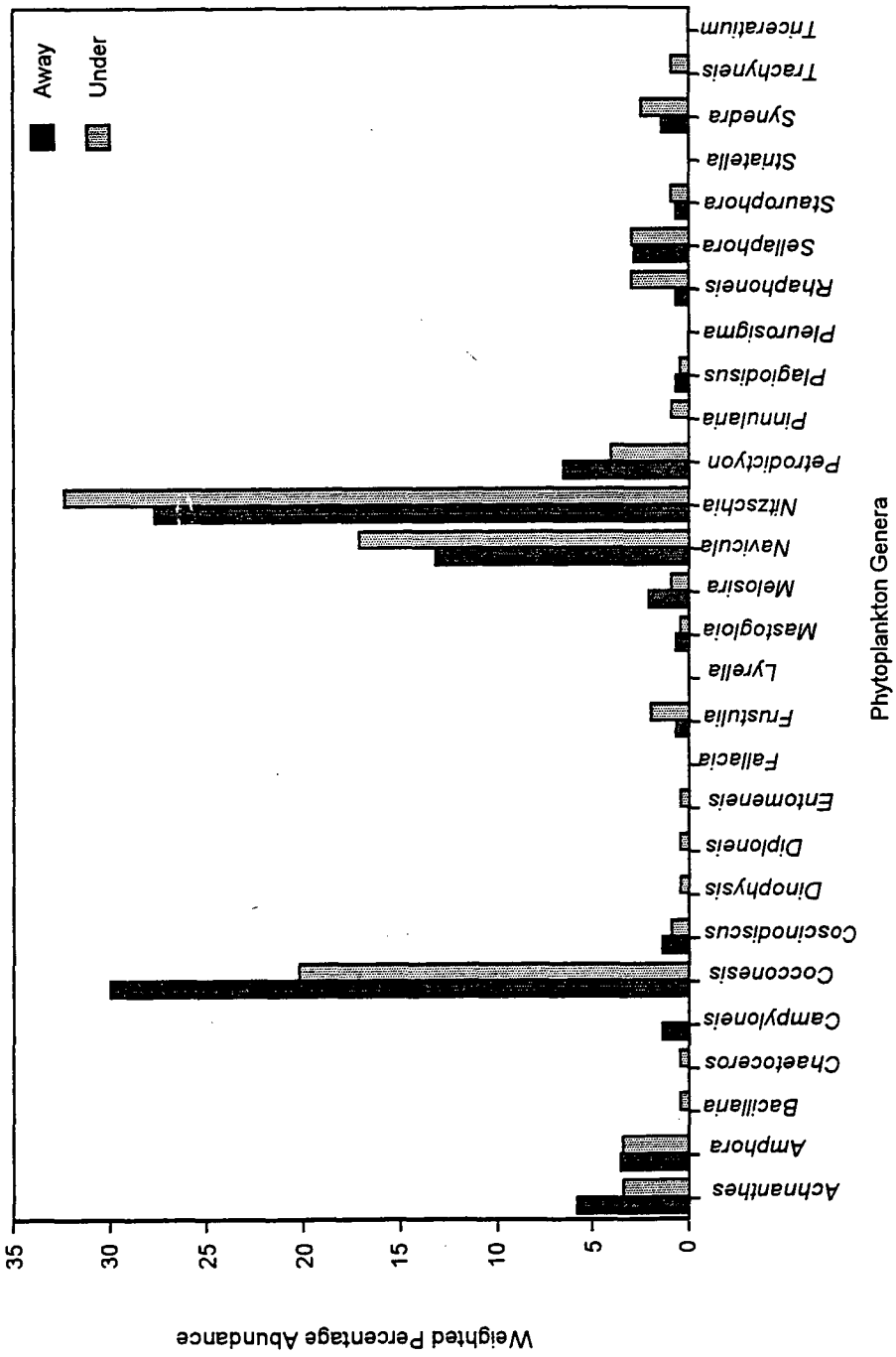

Figure 7.6: Phytoplankton identified in the gut contents of *K. scalarina* on the 20/08/96.

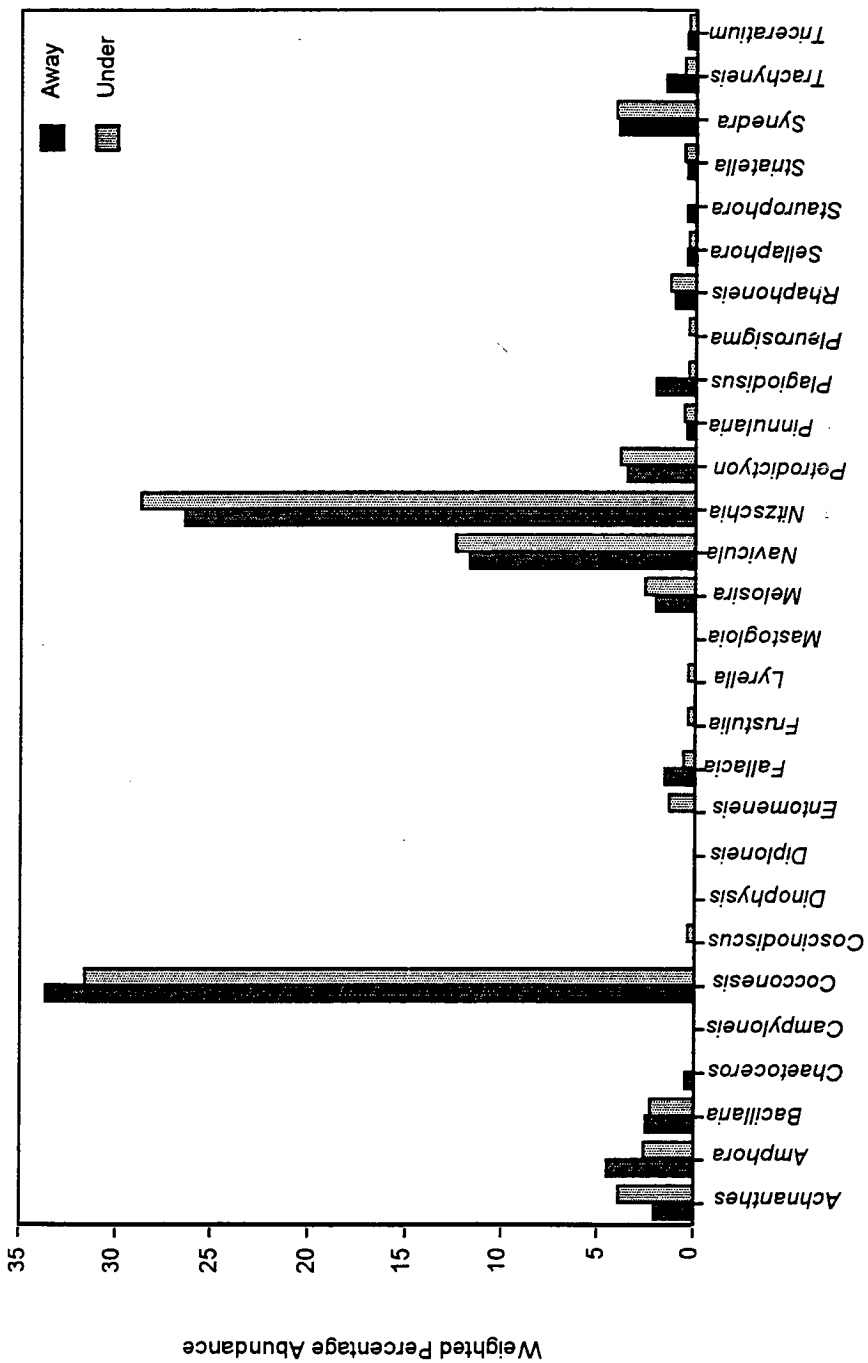

Figure 7.7: Phytoplankton identified in the gut contents of *K. scalarina* on the 13/11/96.

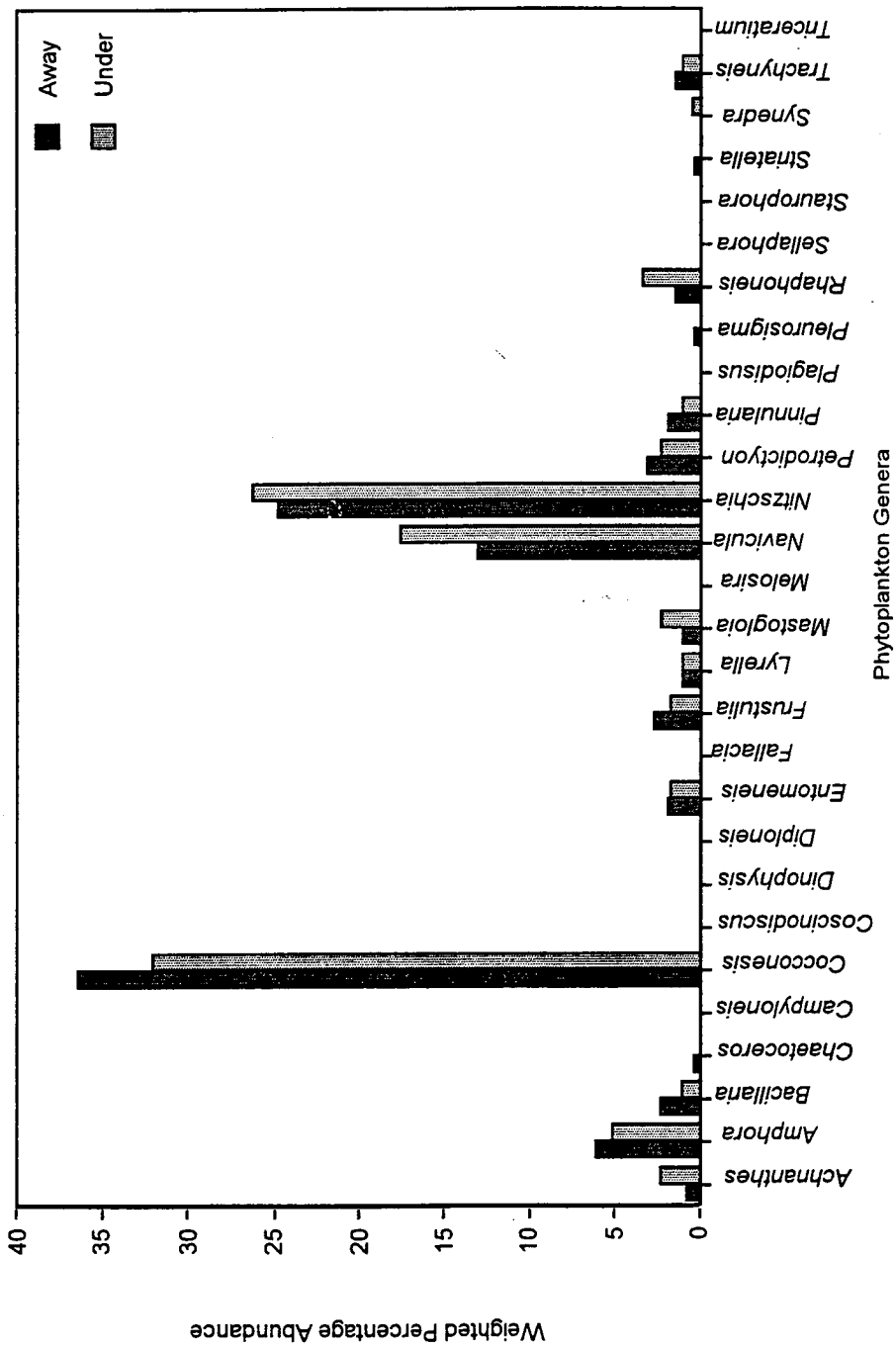

Figure 7.8: Phytoplankton identified in the gut contents of *K. scalarina* on the 22/01/97.

7.4 Discussion

The water column algal assemblage at Duck Bay, Smithton was dominated by benthic pennate diatoms, with over 90% of the species identified belonging to this group. Hallegraeff *et al.* (1986) working at Pittwater estuary, Tasmania, reports that the phytoplankton assemblage of this area is also dominated by diatoms and nanoplankton. The primary diatom component identified by Hallegraeff *et al.* (1986) was *Asterionella glacialis*, *Chaetoceros* spp., *Nitzschia closterium* and *Nitzschia pungens* with a number of benthic or resuspended phytoplankton species which included species from the genera *Amphora*, *Cocconeis, Navicula*, *Pleurosigma* and *Surirella*. Van den Enden (1994), studying another Tasmanian estuary, Little Swanport, reports a similar water column phytoplankton assemblage, dominated primarily by pennate benthic diatoms. Similarly, water samples from Duck Bay were dominated by pennate benthic diatoms from the genera *Cocconeis*, *Navicula*, and *Nitzschia*. In contrast, Hummel (1985) working in the Wadden Sea reports that the water column phytoplankton assemblage is composed primarily of centric diatoms such as *Melosira*, *Thalassiosira*, *Rhaponesis* and *Biddulphia* while the sediment assemblages is composed of pennate diatoms. The Tasmanian estuaries studied by Hallegraeff *et al.*(1986), van den Enden (1994) and this study are areas influenced by wave, tidal and current action which causes the resuspension of benthic diatoms into the water column, thus explaining their abundance in the water column.

The stomach contents of *K. scalarina* displayed a strong resemblance to the algal composition of water samples, and were dominated by benthic diatoms. Newell *et al.* (1989) report that the gut contents of *Mytilus edulis* generally reflected the phytoplankton assemblage of the surrounding water which was dominated by diatoms. Similarly, Kamermans (1994) reports that the stomach contents of *Cerastoderma edule*, *Mya arenaria* and *Mytilus edulis* appear to be a combination of material primarily from the water column with a small proportion from the sediment surface. Fifty three species from 28 genera were identified from the gut contents of *K. scalarina*. Similarly, Hummel (1985) identified 24 species of phytoplankton in the stomach of *Macoma balthica* from the Wadden Sea, of which 8 species were dominant appearing in 50 % of samples with abundances of up to 89%. *Cocconeis*, *Navicula* and *Nitzschia* were the dominant algal species in the stomach of adult *K. scalarina* comprising > 85% of samples. The prevalence of benthic diatoms in the gut contents of *K. scalarina* may be explained by surface deposits and epiphytes on shells, sediment, macrophytes and cultivation trays; being stirred into suspension by wave, current or tidal action and thus made

available to suspension-feeders in the form of seston (Shumway *et al.*, 1987). Newell *et al.* (1989) identified over 38 items from the stomach contents of *Mytilus edulis* and reports that >65% of the algal species identified were of benthic origin.

Shumway *et al.* (1987) state that some algal species, especially small forms (<10 μm,) may be quickly digested and therefore may not be evident in the stomach contents. Furthermore, much of the food may be too delicate to remain intact after digestion, such as naked and minute nanoplankton. In this study, the presence of both *P. lutheri* and *T. suecica* in the gut contents of *K. scalarina* in algal disappearance trials suggests that the absence of flagellates in the guts of field samples is not due to rapid digestion or inadequate preservation techniques. A more plausible explanation for the absence of flagellates from the gut contents of *K. scalarina* is that they comprised a relatively minor part of the phytoplankton assemblage of this area. This explanation is in part supported by the absence of flagellates in water samples.

Tsikhon- Lukanina (1982) studied the feeding of 12 species of bivalve molluscs and suggest that the main sources of food for bivalves are detritus (72%), unicellular algae (16%) and Protozoa (12%). Previous authors agree that algae, micro organisms and detritus are all potential food for bivalves although algae is regarded as a particularly good food source (Fenchel, 1972; Fenchel & Jørgensen, 1977) while detritus is thought to be of low nutritional value (Hummel, 1985). Despite the suggestions of previous authors that detritus is an important food source for bivalves, the gut contents of *K scalarina* were composed primarily of benthic diatoms with little detritus present. Detritus is likely to be digested slowly and if present would be evident. Therefore, the majority of *K. scalarina* diet appears to reflect the availability of phytoplankton species in the clams' immediate habitat. Hunt (1925) suggested that while detrital material could form a considerable proportion of the stomach contents of a variety of suspension feeders much of the material was the remains of live organisms which when unrecognisable due to the digestive processes. He concluded that diatoms were the primary food source.

Hummel (1985) reports that the stomach contents of the cockle *Macoma balthica* from the Wadden Sea also displayed a strong resemblance to the water column despite the fact that *M. balthica* is a deposit feeder. Unlike *M. balthica,* which has an extendible siphon to aid in deposit feeding, *K. scalarina* has a short siphon. Hummel (1985) reports that the stomach of *M. balthica* contained higher quantities of benthic diatoms than would be expected if the water column was the

only source of food and suggested that currents produced by the inhalant siphons of suspension feeders may have transported the particles towards their inhalant siphons. Rasmussen (1973) also observed this phenomenon for *Mya arenaria*. Shumway *et al.* (1987) report that the surface deposit layer can be stirred in to suspension and thus made available to suspension feeding animals. The predominance of benthic algae in the diet of *K. scalarina* is indicative of resuspension of the benthic layer, common in wave and current dominated areas, rather than an indication that *K. scalarina* is capable of mode switching as seen in other bivalve species. This is supported by the dominance of benthic diatoms in the phytoplankton assemblage of the water column. It is possible that *K. scalarina* relies to some extent on the sediment surface as a food source, but given the predominance of benthic phytoplankton in the water column it is difficult to make any conclusions concerning the importance of the benthic environment in the diet of *K. scalarina.*

Despite expectations that *K. scalarina* may derive some benefit in terms of additional food resources by being placed underneath existing oyster racks, due to biodeposition and pseudofaeces production, the gut contents of *K. scalarina* were consistent between under and away treatments. Stomach contents were consistent in both species composition and abundance, with the dominate species being the pennate benthic diatoms *Cocconeis*, *Navicula,* and *Nitzschia*. The limited amount of detritus in the gut of *K. scalarina* may indicate that detritus is not an important food source to this species. In fact any benefit appeared to be outweighed by the negative effect of anoxic sediments, which accumulated over the duration of the experimental period, on the survival of *K. scalarina* (Figure 1, Appendix 5.3). Newell *et al.* (1989) states that the siting of *Mytilus edulis* bottom leases should consider the quality and quantity of sestonic food available to mussels, as sites adjacent to mudflats subject to wind and wave induced resuspension of inorganic particles may be suboptimal for mussel feeding and growth. Similarly this may be the case with *K. scalarina*.

Previous authors have reported that dense assemblages of suspension filter feeders may significantly deplete phytoplankton food resources (Jensen, 1992). Mussel beds have been shown to take up large amounts of phytoplankton in the field (Frechette & Bourget, 1985b; Frechette *et al.*, 1989; Kamermans, 1993). Caddee & Hedgeman (1974) observed locally lowered values for phytoplankton biomass and primary production during the summer in an extensive mussel culture area in the western Wadden Sea, which they attributed to suspension feeding mussels. Diminished phytoplankton concentrations near mussel beds are expected to reduce

growth rates of other neighbouring suspension feeding bivalves (Kamermans, 1993). It has been suggested that tidal flat suspension feeders other than mussels can also cause a reduction of phytoplankton (Carlson *et al.*, 1984: Peterson & Black, 1991). Kamermans (1993) reports that the results of a laboratory experiment suggest that the presence of *Mytilus edulis* has a negative effect on the growth of *Cerastoderma edule*. These results are supported by data from field populations that indicate that the growth of *C. edule* was higher outside than inside a mussel bed. Similarly dense populations of clams may deplete the natural food resources of the commercially more valuable oysters.

Throughout the duration of this study Pacific oysters were collected, for identification and comparison of stomach contents, from the overlying oyster racks. The stomach contents of oysters revealed a large amount of partially digested matter, that precluded identification of algal species without the use of further cleaning techniques. As the techniques required to adequately identify algal species in oyster stomachs were not amenable to those used for the identification of clam gut contents the results of previous studies are used for the purpose of comparison between the two species. However it must be noted that the techniques used for the identification of algal species in previous studies are not identical to those used here and a degree of caution must be used when making comparisons.

Natural populations of oysters feed on phytoplankton, detritus, bacteria protozoan, zooplankton and dissolved organic nutrients from which they derive different benefits (Nell, 1992). The composition of their diet depends largely on what is available in the water (Thangavelu, 1988; Richardson, 1991; van den Enden, 1994). A number of previous studies have investigated the gut contents of natural populations of oysters at various locations around the world (see Table 7.1). The stomach contents of the oyster *Crassostrea madrasensis* from Pulicat Lake south India was characterised by 52.8% diatoms, 45.7% detritus, and 1.5 % zooplankton (Thangavelu, 1988). However, previous studies have indicated that the high percentage of detritus reported by Thangavelu (1988) is not normal and that typically the majority of oyster gut contents are composed of benthic diatoms (Richardson, 1991; van den Eden, 1994). Many of the phytoplankton species found in the gut contents of oyster species are common components of the diet of other bivalves (Thangavelu, 1988; van den Eden, 1994). Van den Eden (1994) working on *Crassostrea gigas* at Little Swanport, Tasmania, reports that 2/3 of the stomach contents were of benthic origin indicating that the benthic phytoplankton community may be a very important food resource to oysters. With the most

preferred species being from the genera *Bacillaria, Cocconeis, Grammatophora, Licmorphora, Navicula, Pleurosigma, Striatella* and *Synedra* (see Table 7.1). The majority of the species identified were also a common component of the gut contents of *K. scalarina* especially *Cocconeis* and *Navicula*. However, previous authors have reported that oysters consume phytoplankton up to 180 μm in length (Richardson, 1991; van den Enden, 1994) whereas the phytoplankton present in the gut contents of *K. scalarina* were approximately 10 μm in length. Therefore potential food competition between the two species, in terms of dominant algal species, may be avoided to a degree by size selection of food particles. Similarly, Kamermans (1994) suggests that differences in particle selection may reduce competition between species utilising the same food source.

Besides the potential for dietary overlap, farming *K. scalarina* on existing oyster leases may not be a valid option due to increased mortality of clams (Table 1, Appendix 5.4). Clams held in cages on existing oyster leases displayed mortality up to 4 times higher than clams in previous trials, regardless of their position in relation to oyster racks. The increased mortality of clams on oyster farms was attributed to sediment characteristics. The sediment on oyster leases is frequently fine grained anoxic sand which is subject to scouring by tidal currents, whereas natural populations of *K. scalarina* are predominant in sheltered areas with well oxygenated fine to medium grain sand.

Benthic diatoms dominate the gut contents of both Pacific oysters (van den Enden, 1994), Sydney rock oysters (Richardson, 1991) and *K. scalarina*. Therefore, the potential for competition for food resources exists if *K. scalarina* is grown in oyster growing areas in southern Australia. The abundance of benthic diatoms in the stomach contents and water samples suggests that *K. scalarina* is predominantly a suspension feeder rather than a deposit feeder. As such *K. scalarina* may derive little trophic benefit from being located below an oyster rack and any benefits are far outweighed by the accumulation of the anoxic sediments below the racks. As, dense beds of suspension feeding bivalves are capable of substantially decreasing the phytoplankton biomass of an area, outplanting dense beds of *K. scalarina* on existing oyster farms is unlikely to be a viable option for oyster farmers already concerned with the carrying capacity of waterways.

Table 7.1: Dominate algal assemblages of oyster stomachs from around the world.

Species	Location	Algal Species	Reference
Ostrea edulis	Wales	*Nitzschia, Rhizosolenia, Skeletonema, Navicula, Melosira, Coscinodiscus, Pleurosigma* spp.	Savage, 1925
O. edulis	France	*Cocconeis, Coscinodiscus, Melosira, Navicula, Diploneis, Grammataphora* spp.	LeRoux, (in Hendey 1964)
Saccostrea commercialis	Sydney	*Pleurosigma, Navicula, Coscinodiscus, Bacillaria, Amphora* spp.	Roughley, 1926
S. commercialis	Port Stephens	*Melosira, Thalassiosira, Nitzschia, Navicula, Amphora, Pleurosigma* spp.	Richardson, 1991
Crassostrea madrasensis	India	*Navicula, Coscinodiscus, Nitzschia, Pleurosigma, Rhizosolenia, Amphora* spp.	Thangavelu, 1988
C. gigas	Little Swanport, Tasmania	*Amphora, Bacillaria, Cocconeis, Diploneis, Dinophysis, Grammatophora, Gyrosigma, Navicula, Nitzschia, Pleurosigma, Prorocentrum, Striatella, Synedra spp.*	van den Eden 1994
C. virginica	USA	*Coscinodiscus, Melosira, Pleurosigma, Navicula, Amphora, Nitzschia* spp.	Martin, 1923

Chapter 8:

General Discussion.

Due to an abundance of high quality food in shallow coastal waters bivalve molluscs support a number of diverse fisheries around the world (Manzi, 1991). With a few exceptions, these fisheries are in decline due to the pressures of overfishing, environmental degradation and pollution (Manzi & Castagna, 1989; Hooker, 1995). However shallow coastal waters are also areas of naturally fluctuating physical and biological conditions which may cause unpredictable variations in faunal populations, thus estuarine fauna are particularly prone to over-exploitation. Populations of *K. scalarina* also display very large annual variations in abundance due to unpredictable mortality events. A knowledge of the factors causing or contributing to these events is important for natural systems, fisheries and aquaculture.

However, despite their apparent economic value and significance as a major component of southern Australian coastal environments, little research has been conducted on *Katelysia* spp., especially in regard to life history. Thus, insufficient data are presently available to assess whether the productivity of clams around Tasmania's coastline is capable of sustaining a significant commercial fishery. Before expansion is possible, fundamental research on the biology and ecology of *K. scalarina* must be conducted to complement fisheries research such as growth modelling (Riley *et al.*, unpublished data 1998). This thesis aimed to investigate various aspects of the ecology and ecophysiology of *K. scalarina* and significantly contribute to the existing knowledge to ensure that future aquaculture endeavours and fisheries management are based on a sound knowledge of the species. Secondly the study aimed to increase the knowledge of a little studied component of Australia's estuarine fauna and its role within the estuarine ecosystem.

There are a number of important environmental factors to consider in the selection of sites for clam farming that influence survival and growth (Toba *et al.*, 1992). These factors include physical factors such as tidal level, substrate type, wave exposure, temperature, and salinity and biological factors such as food availability, dispersal, predation and recruitment. All these factors will affect the profitability and sustainability of harvesting clams.

Food supply is an important factor in determining the growth rate of suspension-feeding bivalves (Seed & Suchanek, 1992). Clams feed only when submerged, so at some point along the tidal gradient the energy required for metabolism during aerial exposure will exceed that available during the feeding period (Seed & Suchanek, 1992). The effects of tidal height may be further confounded by the depletion of food resources due to the presence of large aggregations of suspension feeding bivalves at lower tidal levels (Frechette *et al.*, 1989; Peterson & Black, 1991). Density and tidal position had a significant effect on the survival and growth of *K. scalarina*, with a decrease in survival and growth evident with increasing distance from the low tide mark (Chapter 2). Clearly, lower tidal positions offer some advantage in terms of growth and survival of the species which is supported by the fact that natural populations of *K. scalarina* tend to reach their highest abundance and size in low to mid shore positions (Bellchambers, 1993). Similarly, Peterson & Black (1987, 1988b) transplanted low shore clams (*Circe lenticularis* and *Placamen gravescens*) to high shore levels in Shark Bay, Western Australia and monitored their performance. Both species grew more slowly than in their usual zone and *C. lenticularis* suffered higher mortality

While the exact mechanisms inhibiting the growth of *K. scalarina* at higher tidal positions remain unclear, both shore position and stocking density are important management variables in clam culture (Toba *et al.*, 1992). Further research is required to determine the factors determining the abundance and distribution of *K. scalarina* as these factors are important not only in terms of improving yields for aquaculture but to provide an explanation for patterns observed in natural populations and to ensure sustainable practices are implemented. However, if declining populations are to be supplemented by the addition of cultured stock, clearly stock transplanted to sites low in the intertidal zone has an increased chance of survival and increased growth rates. Conversely meat to shell ratio is improved at higher tidal levels. Pacific oyster farmers in Tasmania manipulate intertidal growing height to achieve an appropriate balance between meat and shell growth (O'Meley, 1995). Tidal exposure may also provide an explanation for the mass mortality events observed in natural populations of *K. scalarina*, especially during periods of hot weather. Clams at high tidal levels may be exposed to unfavourable weather conditions for extended periods causing mass mortality due to heat stress.

An understanding of the effects of stocking density on growth rate is essential to the success of shellfish aquaculture (Newell *et al.*, 1989). Artificially increasing the density of natural populations of *K. scalarina* has been suggested as a means of both sustaining and increasing the productivity of natural populations. The lack of pronounced density-dependent effects on the survival, growth or condition index of *K. scalarina* (Chapter 3) is encouraging for culture of this species even though shell length increase was relatively slow (average = 0.324 mm/month, Chapter 3) compared to those for the commercially farmed clams *Mercenaria mercenaria* (average = 1.19 mm/month) (Ansell 1968; Eldridge *et al.*, 1979; Menzel, 1977) and *Ruditapes philippinarium* (average = 2 mm/month) (Nosho & Chew, 1972; Quayle & Bourne, 1972; Rodde *et al.*, 1976). Similarly, tagging and shell ageing studies indicate that growth rates of *K. scalarina* within Tasmanian fishing grounds are relatively low for this low lived species (> 20 years) (Riley *et al.*, unpublished data 1988). The lack of density dependent effects in experimental cages situated low in the intertidal zone confirms the previous assessment that tidal position is likely to have a major effect on the profitability and success of marine farming. In heavily fished areas recruitment may become limited as adult populations are depleted (Peterson & Summerson, 1992) due either to lack of reproductive adults or disturbance. Enhancement or reseeding of populations may be a valid option as juvenile can be out planted at high density with no deleterious effects on survival or growth.

The absence of intra-specific competition in stocking density trials indicates that high mortality events in natural populations are not due to overcrowding, although density did influence mortality at the highest position in tidal position trials (Chapter 2, Figure 2.2) . However, the physiological stress caused by prolonged exposure to high densities may manifest itself in ways not examined in the scope of this study. *Katelysia* spp. with a history of high density may be physiologically stressed from crowding thereby enhancing their susceptibility to other stresses (Peterson & Black, 1988a). Clams exposed to high densities for prolonged periods may alter their ability to withstand or survive environmental fluxes (Peterson & Black, 1988a) such as temperature and salinity fluctuations which prevail in estuarine systems. Peterson & Black (1988a) suggested that prolonged physiological stress caused by density manipulations may also make clams more prone to pollution, disease and harmful algal blooms.

Many species of estuarine bivalves have a distribution pattern closely correlated with salinity (Castagna & Chanley, 1973). Therefore, both salinity tolerance

(Nell & Gibbs, 1986) and optimum salinity for growth (Nell & Holliday, 1988) are important environmental criteria in the selection of growing sites for bivalves. *K. scalarina* is essentially an osmo- and ionic conformer, that is intolerant of low (<25‰) or high (>50‰) salinity regimes (Chapter 4). A clear relationship between natural habitat preference and optimum aquaculture conditions is complicated by different optima for various life stages. For example, the embryos and larvae of many bivalves have narrower environmental tolerances than the adults (Nell & Holliday, 1988). However juvenile *K. scalarina* displayed a wider salinity tolerance range than adults with only salinities >50‰ causing substantial mortality.

Shumway (1977b) suggests that cell volume regulation by solute extrusion is a long term emergency phenomenon that periodic valve closure renders unnecessary under conditions of fluctuating salinity. *K. scalarina* is an ionic conformer, with the possible exception on K^+ which displays some degree of regulation. In the short term, *K. scalarina* avoids fluctuations in external salinity by shell valve closure. Davenport (1979a) reports that *M. edulis* can effectively isolate itself from low salinity by closing its valves and maintaining a relatively high osmotic concentration within the mantle fluid. Similar results were obtained for *K. scalarina* held in salinities outside its salinity tolerance range. Cell volume regulation over the tolerated salinity range appears to be controlled by fluctuations in the free amino acid pool in particular adjustments in the concentration of taurine/arginine (Chapter 5).

Under extreme conditions lowered salinity can be lethal, however more commonly sub-optimal salinity may have a detrimental effect on growth (Almada-Villela, 1984). Thus, although *K. scalarina* is capable of surviving a salinity range of 25-50‰, the zone for optimal growth may be a narrower band within this range. Results from reciprocal transplant experiments of *Mytilus edulis* suggest that differences in growth rate and maximum size between North Sea and Baltic mussels are mainly due to physiological adaptations to environmental salinity (Kautsky *et al.*, 1990). Bøhle (1972) reports that *M. edulis* exposed to various steady state salinities gradually acclimated to lowered salinity levels. In this study oxygen consumption trials indicated that *K. scalarina* undergoes a period of osmotic shock accompanied by shell closure but resumes a stable pattern of oxygen consumption after 24 h (Chapter 6). However oxygen consumption was reduced in all salinities outside 35‰. Algal clearance trials indicate that juveniles display a decrease in algal consumption in salinities >

45‰ and < 30‰. As feeding is suspended or reduced while the valves remain closed, growth rate will inevitably be depressed. Thus the major influence of salinity on growth may be due to reduce metabolic efficiency (Seed & Suchanek, 1992).

The salinity requirements of clams should be carefully considered when selecting farming sites (Malouf & Bricelj, 1989). Due to an intolerance of low salinity regimes *K. scalarina* can only be successfully grown in the low energy, high salinity areas of estuaries. Nell & Patterson (1997) report similar findings for a closely related species *K. rhytiphora*. However, these areas are often difficult to locate in the low energy environments that are used for aquaculture. Many of the bays and estuaries of Tasmania's east coast where populations of *Katelysia* are dominant are subject to periods of prolonged freshwater influx. However sites near the mouths of estuaries that have stable salinity profiles would be suitable (Nell & Patterson, 1997). The more important factors may be the periodicity and duration of salinity fluxes. *K. scalarina* exposed to low salinity exhibited an extended tolerance e.g. survived 8 days at 15‰ and if returned to 35‰ after extended exposure to low salinity survived well (Bellchambers & Maguire, unpublished data).

Although *K. scalarina* has a wide salinity tolerance range it is essentially a marine species, which may explain their natural distribution towards mouths of estuaries and avoidance of areas of freshwater input. The intolerance of *K. scalarina* to low salinity regimes may also provide an explanation for large scale mortality events that occur periodically in natural populations, especially when coupled with the additional stress of temperature (Kinne, 1964; Shumway, 1996) or high density (Peterson & Black, 1988a).

The potential for *K. scalarina* to be grown as a by-crop on existing oyster farms in Tasmania promoted the investigation of the natural food sources of the species (Chapter 7). There is interest in clam farming in the oyster industry as a means of diversification. Pacific oyster farming can be very profitable but it is vulnerable to changes in market outlook and thus could benefit from diversification (Treadwell *et al.*, 1991). In Tasmania oysters are grown primarily on intertidal racks therefore oyster farms offer not only the opportunity to diversify but potentially an ideal location for clam farming. Besides using the existing infrastructure of established marine farms these areas offer a number of additional food sources due to organic enrichment from pseudofaeces and biodeposition.

However, the stomach contents of clams situated both below existing oyster racks and away from oyster racks indicated that *K. scalarina* is a suspension feeder that relies primarily on phytoplankton present in the water column for nutrition. Therefore the possibility of competition for food resources exists if dense aggregations of *K. scalarina* are located within oyster growing areas in southern Australia. Large aggregations of suspension of shellfish may have a significant impact on nutrient and energy cycling in shallow marine ecosystems (Dame *et al.*, 1980; Carver & Mallet, 1990). Natural beds of filter feeding bivalves are known to substantially deplete particle concentrations, in particular phytoplankton abundance, in the overlying water and are thought to act as natural eutrophication control in shallow enclosed bays (Frechette & Bourget, 1985; Rice *et al.*, 1989). Thus despite initial indications that oyster farms may provide ideal location of grow out of clams it is unlikely that this will be a viable option with oyster farmers already concerned about the carrying capacity of their waterways.

Results of this study indicate that high mortality events due to starvation are unlikely *as K. scalarina* is not a specialised feeder but primarily consumes benthic phytoplankton which dominate the surrounding environment. *K. scalarina* can also be held in aquaria unfed for extended periods without mortality (pers. obs.) There are several other possible explanations for the high mortality events periodically observed in natural populations. Although predation was not a major contributor to mortality in this study previous studies have suggested that a number of guilds consume *K. scalarina* (Taylor, 1995; Chilcott, 1996; Mackinnon, 1997). However the absence of predation may be due to experiments being enclosed in cages coupled with the fact that the clams were outside the previously reported optimal prey size (Chilcott, 1996; Mackinnon, 1997). An alternative explanation is that *K. scalarina* simply expend too much of their energy reserves in spawning causing mass mortality. However this explanation is unlikely on the basis of current reproductive data for the species which indicates that changes in condition index, associated with spawning, are small relative to those for Pacific oysters which still survive spawning (Maguire *et al.*, unpublished data).

The information in this thesis has provided basic information for the establishment of aquaculture, in particular the selection of potential marine farming sites and will ensure informed management of natural populations of *Katelysia* in southern Australia. While a definitive explanation for the high mortality events observed in natural populations was not discovered the thesis has

also addressed questions concerning the factors that govern the abundance and distribution of these small suspension feeders in the estuarine environment. Ecologically, this species can attain very high densities in what are relatively unpredictable habitats. However, at times they can be prone to unfavourable combinations of biotic and abiotic factors which contribute to these ecological zones being characterised by low species diversity (Wolff, 1983; Day *et al.*, 1989).

Akberali, H. B., Marriott, K. R. M. and Trueman, E. R. 1977. Calcium utilisation during anerobiosis induced by osmotic shock in a bivalve mollusc. *Nature* **266**, 852-853.

Allen, S. E. 1989. Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford.

Almanda-Villela, P. C. 1984. The effects of reduced salinity on the shell growth of small *Mytilus edulis*. *J. Mar. Biol. Assoc. UK.* **64**, 171-182.

Ambrose, W. G. J. 1984. Increased emigration of the amphipod *Rhepoxynius abronius* (Barnard) and the polychaete *Nephtys caeca* (Fabricius) in the presence of invertebrate predators. *J. Exp. Mar. Biol. Ecol.* **80**, 67-75.

Amende, L. M. and Pierce, S. K. 1980. Free amino acid mediated volume regulation of isolated *Noetia ponderosa* red blood cells: control by Ca^{2+} and ATP. *J. Comp. Physiol.* **138**, 291-298.

Andre, C., Jonsson, P. R. and Lindegarth, M. 1993. Predation on settling bivalve larvae by benthic suspension in feeders: the role of hydrodynamics and larval behaviour. *Mar. Ecol. Prog. Ser.* **97**, 183-192.

Andre, C. and Rosenberg, R. 1991. Adult-larval interactions in the suspension-feeding bivalves *Cerastoderma edule* and *Mya arenaria*. *Mar. Ecol. Prog. Ser.* **71**, 227 - 234.

Ansell, A. D. 1968. The rate of growth of the hard clam (*Mercenaria mercenaria* (L)) throughout the geographic range. *J. Cons.* **31**, 362-408.

Armesto, J. J. and Picket, S. T. A. 1985. Experiments on disturbance in old-field plant communities: impact on species richness and abundance. *Ecology* **66**, 230-240.

Baginski, R. M. and Pierce Jr., S. K. 1975. Anaerobiosis: a possible source of osmotic solute for high salinity acclimation in marine molluscs. *J. Exp. Biol.* **62**, 589-598.

Baginski, R. M. and Pierce, S. K. 1977. The time course of intracellular free amino acid accumulation in tissues of *Modiolus demissus* during high salinity adaption. *Comp. Biochem. Physiol.* **57A**, 407-412.

Baginski, R. M. and Pierce, S. K. 1978. A comparison of amino acid accumulation during high salinity adaption with anaerobic metabolism in the ribbed mussel, *Modiolus demissus demissus*. *J. Exp. Zool.* **203**, 419-428.

Baillie, P. J. 1986. Oxygenation of intertidal estuarine sediments by benthic microalgal photosynthesis. *Estuarine Coastal Shelf Sci.* **22**, 143 - 159.

Baird, D., Evens, P. R., Milne, H. and Pienkowski, M. W. 1985. Utilisation by shorebirds of benthic invertebrate production in intertidal areas. *Oceanogr. Mar. Biol. Annu. Rev.* **23**, 575-597.

Bally, R. 1983. Intertidal zonation on sandy beaches of the west coast of South Africa. *Cah. Biol. Mar.* **24**, 83-103.

Bartberger, C. A. and Pierce Jr., S. K. 1976. Relationship between ammonia excretion rates and haemolymph nitrogenous compounds of a euryhaline bivalve during low salinity acclimation. *Biol. Bull.* **150**, 1-14.

Bayne, B. L. 1971. Oxygen consumption by three species of lamellibranch mollusc in declining ambient oxygen tension. *Comp. Biochem. Physiol.* **40A**, 955-970.

Bayne, B. L. 1973. The responses of three species of bivalve mollusc to declining oxygen tension at reduced salinity. *Comp. Biochem. Physiol.* **45A**, 793-806.

Bayne, B. L. 1975. Aspects of physiological condition in *Mytilus edulis* (L.), with special reference to the effects of oxygen tension and salinity. Proceedings of the Ninth

European Marine Biology Symposium. H. Barnes. Aberdeen, Aberdeen University Press. 213-238.

Bayne, B. L. and Newell, R. C. 1983. Physiological energetics of marine molluscs. In *The Mollusca, Physiology Part 1.* Ed. by S. M. Saleudin and K. M. Wilbur, pp 407-515. Academic Press, New York.

Bayne, B. L., Thomson, R. J. and Widdows, J. 1976. Physiology: I. In *Marine Mussels: Their Ecology and Physiology.* Ed. by B. L. Bayne, pp 1231-206. Cambridge University Press, London.

Bedford, W. B. and Anderson, J. W. 1972. The physiological response of the estuarine clam *Rangia cuneata* (Grey) to salinity I. Osmoregulation. *Physiol. Zool.* **45**, 255-260.

Bellchambers, L. M. 1993. Aspects of the ecology of *Katelysia scalarina* (Bivalvia: Veneridae), a commercially exploited cockle. unpublished Hons. thesis University of Tasmania.

Bellchambers, L. M. and Richardson, A. M. M. 1995. The effect of substrate disturbance and burial depth on the venerid clam, *Katelysia scalarina* (Lamarck, 1818). *J. Shellf. Res.* **14**, 41-44.

Berger, V. Y., Khlebovich, V. V., Kovaleva, N. M. and Natochin, Y. V. 1978. The changes of ionic composition and cell volume during adaption of molluscs (Littorina) to lowered salinity. *Comp. Biochem. Physiol.* **60A**, 447-452.

Bertness, M. D. and Grosholz, E. 1985. Population dynamics of the ribbed mussel, *Geukensia demissa*: the costs and benefits of an aggregated distribution. *Oecologia* **67**, 192-204.

Beukema, J. J. and Cadee, G. C. 1986. Zoobenthos responses to eutrophication of the Dutch Wadden Sea. *Ophelia* **26**, 55-64.

Beukema, J. J. 1993. Successive changes in distribution patterns as an adaptive strategy in the bivalve *Macoma balthica* (L.) in the Wadden Sea. *Helgol. Wiss. Meeresunters.* **47**, 287-304.

Beukema, J. J. and Flach, E. C. 1995. Factors controlling the upper and lower limits of the intertidal distribution of two *Corophium* species in the Wadden Sea. *Mar. Ecol. Prog. Ser.* **125**, 117-126.

Bishop, S. H., Ellis, L. L. and Burcham, J. M. 1983. Amino acid metabolism in molluscs. In *The Mollusca, Metabolic Biochemistry and Molecular Biomechanics.* Ed. by P. Hochachka, pp 243-327. Academic Press, New York.

Bishop, S. H., Greenwalt, D. E. and Burcham, J. M. 1981. Amino acid cycling in ribbed mussel tissues subjected to hyperosmotic shock. *J. Exp. Zool.* **215**, 277-287.

Black, R. and Peterson, C. H. 1988. Absence of preemption and interference competition for space between large suspension-feeding bivalves and smaller infaunal macroinvertebrates. *J. Exp. Mar. Biol. Ecol.* **120**, 183-198.

Blackhall, S. A. 1986. Moulting Lagoon, Proposed Game Reserve. National Parks and Wildlife Service Tasmania, Hobart, Tasmania.

Bøhle, B. 1972. Effects of adaption to reduced salinity on the filtration activity and growth of mussels (*Mytilus edulis*). *J. Exp. Mar. Biol. Ecol.* **10**, 41-49.

Boss, K. J. 1982. Mollusca. In *Synopsis and Classification of Living Organisms Vol 1.* Ed by S. P. Parker, pp . McGraw-Hill Inc., New York, NY.

Branch, G. M. 1982. The biology of limpets: Physical factors, energy flow and ecological interactions. *Oceanogr. Mar. Biol. Annu. Rev.* **19**, 235-380.

Bricelj, V. M., Malouf, R. E. and De Quillfeldt, C. 1984. Growth of juvenile *Mercenaria mercenaria* and the effect of resuspended bottom sediments. *Mar. Biol.* **84**, 167-173.

Brown, A. C. 1979. Respiration and activity in *Bullia digitalis* (Dillwyn). *South. Afr. J. Sci.* **75**, 451-452.

Brown, A. C. and Meredith, F. L. 1981. The effects of salinity changes on respiration in the sandy-beach whelk *Bullia digitalis* (Dillwyn). *Comp. Biochem. Physiol.* **69A**, 599-601.

Burton, R. F. 1983. Ionic Regulation and Water Balance. In *The Mollusca, Physiology Part 2.* Ed. by K. M. Wilbur, pp 291-352. Academic Press, New York, NY.

Buss, L. W. and Jackson, J. B. C. 1981. Planktonic food availability and suspension-feeder abundance: evidence of in situ depletion. *J. Exp. Mar. Biol. Ecol.* **49**, 151-162.

Butman, C. A. 1987. Larval settlement of soft-sediment invertebrates: the spatial scales of pattern explained by active habitat selection and the emerging role of hydrodynamical processes. *Oceanogr. Mar. Biol. Annu. Rev.* **25**, 113-165.

Butman, C. A., Grassle, J. P. and Webb, C. M. 1988. Substrate choices made by marine larvae in still water and in a flume flow. *Nature* **333**, 771-773.

Buxton, C. D., Newell, R. C. and Field, J. G. 1981. Response-surface analysis of the combined effects of exposure and acclimation temperatures on filtration, oxygen consumption and scope for growth in the oyster *Ostrea edulis*. *Mar. Ecol. Prog. Ser.* **6**, 73-82.

Cadee, G. C. 1990. Feeding traces and bioturbation by birds on a tidal flat, Dutch Wadden Sea. *Ichnos* **1**, 23-30.

Cadee, G. C. and Hegeman, J. 1974. Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea. *Neth. J. Sea. Res.* **8**, 260-291.

Carlson, D. J., Townsend, D. W., Hilyard, A. L. and Eaton, J. F. 1984. Effect of an intertidal mudflat on plankton of the overlying water column. *Can. J. Fish. Aquat. Sci.* **41**, 1523 - 1528.

Carver, C. E. A. and Mallet, A. L. 1990. Estimating the carrying capacity of a coastal inlet for mussel culture. *Aquaculture* **88**, 39-53.

Castagna, M. and Chanley, P. 1973. Salinity tolerances of some marine bivalves from inshore and estuarine environments in Virginia waters on the western mid-Atlantic coast. *Malacol.* **12**, 46-96.

Chilcott, N. 1996. Predation by Drilling Gastropods on Tasmanian Intertidal Bivalves. unpublished BSc Hons. Thesis. University of Tasmania.

Coleman, N. 1982. Population density and biomass of the bivalves *Tellina mariae* and *Katelysia rhytiphora* from a seagrass bed in Western Port, Victoria. *J. Macol. Soc. Aust.* **5**, 141-149.

Collier, A. 1959. Some observations on the respiration of the American oyster *Crassostrea virginica* (Gmelin). *Publ. Inst. Mar. Sci. Univ. Tex.* **6**, 92-108.

Connell, J. H. 1961a. Effects of competition, predation by *Thais lapillus*, and other factors on natural populations of the barnacle *Balanus balanoides*. *Ecol. Monogr.* **31**, 61 - 104.

Connell, J. H. 1961b. The influence of interspefic competition and other factors on the distribution of the benthic barnacle *Chthamalus stellatus*. *Ecology* **42**, 710-723.

Connell, J. H. 1972. Community interactions on marine rocky intertidal shores. *Annu. Rev. Ecol. Syst.* **3**, 169-192.

Conway, E. J. 1960. Principals underlying the exchange of sodium and potassium ions across cell membranes. *Physiol.* **43**, 17-41.

Creese, R. G. and Underwood, A. J. 1982. Analysis of inter- and intra-specific competition amongst intertidal limpets with different methods of feeding. *Oecologia* **53**, 337-346.

Dame, R. F. 1996. *Ecology of Marine Bivalves an Ecosystem Approach.* CRC, Boca Raton, FL.

Dame, R. F., Zingmark, R., Stevenson, H. and Nelson, D. 1980. Filter feeding coupling between the estuarine water column and benthic subsystems. In *Estuarine Perspectives.* Ed. by V. S. Kennedy, pp 521-526. Academic Press, New York.

Davenport, J. 1979a. The isolation response of mussels (*Mytilus edulis* L.) exposed to rising seawater concentrations. *J. Mar. Biol. Assoc. U.K.* **59**, 123-132.

Davenport, J. 1979b. Is *Mytilus edulis* a short term regulator? *Comp. Biochem. Physiol.* **64A**, 91-95.

Davenport, J. 1981. The opening response of mussels (*Mytilus edulis* L.) exposed to rising seawater concentrations. *J. Mar. Biol. Assoc. U.K.* **61**, 667-678.

Day, J. W. J., Hall, C. A. S., Kemp, W. M. and Yanez, A. A. 1989. *Estuarine Ecology.* Wiley Interscience, New York. NY.

Dayton, P. K. 1971. Composition disturbance and community organization. The provision and consequent utilisation of space. *Ecol. Monogr.* **41**, 357-389.

Dayton, P. K. 1984. Patch dynamics and stability of some California kelp communities. *Ecol. Monogr.* **54**, 253-289.

de Franssu, L. 1990. The world market for bivalves (Oyster-Mussel-Clam-Scallop). *Fish Utilization and Marketing Service FAO - Globefish*

de Valence, P. and Peyre, R. 1990. Clam Culture. In *Aquaculture.* Ed. by G. Barnabe, pp 388-407. Ellis Horwood, New York.

de Vooys, C. G. N. 1991. Anaerobic metabolism in sublittoral living *Mytilus galloprovincialis* in the Mediterranean - IV. role of amino acids in adaption to low salinities during anaerobiosis and aerobiosis. *Comp. Biochem. Physiol.* **100A**, 423 - 431.

De Zwaan, A. 1983. Carbohydrate metabolism in bivalves. In *Metabolic Biochemistry and Molecular Biomechanics.* Ed. by P. Hochachka, Academic Press, New York, NY.

Deaton, L. E. 1981. Ion regulation in freshwater and brackish water bivalve mollusks. *Physiol. Zool.* **54**, 109-121.

Deaton, L. E. 1992. Osmoregulation and epithelial permeability in two euryhaline bivalve molluscs: *Mya arenaria* and *Geukensia demissa. J. Exp. Mar. Biol. Ecol.* **158**, 167-177.

Deaton, L. E. 1994. Hypo-osmotic volume regulation in bivalves: protein kinase C and amino acid release. *J. Exp. Zool.* **268**, 145-150.

Deaton, L. E. and Greenberg, M. J. 1991. The adaption of bivalve molluscs to oligohaline and freshwaters phylogenetic and physiological aspects. *Malacol. Rev.* **24**, 1-18.

Deaton, L. E., Hilbish, T. J. and Koehn, R. K. 1985. hyperosmotic volume regulation in the tissues of the mussel *Mytilus edulis. Comp. Biochem. Physiol.* **80A**, 571-574.

Deaton, L. E., Hilbish, T. L. and Koehn, R. K. 1984. Protein as a source of nitrogen during hyperosmotic volume regulation in the mussel *Mytilus edulis*. *Physiol. Zool.* **56**, 609-619.

Denley, E. J. and Underwood, A. J. 1979. Experiments on factors influencing settlement, survival and growth of two species of barnacles in New South Wales. *J. Exp. Mar. Biol. Ecol.* **36**, 269-293.

Djangmah, J. S., Shumway, S. E. and Davenport, J. 1979. Effects of fluctuating salinity on the behaviour of the West African blood clam *Anadara senilis* and on the osmotic pressure and ionic concentrations of the haemolymph. *Mar. Biol.* **50**, 209-213.

Dobbinson, S. J., Barker, M. F. and Jillet, J. B. 1989. Experimental shore level transplantation of the New Zealand cockle *Chione stutchburyi*. *J. Shellf. Res.* **8**, 197 - 212.

Du Paul, W. E. and Webb, K. L. 1970. The effect of temperature on salinity induced changes in the free amino acid pool of *Mya arenaria*. *Comp. Biochem. Physiol.* **32**, 785-801.

DuPaul, W. D. and Webb, K. L. 1974. Salinity induced changes in the alanine and aspartic aminotransferase activity in three marine bivalve molluscs. *Arch. Int. Physiol. Biochim.* **82**, 817-822.

Eagle, R. A. 1975. Natural fluctuations in a soft bottom benthic community. *J. Mar. Biol. Assoc. U.K.* **55**, 865-878.

Eckman, J. E. 1987. The role of hydrodynamics in recruitment, growth and survival of *Argopevten irradians* (L.) and *Anomia simplex* (D'Orbigny) within eelgrass meadows. *J. Exp. Mar. Biol. Ecol* **106**, 165-191.

Edgar, G. J. 1997. *Australian Marine Life*. Reed Books, Kew

Edwards, S., Harris, J. O., Maguire, G. B. and Hindrum, S. M. 1997. Respirometry and energy balance of abalone under stress. Proceedings of the 4th FRDC/CRC Abalone Aquaculture Workshop. P. Hone. Port Fairy, Victoria, SARDI, Adelaide. 37-41.

Eldridge, P. J., Eversole, A. G. and Whetstone, J. M. 1979. Comparative survival and growth rates of hard clams *Mercenaria mercenaria*, planted in trays subtidally and intertidally at varying densities in a South Carolina estuary. *Proc. Nat. Shellf. Assoc.* **69**, 30-39.

Ertman, S. C. and Jumars, P. A. 1988. Effects of bivalve siphonal currents on the settlement of inert particles and larvae. *J. Mar. Res.* **46**, 797-813.

Fenchel, T. 1972. Aspects of decomposer food chains in marine benthos. *Verh. dt. zool. Ges.* **65**, 14-23.

Fenchel, T. 1975a. Factors determining the distribution patterns of mud snails (Hydrobidae). *Oecologia* **20**, 1-17.

Fenchel, T. 1975b. Character displacement and coexistence in mud snails (Hydrobidae). *Oecologia* **20**, 19-32.

Fenchel, T. M. and Jørgensen, B. B. 1977. Detritus food chains or aquatic ecosystems: the role of bacteria. *Adv. Microb. Ecol.* **1**, 1-58.

Flach, E. C. 1992. Disturbance of benthic infauna by sediment-reworking activities of the lugworm *Arenicola marina*. *Neth. J. Sea Res.* **30**, 81-89.

Frechette, M. and Bourget, E. 1985a. Energy flow between the pelagic and benthic zones; factors controlling particulate organic matter available to an intertidal mussel bed. *Can. J. Fish. Aquat. Sci.* **42**, 1158-1165.

Frechette, M. and Bourget, E. 1985b. Food-limited growth of *Mytilus edulis* in relation to the benthic boundary layer. *Can. J. Fish. Aquat. Sci.* **42**, 1166-1170.

Frechette, M., Bulman, C. A. and Geyer, W. R. 1989. The importance of boundary-layer flow processes in supplying phytoplankton to the benthic suspension-feeder *Mytilus edulis*. *Limnol. Oceanogr.* **34**, 19-36.

Gainey, L. F. 1978. The response of Corbiculidae (Mollusca: Bivalvia) to osmotic stress: the cellular response. *Physiol. Zool.* **51**, 79-91.

Galtsoff, P. S. 1964. The American Oyster. *U.S. Fish. Wildl. Serv., Bur. Comm. Fish.* **64**, 1-476.

Gee, J. M., Warwick, R. M., Davey, J. T. and George, C. L. 1985. Field experiments on the role of epibenthic predators in determining prey densities in an estuarine mudflat. *Estuarine Coastal Shelf Sci.* **21**, 429-448.

Gilles, R. 1969. Effect of various salts on the activity of enzymes implicated in amino acid metabolism. *Arch. Int. Physiol. Biochem.* **77**, 441-464.

Gilles, R. 1972. Osmoregulation in three molluscs: *Acanthochitona discrepans* (Brown), *Glycymeris Glycymeris* (L.) and *Mytilus edulis* (L.). *Biol. Bull. Mar. Biol. Lab. Woods Hole* **142**, 25-35.

Gilles, R. 1979. Intracellular organic effectors. In *Mechanisms of Osmoregulation in Animals*. Ed. by R. Gilles, pp 11-154. Wiley, New York, NY.

Grassle, J. F. and Sanders, H. L. 1973. Life histories and the role of disturbance. *Deep Sea Res.* **20**, 643-659.

Gray, J. S. 1981. *The Ecology Of Marine Sediments*. Cambridge University Press, Cambridge.

Hall, S. J., Basford, D. J., Robertson, M. R., Raffaelli, D. G. and Tuck, I. 1991. Patterns of recolonisation and the importance of pit-digging by the edible crab *Cancer pagurus* in a subtidal sand habitat. *Mar. Ecol. Prog. Ser.* **72**, 93-102.

Hall, S. J., Raffaelli, D. and Turrell, W. R. 1990. Predator-caging experiments in marine systems: A re-examination of their value. *Am. Nat.* **136**, 657-672.

Hall, S. J., Robertson, M. R., Basford, D. J. and Fryer, R. 1993. Pit-digging by the crab *Cancer pagurus*: a test for long term, large-scale effects on infaunal community structure. *J. Anim. Ecol.* **62**, 59 - 66.

Hallegraeff, G. M., Tyler, H. L. and Sumner, C. E. 1986. Phytoplankton abundance and growth of the Pacific oyster (*Crassostrea gigas*) in Pittwater (Tasmania). *unpublished*

Harvey, M. and Vincent, B. 1990. Density, size distribution, energy allocation and seasonal variations in shell and soft tissue growth at two tidal levels of a *Macoma balthica* (L.) population. *J. Exp. Mar. Biol. Ecol.* **142**, 151-168.

Hawkins, A. J. S. and Hilbish, T. J. 1992. The cost of cell volume regulation: protein metabolism during hyperosmotic adjustment. *J. Mar. Biol. Assoc. UK* **72**, 569-578.

Hendey, N. I. 1964. An introductory account of the smaller algae of British coastal waters. *Fish. Inves. Ser. IV* 1-298.

Henry, R., Mangum, C. and Webb, K. 1980. Salt and water balance in the oligohaline clam, *Rangia cuneata*. *J. Exp. Zool.* **211**, 11-24.

Henry, R. P. and Mangum, C. P. 1980. Salt and water balance in the oligohaline clam *Rangia cuneata*. III Regulation of the free amino acid pool during low salinity adaption. *J. Exp. Zool.* **211**, 25-32.

He, J. and Wei, X. 1984. A study on food and feeding habit of the clam spat. *J. Fish. China.* **8**, 99-106.

Higgins, P. J. 1980. Effects of food availability on the valve movements and feeding behavior of juvenile *Crassostrea virginica* (Gmelin). I. Valve movements and periodic activity. *J. Exp. Mar. Biol. Ecol.* **45**, 229-244.

Hilbish, T. J. 1986. Growth trajectory of shell and soft tissue in bivalves: seasonal variations in *Mytilus edulis* L. *J. Exp. Mar. Biol. Ecol.* **96**, 103-113.

Hooker, S. H. 1995. Life history and demography of the pipi - *Paphies australis* (Bivalvia: Mesodesmatidae) in northeastern New Zealand. unpublished PhD. Thesis. University of Auckland, Auckland, New Zealand.

Hoyaux, J., Gilles, R. and Jeuniaux, C. 1976. Osmoregulation in molluscs of the intertidal zone. *Comp. Biochem. Physiol.* **53A**, 361-365.

Hulberg, L. W. and Oliver, J. S. 1980. Caging manipulations in marine soft-bottom communities: Importance of animal interactions or sedimentary habitat modifications. *Can. J. Fish. Aquat. Sci.* **37**, 1130-1139.

Hummel, H. 1985. Food intake of *Macoma balthica* (Mollusca) in relation to seasonal changes in its potential food on a tidal flat in the Dutch Wadden Sea. *Neth. J. Sea. Res.* **19**, 52-76.

Hunt, J. H., Ambrose, W. G. and Peterson, C. H. 1987. Effects of the gastropod, *Ilyanassa obsoleta* (Say), and the bivalve, *Mercenaria mercenaria* (L.), on larval settlement and juvenile recruitment of infauna. *J. Exp. Mar. Biol. Ecol.* **108**, 229-240.

Hunt, O. D. 1925. The food of the bottom fauna of the Plymouth fishing grounds. *J. Mar. Biol. Assoc. U.K.* **13**, 560-599.

Hylleberg, I. and Gallucci, V. F. 1975. Selectivity in feeding by the deposit-feeding bivalve *Macoma nasuta*. *Mar. Biol.* **32**, 167-178.

Inglesias, J. I. P., Navarro, E., Alvarez-Jorna, P. and Armentia, I. 1992. Feeding, particle selection and absorption in cockles *Cerastoderma edule* (L.) exposed to variable conditions of food concentrations and quality. *J. Exp. Mar. Biol. Ecol.* **162**, 177-198.

Ivanovici, A. M., Rainer, S. F. and Wadley, V. A. 1981. Free amino acids in three species of mollusc: responses to factors associated with reduced salinity. *Comp. Biochem. Physiol.* **70A**, 17-22.

Jaramillo, E. 1987. Sandy beach macrofauna from the Chilean coast: zonation patterns and zoogeography. *Vie Milieu* **37**, 165-174.

Jaramillo, E., McLachlan, A. and Coetzee, P. 1993. Intertidal zonation patterns of macroinfauna over a range of exposed sandy beaches in south-central Chile. *Mar. Ecol. Prog. Ser.* **101**, 105-118.

Jaramillo, E., McLachlan, A. and Dugan, J. 1995. Total sample area and estimates of species richness in exposed sandy beaches. *Mar. Ecol. Prog. Ser.* **119**, 311-314.

Jensen, K. T. 1992. Dynamics and growth of the cockle, *Cerastoderma edule*, on a intertidal mud flat in the Danish Wadden Sea: effects of submersion time and density. *Neth. J. Sea Res.* **28**, 335-345.

Jensen, K. T. 1993. Density-dependent growth in cockles (*Cerastoderma edule*): evidence from interannual comparisons. *Journal of Marine Biology Association of the UK* **73**, 333 - 342.

Jensen, K. T. and Jensen, J. N. 1985. The importance of some epibenthic predators on the density of juvenile benthic macrofauna in the Danish Wadden Sea. *Journal of Experimental Marine Biology and Ecology* **89**, 157-174.

Sall, J., Ng, K. and Hecht, M. 1995. JMP Version 2.02 SAS Institute, Cary NC.

Jones, S. E. and Jago, C. F. 1993. In situ assessment of modification of sediment properties by burrowing invertebrates. *Mar. Biol.* **115**, 133-142.

Jørgensen, C. B. 1966. *Biology of Suspension Feeding.* Pergamon Press, Oxford.

Jørgensen, C. B. 1983. Patterns of uptake of dissolved amino acids in mussels (*Mytilus edulis*). *Mar. Biol.* **73**, 177-182.

Jørgensen, C. B. 1990. *Bivalve Filter Feeding: Hydrodynamics, Bioenergetics, Physiology and Ecology.* Olsen & Olsen, Fredensborg.

Jørgensen, C. B., Larsen, P. S., Møhlenberg, F. and Riisgård, H. U. 1986. The bivalve pump. *Mar. Ecol. Prog. Ser.* **34**, 69-77.

Kamermans, P. 1993. Food limitation in cockles (*Cerastoderma edule* (L.)): influences of location on tidal flat and of nearby presence of mussel beds. *Neth. J. Sea. Res.* **31**, 71-81.

Kamermans, P. 1994. Similarity in food source and timing of feeding in deposit- and suspension-feeding bivalves. *Mar. Ecol. Prog. Ser.* **104**, 63-75.

Kamermans, P., van der Veer, H. W., Karczmarski, L. and Doeglas, G. W. 1992. Competition in deposit- and suspension-feeding bivalves: experiments in controlled outdoor environments. *J. Exp. Mar. Biol. Ecol.* **162**, 113-135.

Kautsky, N., Johannesson, J. and Tedengren, M. 1990. Genotypic and phenotypic differences between Baltic and North Sea populations of *Mytilus edulis* evaluated through reciprocal transplantations. 1. Growth and morphology. *Mar. Ecol. Prog. Ser.* **59**, 203-210.

Kennedy, V. S., Roosenberg, W. H., Castagna, M. and Mihursky, J. A. 1974. *Mercenaria mercenaria* (Mollusca: Bivalvia): Temperature-time relationships for survival of embryos and larvae. *Fish. Bull.* **74**, 1160-1166.

Kent, A. C. and Day, R. W. 1983. Population dynamics of an infaunal polychaete: the effect of predators and an adult-recruit interaction. *J. Exp. Mar. Biol. Ecol.* **73**, 185-203.

Ketchum, B. H. 1983. Estuaries and Enclosed Seas. In *Ecosystems of the World.* Ed. by D. W. Goodall, pp 500. Elsevier Scientific Publishing Company, New York, NY.

Kinne, O. 1964. The effects of temperature and salinity on marine and brackish-water animals. II Salinity and temperature-salinity combinations. *Oceanogr. Mar. Biol. Annu. Rev.* **2**, 281-339.

Kinne, O. 1971. Salinity: animals - invertebrates. In *Marine Ecology.* Ed. by O. Kinne, pp 821-995. Wiley Interscience, London.

Kiørboe, T., Møhlenberg, F. and Nøhr, O. 1981. Effect of suspended bottom material on growth and energetics in *Mytilus edulis*. *Mar. Biol.* **61**, 283-288.

Langdon, C. J. and Newell, R. I. E. 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa*. *Mar. Ecol. Prog. Ser.* **58**, 299-310.

Lange, R. 1963. The osmotic function of amino acids and taurine in the mussel, *Mytilus edulis*. *Comp. Biochem. Physiol.* **10**, 173-179.

Lange, R. 1972. Some recent work on osmotic, ionic, and volume regulation in marine animals. *Oceanogr. Mar. Biol. Annu. Rev.* **10**, 97-137.

Last, P. 1983. Ecology and zoogeography of Tasmania shore fishes. unpublished PhD. Thesis. University of Tasmania, Hobart, Tasmania.

Lee, S. Y. 1996. Distribution pattern and interaction of two infaunal bivalves, *Tapes philippinarum* (Adams and Reeve) and *Anomalocardia squamosa* (Linnaeus) (Bivalvia: Veneridae). *J. Exp. Mar. Biol. Ecol.* **201**, 253-273.

Lehman, L. E., Burchman, J. M., Paynter, K. T. and Bishop, S. H. 1985. Amino acid metabolism in euryhaline bivalves: Regulation of glycine accumulation in ribbed mussel gills. *J. Exp. Zool.* **233**, 347-358.

Levin, L. A. 1984. Life history and dispersal patterns in a dense infaunal polychaete assemblage: community structure and response to disturbance. *Ecology* **65**, 1185-2000.

Levin, S. 1981. The role of theoretical ecology in the description and understanding of populations in heterogeneous environments. *Am. Zool.* **21**, 865-875.

Levinton, J. 1972. Stability and trophic structure in deposit-feeding suspension-feeding communities. *Am. Nat.* **106**, 472 - 486.

Levinton, J. S. 1977. Ecology of shallow water deposit-feeding communities, Quisset Harbour, Massachusetts. In *Ecology of Marine Benthos*. Ed. by B. C. Coull, pp 191-227. University of South Carolina Press, Columbia.

Livingstone, D. R., Widdows, J. and Fieth, P. 1979. Aspects of nitrogen metabolism of the common mussel *Mytilus edulis*: adaption to abrupt and fluctuating changes in salinity. *Mar. Biol.* **53**, 41-55.

Loo, L. O. 1992. Filtration, assimilation, respiration and growth of *Mytilus edulis* L. at low temperatures. *Ophelia* **35**, 123-131.

Lukanina, V. V. and Gurina, V. I. 1977. Adaptive response of White Sea mussels to changes in salinity and temperature. *Physiol. Ecol.* 118-121.

Mackinnon, C. 1997. Impact of the Introduced European Green Crab, *Carcinus maenas* on Tasmanian Bivalve Populations. unpublished BSc Hons. thesis University of Tasmania, Hobart, Tasmania.

Maguire, G. 1991. Small clams and cockles - are they valuable for fisheries and aquaculture ? Austasia Aquaculture Magazine. **5:** 48 - 49.

Maguire, G. 1992. What does the public think of oyster farms? Austasia Aquaculture Magazine. **6:** 45-46.

Maguire, G. B., Edwards, S., Hindrum, S. M., Harris, J. O. and Bellchambers, L. M. submitted. Bioassay, digestibility and respirometry systems for use with juvenile greenlip abalone *Haliotis laevugata*. *J. Shellf. Res.*

Mahasneh, D. and Pora, E. 1981. The effect of salinity on the osmotic and ionic regulation in *Mytilus galloprovincialis* and *Modiolus auriculatus*. *Tethys* **10**, 129-134.

Maidment, D. 1997. Technical notes about the present developments of clam (*Tapes doratus* and *Katelysia rhytiphora*) and flat oyster (*Ostrea angasi*) farming in New South Wales. Proceedings of the Field Day 1997, NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW.

Malouf, R. E. and Bricelj, V. M. 1989. Comparative biology of clams: environmental tolerances, feeding, and growth. In *Clam Mariculture in North America*. Ed. by J. J. Manzi and M. Castagna, pp 23-75. Elsevier, Amsterdam.

Mane, U. H. 1975. Oxygen consumption of the clam, *Katelysia opima* in relation to environmental conditions. *Brotéria* **44**, 33-58.

Manzi, J. J. 1991. Clams, Cockles and Arkshells. In *Production of Aquatic Animals Crustaceans, Molluscs, Amphibians and Reptiles*. Ed. by C. E. Nash, pp 139-157. Elsevier, Amsterdam.

Manzi, J. J. and Castagna, M. 1989. Introduction. In *Clam Mariculture in North America*. Ed. by J. J. Manzi and M. Castagna, pp 1-21. Elsevier, New York, NY.

Martin, G. W. 1923. Food of the oyster. *Bot. Gazette* **75**, 143-169.

Matsushima, O., Katayama, H. and Yamada, K. 1987. The capacity for intracellular osmoregulation mediated by free amino acids in three bivalve molluscs. *J. Exp. Mar. Biol. Ecol.* **109**, 93-99.

McGrorty, S., Clarke, R. T., Reading, C. J. and Goss-Custard, J. D. 1990. Population dynamics of the mussel *Mytilus edulis*: density changes and regulation of the population in the Exe estuary, Devon. *Mar. Ecol. Prog. Ser.* **67**, 157-169.

McHugh, J. L. 1976. Estuarine fisheries: are they doomed? In *Estuarine Processes Vol 1: Uses, Stresses, and Adaptations to the Estuary*. Ed. by M. Wiley, pp 15-27. Academic Press, New York.

McLachlan, A. 1983. Sandy beach ecology - a review. In *Sandy Beaches as Ecosystems*. Ed. by A. McLachlan and T. Erasmus, pp 321-380. The Hauge, Netherlands.

McLusky, D. S. 1981. *The Estuarine Ecosystem*. Thomson Litho Ltd., East Kilbride.

McLusky, D. S. and Elliot, M. 1981. The feeding and survival strategies of estuarine molluscs. In *Feeding and Survival Strategies of Estuarine Organisms*. Ed. by N. V. Jones and W. J. Wolff, pp 109-121. Plenum Press, New York.

McMahon, R. F. 1988. Respiratory response to periodic emergence in intertidal molluscs. *Am. Zool.* **28**, 97-114.

Menge, B. A. and Sutherland, J. P. 1987. Community regulation: variation in disturbance, competition , and predation in relation to environmental stress and recruitment. *Am. Nat.* **130**, 730-757.

Menzel, R. W. 1977. Selection and hybridization in quahog clams. *Proc. WMS* **VIII**, 507-521.

Møller, P. 1986. Physical factors and biological interactions regulating infauna in shallow boreal areas. *Mar. Ecol. Prog. Ser.* **30**, 33-47.

Monismith, S. G., Koseff, J. R., Thompson, J. K., O'Riordon, C. A. and Nepf, H. M. 1990. A study of model bivalve siphonal currents. *Limnol. Oceanogr.* **35**, 680-696.

Morrisey, D. 1996. Estuaries. In *Coastal Marine Ecology of Temperate Australia*. Ed. by A. J. Underwood and M. G. Chapman, pp 152-170. UNSW Press, Sydney.

Morton, B. 1983. Feeding and Digestion in Bivalvia. In *The Mollusca, Physiology, Part 2*. Ed. by A. S. M. Saleuddin and K. M. Wilbur, pp 65-148. Academic Press, New York.

Nell, J. A. 1992. Oyster Nutrition. Proceedings of Aquaculture Nutrition Workshop. G. L. Allan and W. Dall. NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, Australia.

Nell, J. A. and Dunkley, P. R. 1984. Effects of temperature, nutritional factors and salinity on the uptake of L-Methionine by the Sydney rock oyster *Saccostrea commercialis*. *Mar. Biol.* **80**, 335-339.

Nell, J. A. and Gibbs, P. J. 1986. Salinity Tolerance and Absorbtion of L-Methionine by some Australian Bivalve Molluscs. *Aust. J. Mar. Freshwater Res.* **37**, 721-727.

Nell, J. A. and Holliday, J. E. 1988. Effects of salinity on the growth and survival of Sydney Rock Oyster (*Saccostrea commercialis*) and Pacific Oyster (*Crassostrea gigas*) larvae and spat. *Aquaculture* **68**, 39-44.

Nell, J. A. and Patterson, K. J. 1997. Salinity studies on the clams *Katelysia rhytiphora* (Lamy) and *Tapes dorsatus* (Lamarck). *Aquaculture Res.* **28**, 115-119.

Neufeld, D. S. and Wright, S. H. 1996. Response of cell volume in *Mytilus* gill to acute salinity change. *J. Exp. Biol.* **199**, 473-484.

Newell, C. R. and Shumway, S. E. 1993. Grazing of natural particulates by bivalve molluscs: a spatial and temporal perspective. In *Bivalve Filter Feeders in Estuarine and Coastal Ecosystem Processes*. Ed. by R. F. Dame, pp 85-148. Spriger-Verlag, Berlin.

Newell, C. R., Shumway, S. E., Cucci, T. L. and Selvin, R. 1989. The effects of natural seston particle size and type on the feeding rates, feeding selectivity and food resource availability for the mussel *Mytilus edulis* Linnaeus, 1758 at bottom culture sites in Maine. *J. Shellf. Res.* **8**, 187-196.

Newell, R. C. 1970. *The Biology of Intertidal Animals*. Logos Press, London.

Newell, R. C. 1973. Factors affecting the respiration of intertidal invertebrates. *Am. Zool.* **13**, 513-528.

Newell, R. C. 1979. *Biology of Intertidal Animals*. Marine Ecological Surveys, Faversham, Kent.

Newell, R. C. and Branch, G. M. 1980. The influence of temperature on the maintenance of metabolic energy balance in marine invertebrates. *Adv. Mar. Biol.* **17**, 329-396.

Nielsen, B. J. 1963. Studies of the genus *Katelysia* Romer 1857 (Mollusca, Lamellibranchiata). *Mem. Nat. Mus. Vic.* **26**, 219-257.

Nosho, T. Y. and Chew, K. K. 1972. The setting and growth of the Manila clam *Venerupis japonica* (Deshayes), in Hood Canal, Washington. *Proc. Natl. Shellf. Assoc.* **62**, 50-58.

Nossier, M. A. 1986. Ecophysiological responses of *Cerastoderma edule* (L.) and *C. glaucum* (Bruguiere) to different salinity regimes and exposure to air. *J. Moll. Stud.* **52**, 110-119.

O'Meley, C. M. 1995. Effects of shell abrasion and aerial exposure on the performance of Pacific oysters *Crassostrea gigas* (Thunberg, 1793) cultured in Tasmania, Australia. unpublished M.Appl.Sc. Thesis. University of Tasmania.

Olafsson, E. B. 1986. Density dependence in suspension-feeding and deposit-feeding populations of the bivalve *Macoma balthica*: a field experiment. *J. Anim. Ecol.* **55**, 517-526.

Olafsson, E. B. and Persson, L. 1986. The interaction between *Nereis diversicolor* O.F. Muller and *Corophium volutator* Pallas as a structuring force in a shallow brackish environment. *J. Exp. Mar. Biol. Ecol.* **103**, 103-117.

Olafsson, E. B., Peterson, C. H. and Ambrose, W. G. 1994. Does recruitment limitation structure populations and communities of macro-invertebrates in marine soft sediments: the relative significance of pre- and post-settlement processes. *Oceanogr. Mar. Biol. Annu. Rev.* **32**, 65-109.

Oliver, J. S. and Slattery, P. N. 1985. Destruction and opportunity on the sea floor: effects of gray whale feeding. *Ecology* **66**, 1965-1975.

Oliver, J. S., Slattery, P. N., Silberstein, M. A. and O'Connor, E. F. 1984. Gray whale feeding on dense ampeliscid amphipod communities near Bamfield, British Columbia. *Can. J. Zool.* **62**, 41-49.

Paine, R. T. 1966. Food web complexity and species diversity. *Am. Nat.* **100**, 65-75.

Pamatmat, M. M. 1983. Measuring aerobic and anaerobic metabolism of benthic infauna under natural conditions. *J. Exp. Zool.* **228**, 405-413.

Patterson, K. J. and Nell, J. A. 1997. Effect of different growing techniques and substrate types on he growth and survival of the clams *Tapes dorsatus* (Lamarck) and *Katelysia rhytiphora*. *Aquaculture Res.* **28**, 707-715.

Peterson, C. H. 1979. The importance of predation and competition in organising the intertidal epifaunal communities of Barnegat Inlet, New Jersey. *Oecologia* **39**, 1-24.

Peterson, C. H. 1980. Approaches to the study of competition in benthic communities in soft sediments. In *Estuarine Perspectives*. Ed. by V. S. Kennedy, pp 291-302. Academic Press, New York, NY.

Peterson, C. H. 1982a. The importance of predation and intra- and interspeific competition in the population biology of two infaunal suspension feeding bivalves, *Protothaca staminea* and *Chione undatella*. *Ecol. Monogr.* **52**, 437-475.

Peterson, C. H. 1982b. Clam predation by whelks (*Busycon* spp.): Experimental tests of the importance of prey size, pry density, and seagrass cover. *Mar. Biol.* **66**, 159-170.

Peterson, C. H. 1992. Competition for food and its community-level implications. *Benthos Res.* **42**, 1-11.

Peterson, C. H. and Andre, S. V. 1980. An experimental analysis of interspecific competition among marine filter feeders in a soft-sediment environment. *Ecology* **61**, 129-139.

Peterson, C. H. and Beal, B. F. 1989. Bivalve growth and higher order interactions: importance of density, site and time. *Ecology* **70**, 1390 - 1404.

Peterson, C. H. and Black, R. 1987. Resource depletion by active suspension feeders on tidal flats: Influence of local density and tidal elevation. *Limnol. Oceanogr.* **32**, 143-166.

Peterson, C. H. and Black, R. 1988a. Density- dependent mortality caused by physical stress interacting with biotic history. *Am. Nat.* **131**, 257-270.

Peterson, C. H. and Black, R. 1988b. Responses of growth to elevation fail to explain vertical zonation of suspension feeding bivalves on a tidal flat. *Oecologia* **76**, 423-429.

Peterson, C. H. and Black, R. 1991. Preliminary evidence for progressive sestonic food depletion in incoming tide over a broad tidal sand flat. *Estuarine Coastal Shelf Sci.* **32**, 405-413.

Peterson, C. H. and Black, R. 1993. Experimental tests of the advantages and disadvantages of high density for two coexisting cockles in a southern ocean lagoon. *J. Anim. Ecol.* **62**, 614-633.

Peterson, C. H., Irlandi, E. A. and Black, R. 1994. The crash in suspension-feeding bivalve populations (*Katelysia* spp.) in Princess Royal Harbour: an unexpected consequence of eutrophication. *J. Exp. Mar. Biol. Ecol.* **176**, 39-52.

Peterson, C. H. and Summerson, H. C. 1992. Basin-scale coherence of population dynamics of an exploited marine invertebrate, the bay scallop: implications of recruitment limitation. *Mar. Ecol. Prog. Ser.* **90**, 257-272.

Pierce, S. K. 1970. The water balance of *Modiolus* (Mollusca: Bivalvia: Mytilidae): osmotic concentrations in changing salinities. *Comp. Biochem. Physiol.* **36**, 521-533.

Pierce, S. K. 1971. Volume regulation and valve movements by marine mussels. *Comp. Biochem. Physiol.* **39A**, 103-117.

Pierce, S. K. and Amende, L. M. 1981. Control mechanisms of amino acid-mediated cell volume regulation in salinity- stressed molluscs. *J. Exp. Zool.* **215**, 247-257.

Pierce, S. K. and Greenberg, M. J. 1972. The nature of cellular volume regulation in a marine bivalve. *J. Exp. Biol.* **57**, 681-692.

Pierce, S. K., Rowland-Faux, L. M. and O'Brien, S. M. 1992. Different salinity tolerance mechanisms in Atlantic and Chesapeake conspecific oysters: glycine betaine and amino acid pool variations. *Mar. Biol.* **113**, 107-115.

Pimentel, R. A. and Smith, J. D. 1990. Biostat II: A multivariate toolbox. Placentia, CA.

Posey, M. 1987. Effects of lowered salinity on activity of the ghost shrimp, *Callianassa californiensis*. *Northwest Sci.* **61**, 93-96.

Pritchard, D. W. 1967. What is an estuary: physical viewpoint. In *Estuaries*. Ed. by G. Lauff, pp 3-5. AAAS, Washington DC.

Quammen, M. L. 1984. Predation by shorebirds, fish and crabs on invertebrates in intertidal mudflats: an experimental test. *Ecology* **65**, 529-537.

Quayle, D. B. and Bourne, N. 1972. The clam fisheries in British Columbia. *Fish. Res. Bd. Canada* **179**, 70.

Raffaelli, D., Falcy, V. and Galbraith, C. 1990. Eider predation and the dynamics of mussel-bed communities. In *Trophic Relationships in the Marine Environment*. Ed. by M. Barnes and R. N. Gibson, pp 157-169. Aberdeen University Press, Aberdeen.

Raffaelli, D. and Hawkins, S. 1996. *Intertidal Ecology*. Chapman & Hall, London.

Raffaelli, D. and Milne, H. 1987. An experimental investigation of the effects of shorebird and flatfish predation on estuarine invertebrates. *Estuarine Coastal Shelf Sci.* **24**, 1-13.

Rasmussen, E. 1973. Systematics and ecology of the Isefjord marine fauna (Denmark). *Ophelia* **11**, 1-495.

Reise, K. 1985. *Tidal Flat Ecology : An Experimental Approach to Species Interactions*. Springer-Verlag, Berlin.

Rhodes, D. C. 1974. Organism-sediment relations on the muddy sea floor. *Oceanogr. Mar. Biol. Annu. Rev.* **12**, 263-300.

Rhodes, D. C., McCall, P. L. and Yingst, J. Y. 1978. Disturbance and production on the estuarine seafloor. *Am. Sci.* **66**, 577-586.

Rhodes, D. C. and Young, D. K. 1970. The influence of deposit feeding organisms on sediment stability and community trophic structure. *J. Mar. Res.* **28**, 150-178.

Rice, M. A., Hickok, C. and Zehra, I. 1989. Effects of intensive fishing effort on the population structure of Quahogs, *Mercenaria* in Narragansett Bay. *J. Shellf. Res.* **8**, 345 - 354.

Richardson, D. L. 1991. Some Aspects of Food and Feeding in the Sydney Rock Oyster *Saccostrea commercialis* (Iredale & Roughley) in Port Stephens, NSW. unpublished B Appl.Sc Hons. Thesis. University of New England, Armidale, NSW.

Riisgård, H. U. 1991. Filtration rate and growth in the blue mussel, *Mytilus edulis* Linneaus, 1758: dependence on algal concentration. *J. Shellf. Res.* **10**, 29-35.

Roberts, D. 1981. The effects of substrate and shell form on burrowing in the Genus *Katelysia* (Bivalvia: Veneridae). *J. Malacol. Soc. Aust.* **5**, 33-40.

Roberts, D. 1983. The genus *Katelysia* (Bivalvia: Veneridae) in southern Australia. *J. Malacol. Soc. Aust.* **6**, 191-204.

Rodde, K. M., Sunderlin, J. B. and Roels, O. A. 1976. Experimental cultivation of *Tapes japonica* (Deshayes) (Bivalvia: Veneridae) in an artificial upwelling culture system. *Aquaculture* **9**, 203-215.

Rodhouse, P. G. 1978. A note on the energy budget for an oyster population in a temperate estuary. *J. Exp. Mar. Biol. Ecol.* **37**, 205-212.

Roegner, G. C. and Mann, R. 1995. Early recruitment and growth of the American oyster *Crassostrea virginica* (Bivalvia: Ostreidae) with respect to tidal zonation and season. *Mar. Ecol. Prog. Ser.* **117**, 91-101.

Roughly, T. C. 1926. An investigation of the cause of an oyster mortality on the Georges River, N.S.W. *Proc. Linnean Soc. NSW.* **51**, 473-481.

Round, F. E., Crawford, R. M. and Mann, D. E. 1990. *The Diatoms. Biology and Morphology of the Genera.* Cambridge University Press. Cambridge.

Sadok, S., Uglow, R. F. and Haswell, S. J. 1997. Haemolymph and mantle fluid ammonia and ninhydrin positive substances variations in salinity-challenged mussels (*Mytilus edulis* L.). *J. Exp. Biol. Ecol.* **211**, 195-212.

Sanchez-Salazar, M. E., Griffiths, C. L. and Seed, R. 1987. The interactive roles of predation and tidal elevation in structuring populations of the edible cockle, *Cerastoderma edule*. *Estuarine Coastal Shelf Sci.* **25**, 245-260.

Savage, R. C. 1925. Food of the oyster. *Fishery Investigation, Series II* **8**, 1-50.

Savidge, W. B. and Taghon, G. L. 1988. Passive and active components of colonisation following two types of disturbance on an intertidal sandflat. *J. Exp. Mar. Biol. Ecol.* **115**, 137-155.

Scarratt, A. 1994. The effect of changes in quality and quantity of food on the feeding behaviour of the soft shelled clam, *Mya arenaria*, using a new technique to determine gut retention time. unpublished Masters Thesis. Memorial University of Newfoundland.

Schoffeniels, E. and Gilles, R. 1972. Ionoregulation and osmoregulation in molluscs. In *Chemical Zoology*. Ed. by M. Florkin and B. T. Scheer, pp 393-420. Academic Press, New York.

Schulte, E. H. 1975. Influence of algal concentration and temperature on the filtration rate of *Mytilus edulis*. *Mar. Biol.* **30**, 331-341.

Seed, R. 1993. Invertebrate predators and their role in structuring coastal and estuarine populations of filter feeding bivalves. In *Bivalve Filter Feeders in Estuarine and Coastal Ecosystem Processes*. Ed. by R. Dame, pp 149-195. Springer-Verlag, Heidelberg.

Seed, R. and Suchanek, T. H. 1992. Population and community ecology of *Mytilus*. In *The Mussel Mytilus: Ecology, Physiology, Genetics and Culture*. Ed. by E. Gosling, pp 87-169. Elsevier, Amsterdam.

Shumway, S. E. 1977a. Effect of salinity fluctuation on the osmotic pressure and Na^+ Ca^{2+} and Mg^{2+} ion concentrations in the hemolymph of bivalve molluscs. *Mar. Biol.* **41**, 153-177.

Shumway, S. E. 1977b. The effect of fluctuating salinity on the tissue water content of eight species of bivalve mollusc. *J. Comp. Physiol. B* **116**, 269-285.

Shumway, S. E. 1979. The effects of fluctuating salinity on respiration in gastropod molluscs. *Comp. Biochem. Physiol.* **63A**, 279-283.

Shumway, S. E. 1981. Factors affecting oxygen consumption in the marine pulmonate *Amphibola crenata* (Gmelin, 1791). *Biol. Bull.* **160**, 332-347.

Shumway, S. E. 1982. Oxygen consumption in oysters: an overview. *Mar. Biol. Lett.* **3**, 1-23.

Shumway, S. E. 1996. Natural Environmental Conditions. In *The Eastern Oyster Crassostrea virginica.* Ed. by V. S. Kennedy, I. E. Newell and A. F. Eble, pp 467-513. Maryland Sea Grant College, College Park, Maryland, MD.

Shumway, S. E., Gabbott, P. A. and Youngson, A. 1977c. The effect of fluctuating salinity on the concentrations of free amino acids and ninhydrin-positive substances in the adductor muscles of eight species of bivalve molluscs. *J. Exp. Mar. Biol. Ecol.* **29**, 131-150.

Shumway, S. E. and Koehn, R. K. 1982. Oxygen consumption in the American oyster *Crassostrea virginica. Mar. Ecol. Prog. Ser.* **9**, 59-68.

Shumway, S. E. and Marsden, I. D. 1982. The combined effects of temperature, salinity, and declining oxygen tension on oxygen consumption in the marine pulmonate *Amphibola crenata* (Gmelin, 1791). *J. Exp. Mar. Biol. Ecol.* **61**, 133-146.

Shumway, S. E., Selvin, R. and Schick, D. F. 1987. Food resources related to habitat in the scallop *Placopecten magellanicus* (Gmelin 1791): a quantitative study. *J. Shellf. Res.* **6**, 89-95.

Shumway, S. E. and Youngson, A. 1979. The effects of fluctuating salinity on the physiology of *Modiolus demissus* (Dillwyn). *J. Exp. Mar. Biol. Ecol.* **40**, 167-181.

Silva, A. L. and Wright, S. H. 1992. Integumental taurine transport in *Mytilus* gill: short term adaption to reduced salinity. *J. Exp. Biol.* **162**, 265-279.

Singnoret-Brailovsky, G., Maeda-Martinez, A. N., Reynoso-Granados, T., Soto-Galera, E., Monsalvo-Spencer, P. and Valle-Meza, G. 1996. Salinity tolerance of the Catarina scallop *Argopecten ventricosus-circularis* (Sowerby II, 1842). *J. Shellf. Res.* **15**, 623-626.

Skilleter, G. A. and Peterson, C. H. 1994. Control of foraging behaviour of individuals within an ecosystem context: the clam *Macoma balthica* and the interactions between competition and siphon cropping. *Oecologia* **100**, 268-278.

Smaal, A. C., Vonck, A. P. M. A. and Bakker, M. 1997. Seasonal variation in physiological energetics of *Mytilus edulis* and *Cerastoderma edule* of different size classes. *J. Mar. Biol. Assoc. UK.* **77**, 817-838.

Smaal, A. C., Wagenvoort, A., Hemelraad, J. and Akkerman, I. 1991. Response to stress of mussels (*Mytilus edulis*) exposed in Dutch tidal waters. *Comp. Biochem. Physiol.* **100C**, 197-200.

Soh, S. W. L., Maguire, G. B. and Ward, R. D. submitted. Genetic studies of the venerid clam genus *Katelysia. J. Shellf. Res.*

Sokal, R. R. and Rohlf, F. J. 1981. *Biometry.* W. H. Freeman and Company, San Francisco, CA.

Spencer, B. E., Edwards, D. B. and Millican, P. F. 1991. Cultivation of Manila clams. MAFF, Fish Res., Lowestoft.

Stickle, W. B. and Bayne, B. L. 1982. Effects of temperature and salinity on oxygen consumption and nitrogen excretion in *Thais (nacella) lapillus* (L.). *J. Exp. Mar. Biol. Ecol.* **58**, 1-17.

Stirling, H. P. and Okumus, I. 1995. Growth and production of mussels (*Mytilus edulis* L.) suspended at salmon cages and shellfish farms in two Scottish sea lochs. *Aquaculture* **134**, 193-210.

Strange, K. B. and Crowe, J. H. 1979. Acclimation to successive short term salinity changes by bivalve *Modiolus demissus* II Nitrogen metabolism. *J. Exp. Biol.* **210**, 227-236.

SYSTAT: Statistics 1992, Version 5.2, SYSTAT, Inc., Evanston, IL.

Taylor, B. E., Jamieson, G. and Corbett, T. H. 1992. Mussel culture in British Columbia: the influence of salmon farms on growth of *Mytilus edulis*. *Aquaculture* **108**, 51-66.

Taylor, I. R. 1995. Clam harvesting and the conservation of wading birds in Tasmania. Johnstone Centre of Parks, Recreation and Heritage. Charles Stuart University, Armidale, NSW.

Thangavelu, R. 1988. Natural food of the edible oyster *Crassostrea madrasensis* (Preston) of Pulicat lake, south India. *Proc. Indian Nat. Sci. Acad. (Anim. Sci.)* **97**, 463-470.

Thistle, D. 1981. Natural physical disturbances and communities of marine soft bottoms. *Mar. Ecol. Prog. Ser.* **6**, 223-228.

Thrush, S. F. 1986. Spatial heterogeneity in subtidal gravel generated by the pitdigging activities of *Cancer pagurus*. *Mar. Ecol. Prog. Ser.* **30**, 221-227.

Thrush, S. F., Hewitt, J. E., Pridmore, R. D. and Cummings, V. J. 1996. Adult/juvenile interactions of infaunal bivalves: contrasting outcomes in different habitats. *Mar. Ecol. Prog. Ser.* **132**, 83-92.

Toba, D. R., Thompson, D. S., Chew, K. K., Anderson, G. J. and Miller, M. B. 1992. Factors influencing production. In *Guide to Manila Clam Culture in Washington*. Ed. by pp 7-18. University of Washington, Seattle, WA.

Treadwell, R., McKelvie, L. and Maguire, G. B. 1991. Profitability of Selected Aquacultural Species. ABARE. Canberra, ACT

Treadwell, R., McKelvie, L. and Maguire, G. B. 1992. Potential for Australian Aquaculture. ABARE. Canberra, ACT

Tsikhon-Lukanina, Y. A. 1982. Food spectra of bottom molluscs. *Oceanology* **22**, 751-754.

Underwood, A. J. and Denley, E. J. 1984. Paradigms, explanations and generalizations in models for the structure of intertidal communities on rocky shores. In *Ecological Communities, Conceptual Issues and the Evidence*. Ed. by D. R. Strong, D. Simberloff, L. G. Abele and A. B. Thistle, pp 151-180. Princeton University Press, Princeton.

Van Blaricom, G. R. 1982. Experimental analyses of structural regulation in a marine sand community exposed to ocean swell. *Ecol. Monogr.* **52**, 283-305.

van den Enden, R. 1994. The role of phytoplankton in the diet of juvenile and adult Pacific oysters (*Crassostrea gigas*) cultured at Little Swanport Lagoon. unpublished BSc Hons. Thesis. University of Tasmania, Hobart, Tasmania.

Van Winkle, W. 1968. The effects of season, temperature and salinity on the oxygen consumption of bivalve gill tissue. *Comp. Biochem. Physiol.* **26**, 69-80.

Van Winkle, W. 1972. Ciliary activity and oxygen consumption of excised bivalve gill tissue. *Comp. Biochem. Physiol.* **42A**, 473-485.

Vermeij, G. J. 1972. Intraspecific shore-level size gradients in intertidal molluscs. *Ecology* **53**, 693 - 700.

Vernberg, F. J. 1969. Acclimation of intertidal crabs. *Am. Zool.* **9**, 333-341.

Vernberg, F. J. and Vernberg, W. B. 1981. *Functional Adaptions of Marine Organisms.* Academic Press, New York, NY.

Vincent, B., Joly, D. and Harvey, M. 1994. Spatial variation in growth of the bivalve *Macoma balthica* (L.) on a tidal flat : effects of environmental factors and intraspecific competition. *J. Exp. Mar. Biol. Ecol.* **181**, 223-238.

Virkar, R. A. and Webb, K. L. 1970. Free amino acid composition of the soft-shell clam *Mya arenaria* in relation to salinity of the medium. *Comp. Biochem. Physiol.* **32**, 775-783.

Wada, K. 1984. Osmoregulation in Marine Bivalves. *Proceedings of the Ninth and Tenth US-Japan Meetings on Aquaculture* 89-92.

Wallace, J. C. 1980. Growth rates of different populations of the edible mussel, *Mytilus edulis*, in N. Norway. *Aquaculture* **19**, 303-311.

Weinburg, J. R. 1985. Factors regulating population dynamics of the marine bivalve *Gemma gemma*: intraspecific competition and salinity. *Mar. Biol.* **86**, 173-182.

Wells, F. E. and Roberts, D. 1980. Molluscan assemblages on an intertidal sandflat in Princess Royal Harbour, Western Australia. *Aust. J. Mar. Freshwater Res.* **31**, 499-507.

Wells, F. E. and Threlfall, T. J. 1980. A comparison of the molluscan communities on intertidal sand flats in Oyster Harbour and Peel Inlet, Western Australia. *J. Moll. Stud.* **46**, 300-311.

Wendt, G. E. and McLachlan, A. 1985. Zonation and biomass of the intertidal macrofauna along a South African sandy beach. *Cahiers De Biologie Marine* **26**, 1-14.

Widdows, J. and Bayne, B. L. 1971. Temperature acclimation of *Mytilus edulis* with reference to its energy budget. *J. Mar. Biol. Assoc. UK.* **51**, 827-843.

Widdows, J. and Donkin, P. 1992. Mussels and environmental contaminants: bioaccumulation and physiological aspects. In *The Mussels Mytilus: Ecology, Physiology, Genetics and Culture.* Ed. by E. Gosling, pp 383-425. Elsevier, Amsterdam.

Widdows, J., Fieth, P. and Worrall, C. M. 1979. Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. *Mar. Biol.* **85**, 217-232.

Widdows, J., Newell, R. I. E. and Mann, R. 1989. Effects of hypoxia and anoxia on survival, energy metabolism, and feeding of oyster larvae (*Crassostrea virginica*, Gmelin). *Biol. Bull.* **177**, 154-166.

Wilson, J., Crawford, C. and Mitchell, I. 1996. Predictive modelling of carrying capacities of the oyster (*Crassostrea gigas*) farming areas in Tasmania. DPIF. Hobart, Tasmania.

Wilson, J. G. 1988. *The Biology of Estuarine Management.* Croom Helm, London.

Wilson, J. G. and Davis, J. P. 1984. The effect of environmental variables on the oxygen consumption of the protobranch bivalve *Nucula tugida* (Leckenby and Marshall). *J. Moll. Stud* **50**, 73-77.

Wilson, W. H. J. 1983. The role of density dependence in a marine infaunal community. *Ecology* **64**, 295-306.

Wilson, W. H. 1991. Competition and predation in marine soft-sediment communities. *Annu. Rev. Ecol. Syst.* **21**, 221-241.

Wilste, W. I. 1980. Effects of *Polinices duplicatus* (Gastropoda: Naticidae) on infaunal community structure at Barnstable Harbor, Massachusetts, USA. *Mar. Biol.* **56**, 301-310.

Wolff, W. J. 1983. Estuarine Benthos. In *Ecosystems of the World; Estuaries and Enclosed Seas*. Ed. by B. H. Ketchum, Elsevier Scientific Publishing Company, Amsterdam.

Woodin, S. A. 1974. Polychaete abundance patterns in a marine soft-sediment environment: the importance of biological interactions. *Ecol. Monogr.* **44**, 171-187.

Woodin, S. A. 1976. Adult-larval interactions in dense infaunal assemblages; patterns of abundance. *J. Mar. Res.* **34**, 25-41.

Woodin, S. A. 1978. Refuges, disturbance, and community structure: a marine soft - bottom example. *Ecology* **59**, 274-284.

Woodin, S. A. 1985. Effects of defecation by arenicolid polychaete adults on spionid polychaete juveniles in field experiments. Selective settlement or differential mortality? *J. Exp. Mar. Biol. Ecol.* **87**, 119-132.

Woodin, S. A. 1986. Settlement of infauna: larval choice? *Biol. Mar. Sci.* **39**, 401-407.

Woodin, S. A. 1991. Recruitment of infauna: positive or negative cues? *Am. Zool.* **31**, 797-807.

Woodin, S. A. 1997. Shallow Water Benthic Ecology: A North American perspective of sedimentary habitats. Ecology of Estuaries and Soft Sediment Habitats. Deakin University, Warrnambool, Australia, *Aust. J. Ecol.*.

Woodward, I. O. 1985. The structural dynamics of a tidal flat mollusc community. unpublished PhD. Thesis. University of Tasmania.

Young, J. P. W., Koehn, R. K. and Arnhein, N. 1979. Biochemical characterisation of "Lap", a polymorphic aminopeptidase from the mussel *Mytilus edulis*. *Physiol. Zool.* **57**, 609-619.

Zurberg, W. and de Zwaan, A. 1981. Role of amino acids in anaerobiosis and osmoregulation in bivalves. *J. Exp. Zool.* **215**, 315-326.

Appendix 1: Statistical Analysis of Tidal Height x Density Experiment.

Percent Survival (transformed by arcsin $x^{0.5}$)

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Tide Ht	4	4	0.46984401	4.0939	0.0065
Density	2	2	0.31910092	5.5609	0.0069
Tide Ht*Density	8	8	0.48149665	2.0977	0.0559

Shell Length Increase

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Tide Ht	4	4	30.047918	63.6394	0.0000
Density	2	2	0.799724	3.3875	0.0426
Tide Ht*Density	8	8	0.269525	0.2854	0.9674

Shell Height Increase

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Tide Ht	4	4	32.977811	23.1622	0.0000
Density	2	2	3.625469	5.0928	0.0101
Tide Ht*Density	8	8	2.597899	0.9123	0.5151

Meat Ratio

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Tide Ht	4	4	50.468192	3.9807	0.0075
Density	2	2	18.731384	2.9549	0.0623
Tide Ht*Density	8	8	28.296998	1.1160	0.3709

Number of Recruits

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Tidal Ht	4	4	0.33248151	0.6661	0.6188
Density	2	2	0.19807132	0.7937	0.4584
Tidal Ht*Density	8	8	0.64873660	0.6499	0.7317

Total numbers of macrobenthos

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Tidal Ht	4	4	1.9266568	0.4935	0.7405
Density	2	2	0.2179484	0.1117	0.8946
Tidal Ht*Density	8	8	6.4579387	0.8271	0.5833

Number of crabs

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Tidal Ht	4	4	0.94221949	0.7544	0.5604
Density	2	2	0.00482680	0.0077	0.9923
Tidal Ht*Density	8	8	0.66530818	0.2663	0.9736

Number of bivalves

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Tidal Ht	4	4	2.4469070	3.6768	0.0113
Density	2	2	0.4161950	1.2508	0.2960
Tidal Ht*Density	8	8	2.0739031	1.5581	0.1647

Number of gastropods

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Tidal Ht	4	4	0.2261506	0.4378	0.7806
Density	2	2	0.0543542	0.2105	0.8110
Tidal Ht*Density	8	8	1.8009286	1.7434	0.1144

Number of *Carcinus*
Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Tidal Ht	4	4	0.07145312	0.3871	0.8167
Density	2	2	0.11611132	1.2581	0.2940
Tidal Ht*Density	8	8	0.46444527	1.2581	0.2891

Appendix 2: Statistical Analysis of Stocking Density Experiment.

Percent Survival (transformed by arcsin $x^{0.5}$)

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	8	8	0.11042514	1.1059	0.4040
Block	1	1	0.06105688	4.8921	0.0402
Density*Block	8	8	0.10576042	1.0592	0.4317

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	0.11042514	0.013803	0.9520
Error	27	0.39147186	0.014499	**Prob>F**
C Total	35	0.50189700		0.4923

Shell Length Increase

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	8	8	0.59936822	1.6277	0.1857 NS
Block	1	1	0.02998669	0.6515	0.4301 NS
Density*Block	8	8	0.17277456	0.4692	0.8620 NS

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	0.5993682	0.074921	1.9615
Error	27	1.0312807	0.038196	**Prob>F**
C Total	35	1.6306490		0.0912 NS

Shell Height Increase

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	8	8	1.5843196	1.3644	0.2763
Block	1	1	0.2805468	1.9329	0.1814
Density*Block	8	8	0.8014882	0.6903	0.6954

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	1.5843196	0.198040	1.4473
Error	27	3.6946100	0.136837	**Prob>F**
C Total	35	5.2789296		0.2227

Meat Ratio

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	8	8	16.714615	1.0738	0.4229
Block	1	1	3.000979	1.5423	0.2302
Density*Block	8	8	10.253277	0.6587	0.7201

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	16.714615	2.08933	1.1685
Error	27	48.277404	1.78805	**Prob>F**
C Total	35	64.992018		0.3532

Number of Recruits

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	8	8	3.3888889	0.4485	0.8758
Block	1	1	0.4444444	0.4706	0.5015
Density*Block	8	8	6.0555556	0.8015	0.6093

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	3.388889	0.423611	0.4867
Error	27	23.500000	0.870370	**Prob>F**
C Total	35	26.888889		0.8548

Number of Macrofauna

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	8	8	2.4508538	0.7556	0.6444
Block	1	1	0.8921681	2.2004	0.1553
Density*Block	8	8	1.6274863	0.5018	0.8393

Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	8	0.5869733	0.073372	0.4649
Error	27	4.2613631	0.157828	**Prob>F**
C Total	35	4.8483364		0.8698

Number of Crabs

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	8	8	2.0898399	0.8363	0.5832
Block	1	1	1.3031995	4.1719	0.0560
Density*Block	8	8	3.1132185	1.2458	0.3296

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	2.089840	0.261230	0.7026
Error	27	10.039234	0.371823	**Prob>F**
C Total	35	12.129074		0.6865

Number of Gastropods

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	8	8	1.9469292	1.2772	0.3147
Block	1	1	0.0093245	0.0489	0.8274
Density*Block	8	8	0.7691749	0.5046	0.8372

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	1.9469292	0.243366	1.5614
Error	27	4.2082133	0.155860	**Prob>F**
C Total	35	6.1551425		0.1832

Number of Other Bivalves

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	8	8	0.34809174	0.3723	0.9218
Block	1	1	0.08488134	0.7263	0.4053
Density*Block	8	8	0.33881773	0.3624	0.9271

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	0.3480917	0.043511	0.4648
Error	27	2.5273770	0.093607	**Prob>F**
C Total	35	2.8754688		0.8699

Number of Carcinus

Effect Test

Source	Nparm	DF	Sum of Squares	F Ratio	Prob>F
Density	8	8	0.14886066	0.6250	0.7465
Block	1	1	0.00000000	0.0000	1.0000
Density*Block	8	8	0.26794919	1.1250	0.3931

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	8	0.14886066	0.018608	0.6250
Error	27	0.80384758	0.029772	**Prob>F**
C Total	35	0.95270824		0.7495

Appendix 3: Statistical Analysis of Amino Acid Data.

Bartlett Test for Homogenity of Group Variances = 3.641
Approximate F = 0.432 DF = 7, 256 Probability = 0.882

Analysis of Variance

Source	Sum of Squares	DF	Mean Square	F	Probability
Between groups	2.185	7	0.312	8.112	0.0001
Within groups	0.616	16	0.038		

Appendix 4: Statistical Analysis of Shell Closure and Respiration Data.

Shell closure (Adults)

Bartlett Test for Homogenity of Group Variances=8.118
Approximate F=2.417 DF=3, 259 Probability=0.067

Analysis of Variance

Source	Sum of Squares	DF	Mean Square	F	Probability
Between groups	0.776	3	0.259	8.447	0.003
Within groups	0.368	12	0.031		

Shell Closure (Juveniles)

Bartlett Test for Homogenity of Group Variances=6.156
Approximate F=1.820 DF=3, 259 Probability=0.144

Analysis of Variance

Source	Sum of Squares	DF	Mean Sqaure	F	Probability
Between groups	0.862	3	0.287	22.538	0.0001
Within groups	0.153	12	0.013		

Respiration

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	124.76591	62.3830	10.3196
Error	17	102.76665	6.0451	**Prob>F**
C Total	19	227.53256		0.0012

Appendix 5: Gut Contents

Appendix 5.1:Preparation of SEM Specimens

1. Settle 2L water samples for 24hours in 2L measuring cylinders
2. Siphon off supernatant (350ml/2L)

3. Fix in 2.5% Glutaraldehyde (made up in cacodylate buffer + marine salts) 3-24 hrs

4. Three washes of decreasing osmolarity

Buffer #1	10 mins
Buffer #2	10 mins
Buffer #3	10 mins

5. Dehydration

25% (v/v) ethanol + 0.5% (w/v) NaCl	20 mins
50% (v/v) ethanol + 0.1% (w/v) NaCl	20 mins
70% (v/v) ethanol + 0.1% (w/v) NaCl	20 mins
90% (v/v) ethanol + 0.1% (w/v) NaCl	20 mins
100% (v/v) ethanol	

NB. If drying cannot be performed immediately after dehydration, then the dehydration should be stopped at the 70% stage. Specimens can be stored in 70% for at least 2 weeks.

6. Critical point dry

7. Mount specimens onto stubs for gold coating

SEM Fixatives and Buffers

1. <u>0.2 Cacodylate Buffer</u>

Sodium cacodylate	21.4 gm
Distilled water	500 ml
Adjusted to pH 7.4	

2. <u>0.1 Cacodylate Buffer</u>

0.2 M Cacodylate buffer	100 ml
Distilled water	100 ml
Adjusted to pH 7.4	

3. Marine Salts Solution

NaCl	4.0 gm
$MgCl_2$	1.5 gm
$CaCl_2$	0.25 gm
Distilled water	100 ml

Autoclaved (15 psi, 121°C, 15 mins)

4. Buffer # 1 (0.1M Cacodylate buffer)

Marine salts solution	40 ml
0.2 M Cacodylate buffer	50 ml
Distilled water	10 ml

5. Buffer # 2 (0.1M Cacodylate buffer)

Marine salts solution	25 ml
0.2M Cacodylate buffer	50 ml
Distilled water	10 ml

6. Buffer # 3 (0.1M Cacodylate buffer)

Marine salts solution	12.5 ml
0.2M Cacodylate buffer	50 ml
Distilled water	37.5 ml

7. 2.5% Glutaraldehyde fixative

25% Glutaraldehyde	10 ml
0.2M Cacodylate buffer	50 ml
Marine salts solution	40 ml

8. 1% (w/v) Osmium Tetroxide fixative

Osmium tetraoxide	1 gm
0.1M Cacodylate buffer	100 ml

Appendix 5.2: Algal Assembledge at Duck Bay Smithton.

Abbreviations
H - Haptobenthic
Ep - Epiphytic
Ec - Epipelic
P - Planktonic
Eb - Epilithic

Appendix 5.2a: Algal assembledge of gut contents of *K. scalarina* at Duck Bay.

Species present 16/01/96			Away 2a	Away 3a	Away 4a	Total	Under 2u	Under 3u	Under 4u	Total
Achnanthes brevipes	H	Centric	17	4		21	8	11	2	21
Amphora ovals	Ep	Pennate	3	10	2	15	4	4	3	11
Amphora sp. 1	Ep	Pennate	1		4	5		1	1	2
Amphora sp. 2	Ep	Pennate	4	1	1	6	4	6		10
Amphora sp. 3	Ep	Pennate			4	4				0
Bacillaria paradoxa	Ep	Pennate				0				0
Chaetoceros	P	Centric				0				0
Campyloneis	Ep	Pennate	1			1	1			1
Cocconeis scutellum	Ep	Pennate	13	6		19	7	2	7	16
Cocconeis sp. 2	Ep	Pennate	7	5	30	42	3	9	9	21
Cocconesis sp. 3	Ep	Pennate	18	14	20	52	25	15	8	48
Cocconesis sp. 4	Ep	Pennate	6	2	6	14	10	1	2	13
Cocconesis sp. 5	Ep	Pennate				0				0
Cocconesis sp. 6	Ep	Pennate	10	19		29	4	13	1	18
Coscinodiscus		Centric	1			1				0
Dinophysis	P	Dinoflagellate	3			3				0
Diploneis	Ec	Pennate				0				0
Entomeneis	Ec	Centric	2			2	1	1	2	4
Fallacia	Ec	Pennate				0				0
Frustulia	Ep	Pennate				0				0
Lyrella lyra	El	Pennate	1	3		4	2	2	8	12
Mastogloia	Ep,Ec	Pennate	5			5		5		5
Melosira sp. 1	Eb	Centric				0				0
Melosira sp. 2	Eb	Centric				0				0
Navicula tripunctata	Ec	Pennate	1		3	4	3	3	4	10
Navicula sp. 1	Ec	Pennate	5	5	3	13		5		5
Navicula sp. 5	Ec	Pennate	3	2	8	13	9	1	8	18
Navicula sp. 6	Ec	Pennate	14	8	10	32	9	7	6	22
Navicula sp7	Ec	Pennate	1			1	1			1
Navicula sp. 8	Ec	Pennate				0	2			2
Nitzschia sp. 1	Ec,P	Pennate	5	15		20	14	9	10	33
Nitzschia sp. 2	Ec,P	Pennate	6	11	1	18	14	8		22
Nitzschia sp. 3	Ec,P	Pennate				0	7			7
Nitzschia sp. 4	Ec,P	Pennate	14			14	4			4
Nitzschia sp. 5	Ec,P	Pennate				0				0
Nitzschia sp. 6	Ec,P	Pennate	6	10	20	36	8	7	12	27
Petrodictyon	Ec	Pennate				0				0
Pinnularia	Ec	Pennate	8	12		20	1	14	5	20
Plagiodiscus	Ec	Pennate	2	1	2	5				0
Pleurosigma	Ec	Pennate				0				0
Rhaphoneis sp 1	Ec	Pennate	15	3		18	2	14		16
Rhaphoneis	Ec	Pennate		4		4	1			1
Sellaphora	Ec	Pennate			15	15	4			4
Staurophora	Ec	Pennate				0				0
Striatella unipunctata	Ep	Pennate	4	5	17	26		8	3	11
Synedra sp 1	Ec	Pennate	1			1	2		1	3
Synedra sp2	Ec	Pennate				0				0
Synedra sp 3	Ec	Pennate				0	1	1	3	5
Trachyneis	Ec	Pennate				0	2		2	4
Triceratium		Centric				0				0
Total			177	140	146	463	153	147	97	

Species present 1/04/96			Away 2a	Away 3a	Away 4a	Total	Under 2u	Under 3u	Under 4u	Total
Achnanthes brevipes	H	Centric		1	2	3	2	6	3	11
Amphora ovals	Ep	Pennate		1		1		4	2	6
Amphora sp. 1	Ep	Pennate				0				0
Amphora sp. 2	Ep	Pennate		2		2	1	1		2
Amphora sp. 3	Ep	Pennate				0				0
Bacillaria paradoxa	Ep	Pennate				0				0
Chaetoceros	P	Centric				0				0
Campyloneis	Ep	Pennate				0				0
Cocconeis scutellum	Ep	Pennate	2			2	3	3	2	8
Cocconeis sp. 2	Ep	Pennate		2		2	2	1	5	8
Cocconesis sp. 3	Ep	Pennate	2	7	1	10	1	1	6	8
Cocconesis sp. 4	Ep	Pennate				0			1	1
Cocconesis sp. 5	Ep	Pennate				0				0
Cocconesis sp. 6	Ep	Pennate				0				0
Coscinodiscus		Centric				0				0
Dinophysis	P	Dinoflagellate	1	1		2				0
Diploneis	Ec	Pennate				0				0
Entomeneis	Ec	Centric				0	1			1
Fallacia	Ec	Pennate				0				0
Frustulia	Ep	Pennate				0				0
Lyrella lyra	El	Pennate				0		1		1
Mastogloia	Ep,Ec	Pennate				0		3		3
Melosira sp. 1	Eb	Centric				0				0
Melosira sp. 2	Eb	Centric				0				0
Navicula tripunctata	Ec	Pennate				0				0
Navicula sp. 1	Ec	Pennate	3	1		4	1	3	5	9
Navicula sp. 5	Ec	Pennate	1	3	2	6		3	1	4
Navicula sp. 6	Ec	Pennate				0		1		1
Navicula sp7	Ec	Pennate				0				0
Navicula sp. 8	Ec	Pennate	2			2				0
Nitzschia sp. 1	Ec,P	Pennate	3	1	1	5	1	1	3	5
Nitzschia sp. 2	Ec,P	Pennate		2	2	4		2	4	6
Nitzschia sp. 3	Ec,P	Pennate				0				0
Nitzschia sp. 4	Ec,P	Pennate		2		2				0
Nitzschia sp. 5	Ec,P	Pennate	1			1	1			1
Nitzschia sp. 6	Ec,P	Pennate	8	12	4	24	10		10	20
Petrodictyon	Ec	Pennate				0				0
Pinnularia	Ec	Pennate	1	1		2		2		2
Plagiodiscus	Ec	Pennate				0				0
Pleurosigma	Ec	Pennate				0				0
Rhaphoneis sp 1	Ec	Pennate				0				0
Rhaphoneis	Ec	Pennate				0				0
Sellaphora	Ec	Pennate				0			1	1
Staurophora	Ec	Pennate				0		1		1
Striatella unipunctata	Ep	Pennate				0				0
Synedra sp 1	Ec	Pennate				0				0
Synedra sp2	Ec	Pennate				0				0
Synedra sp 3	Ec	Pennate				0				0
Trachyneis	Ec	Pennate		1		1		1		1
Triceratium		Centric				0				0
Total			24	37	12		23	34	43	

Species present 20/08/96			Away 2a	Away 3a	Away 4a	Total	Under 2u	Under 3u	Under 4u	Total
Achnanthes brevipes	H	Centric	3	2	3	8	1	2	4	7
Amphora ovals	Ep	Pennate	2			2		1		1
Amphora sp. 1	Ep	Pennate		1		1		1		1
Amphora sp. 2	Ep	Pennate	1		1	2	2	1	2	5
Amphora sp. 3	Ep	Pennate				0				0
Bacillaria paradoxa	Ep	Pennate				0	1			1
Chaetoceros	P	Centric				0	1			1
Campyloneis	Ep	Pennate		2		2				0
Cocconeis scutellum	Ep	Pennate	3		3	6		1	3	4
Cocconeis sp. 2	Ep	Pennate	10	3	4	17	7	1	3	11
Cocconesis sp. 3	Ep	Pennate	4	6	5	15	5	2	3	10
Cocconesis sp. 4	Ep	Pennate	1		1	2	3	6	2	11
Cocconesis sp. 5	Ep	Pennate				0				0
Cocconesis sp. 6	Ep	Pennate	4	3		7	1		3	4
Coscinodiscus		Centric			2	2	2			2
Dinophysis	P	Dinoflagellate				0			1	1
Diploneis	Ec	Pennate				0		1		1
Entomeneis	Ec	Centric				0			1	1
Fallacia	Ec	Pennate				0				0
Frustulia	Ep	Pennate			1	1			4	4
Lyrella lyra	El	Pennate				0				0
Mastogloia	Ep,Ec	Pennate	1			1			1	1
Melosira sp. 1	Eb	Centric	1			1	1+Y5		1	1
Melosira sp. 2	Eb	Centric		2		2				0
Navicula tripunctata	Ec	Pennate	1	2		3	1			1
Navicula sp. 1	Ec	Pennate	2	2	2	6	2	7	3	12
Navicula sp. 5	Ec	Pennate	1	1	3	5	1	4	3	8
Navicula sp. 6	Ec	Pennate		1	1	2	1	3	2	6
Navicula sp7	Ec	Pennate			1	1			1	1
Navicula sp. 8	Ec	Pennate	1			1	2	1	3	6
Nitzschia sp. 1	Ec,P	Pennate	4	4	2	10	5	1	6	12
Nitzschia sp. 2	Ec,P	Pennate	8	3		11	3	6	8	17
Nitzschia sp. 3	Ec,P	Pennate		1	3	4	3	5	2	10
Nitzschia sp. 4	Ec,P	Pennate				0				0
Nitzschia sp. 5	Ec,P	Pennate			1	1	1		1	2
Nitzschia sp. 6	Ec,P	Pennate	4	5	3	12	5	9	9	23
Petrodictyon	Ec	Pennate	6	2	1	9	4	2	2	8
Pinnularia	Ec	Pennate				0			2	2
Plagiodiscus	Ec	Pennate			1	1			1	1
Pleurosigma	Ec	Pennate				0				0
Rhaphoneis sp 1	Ec	Pennate	1			1			4	4
Rhaphoneis	Ec	Pennate				0		1	1	2
Sellaphora	Ec	Pennate	3		1	4	4		2	6
Staurophora	Ec	Pennate			1	1			2	2
Striatella unipunctata	Ep	Pennate				0				0
Synedra sp 1	Ec	Pennate	1	1		2	1		1	2
Synedra sp2	Ec	Pennate				0		2		2
Synedra sp 3	Ec	Pennate				0			1	1
Trachyneis	Ec	Pennate				0			2	2
Triceratium		Centric				0				0
Total			62	41	40		56	57	84	

Species present 13/11/96			Away 2a	Away 3a	Away 4a	Total	Under 2u	Under 3u	Under 4u	Total
Achnanthes brevipes	H	Centric			4	4	2	7	3	12
Amphora ovalis	Ep	Pennate			2	2		1		1
Amphora sp. 1	Ep	Pennate		1	1	2	1		1	2
Amphora sp. 2	Ep	Pennate			2	2	1		2	3
Amphora sp. 3	Ep	Pennate			3	3	1		1	2
Bacillaria paradoxa	Ep	Pennate		2	3	5	4	3		7
Chaetoceros	P	Centric			1	1				0
Campyloneis	Ep	Pennate				0				0
Cocconeis scutellum	Ep	Pennate	2		1	3	1	2		3
Cocconeis sp. 2	Ep	Pennate	13	11	8	32	21	15	10	46
Cocconesis sp. 3	Ep	Pennate		1		1	4	5	9	18
Cocconesis sp. 4	Ep	Pennate	3	6	3	12	5		9	14
Cocconesis sp. 5	Ep	Pennate			1	1	1		1	2
Cocconesis sp. 6	Ep	Pennate	7	5	5	17	7	2	5	14
Coscinodiscus		Centric				0		1		1
Dinophysis	P	Dinoflagellate				0				0
Diploneis	Ec	Pennate				0				0
Entomeneis	Ec	Centric				0	2	1	1	4
Fallacia	Ec	Pennate		2	1	3		2		2
Frustulia	Ep	Pennate				0	1			1
Lyrella lyra	El	Pennate				0			1	1
Mastogloia	Ep, Ec	Pennate				0				0
Melosira sp. 1	Eb	Centric	2		1	3	1	2	2	5
Melosira sp. 2	Eb	Centric		1		1	2		1	3
Navicula tripunctata	Ec	Pennate				0	1		3	4
Navicula sp. 1	Ec	Pennate	6	3	4	13	3		2	5
Navicula sp. 5	Ec	Pennate	2	1	2	5	4	4	1	9
Navicula sp. 6	Ec	Pennate		1	2	3	2		3	5
Navicula sp7	Ec	Pennate				0				0
Navicula sp. 8	Ec	Pennate			2	2	5	2	8	15
Nitzschia sp. 1	Ec,P	Pennate	6	5	4	15	10	10	5	25
Nitzschia sp. 2	Ec,P	Pennate	7	2	2	11	11	6	3	20
Nitzschia sp. 3	Ec,P	Pennate	5	1	3	9	3	3	1	7
Nitzschia sp. 4	Ec,P	Pennate	1		1	2			3	3
Nitzschia sp. 5	Ec,P	Pennate		1	1	2	1	1		2
Nitzschia sp. 6	Ec,P	Pennate	5	1	7	13	11	11	9	31
Petrodictyon	Ec	Pennate	6	1		7	3	6	3	12
Pinnularia	Ec	Pennate			1	1			2	2
Plagiodiscus	Ec	Pennate	1	1	2	4			1	1
Pleurosigma	Ec	Pennate				0			1	1
Rhaphoneis sp 1	Ec	Pennate			2	2	2			2
Rhaphoneis	Ec	Pennate				0	1	1		2
Sellaphora	Ec	Pennate			1	1		1		1
Staurophora	Ec	Pennate		1		1				0
Striatella unipunctata	Ep	Pennate			1	1	2			2
Synedra sp 1	Ec	Pennate			1	1	1	1		2
Synedra sp2	Ec	Pennate		2		2		7		7
Synedra sp 3	Ec	Pennate	3		2	5	2		2	4
Trachyneis	Ec	Pennate		2	1	3	2			2
Triceratium		Centric			1	1	1			1
Total			69	51	76		119	94	93	

Species present 22/01/97			Away 2a	Away 3a	Away 4a	Total	Under 2u	Under 3u	Under 4u	Total
Achnanthes brevipes	H	Centric	1		1	2	3		1	4
Amphora ovals	Ep	Pennate	2		3	5	2		1	3
Amphora sp. 1	Ep	Pennate	2	1		3	1			1
Amphora sp. 2	Ep	Pennate	3	1		4	1			1
Amphora sp. 3	Ep	Pennate	2		2	4	2	2		4
Bacillaria paradoxa	Ep	Pennate		3	3	6		2		2
Chaetoceros	P	Centric	1			1				0
Campyloneis	Ep	Pennate				0				0
Cocconeis scutellum	Ep	Pennate		4	1	5		1		1
Cocconeis sp. 2	Ep	Pennate	8	11	19	38	5	15	4	24
Cocconesis sp. 3	Ep	Pennate			3	3		1	1	2
Cocconesis sp. 4	Ep	Pennate	3	15	10	28	3	4	10	17
Cocconesis sp. 5	Ep	Pennate			1	1				0
Cocconesis sp. 6	Ep	Pennate	1	7	11	19	1	6	5	12
Coscinodiscus		Centric				0				0
Dinophysis	P	Dinoflagellate				0				0
Diploneis	Ec	Pennate				0				0
Entomeneis	Ec	Centric		1	4	5		1	2	3
Fallacia	Ec	Pennate				0				0
Frustulia	Ep	Pennate			7	7		3		3
Lyrella lyra	El	Pennate		2	1	3		1	1	2
Mastogloia	Ep, Ec	Pennate			3	3	1	2	1	4
Melosira sp. 1	Eb	Centric				0				0
Melosira sp. 2	Eb	Centric				0				0
Navicula tripunctata	Ec	Pennate	2	5	1	8		2	2	4
Navicula sp. 1	Ec	Pennate	4	1	4	9	1	1	1	3
Navicula sp. 5	Ec	Pennate		3		3	1	3	2	6
Navicula sp. 6	Ec	Pennate	2	3	3	8		3	4	7
Navicula sp7	Ec	Pennate				0				0
Navicula sp. 8	Ec	Pennate	3	3		6	4	1	6	11
Nitzschia sp. 1	Ec,P	Pennate	1	19	8	28	2	5	2	9
Nitzschia sp. 2	Ec,P	Pennate	1	3	8	12		3	3	6
Nitzschia sp. 3	Ec,P	Pennate	1		6	7		2	2	4
Nitzschia sp. 4	Ec,P	Pennate			1	1		2		2
Nitzschia sp. 5	Ec,P	Pennate			1	1		1		1
Nitzschia sp. 6	Ec,P	Pennate	3	3	9	15	9	7	8	24
Petrodictyon	Ec	Pennate	4	3	1	8		1	3	4
Pinnularia	Ec	Pennate	2	1	2	5	1	1		2
Plagiodiscus	Ec	Pennate				0				0
Pleurosigma	Ec	Pennate			1	1				0
Rhaphoneis sp 1	Ec	Pennate	1	2		3	1	2		3
Rhaphoneis	Ec	Pennate			1	1		1	2	3
Sellaphora	Ec	Pennate				0				0
Staurophora	Ec	Pennate				0				0
Striatella unipunctata	Ep	Pennate		1		1				0
Synedra sp 1	Ec	Pennate				0	1			1
Synedra sp2	Ec	Pennate				0				0
Synedra sp 3	Ec	Pennate				0				0
Trachyneis	Ec	Pennate	3		1	4			2	2
Triceratium		Centric				0				0
Total			50	92	116		39	73	63	

Appendix 5.2b: Algal assembledge of water column at Duck Bay.

Species present			Total	Grouped Totals	Percent Abundance
Achnanthes brevipes	H	Centric	182	93	2.316313823
Amphora ovals	Ep	Pennate	91		
Amphora sp. 1	Ep	Pennate	33		
Amphora sp. 2	Ep	Pennate	73		
Amphora sp. 3	Ep	Pennate	30	227	5.653798257
Bacillaria paradoxa	Ep	Pennate	40	21	0.523038605
Chaetoceros	P	Centric	6	3	0.074719801
Campyloneis	Ep	Pennate	8	4	0.099626401
Cocconeis scutellum	Ep	Pennate	133		
Cocconeis sp. 2	Ep	Pennate	458		
Cocconesis sp. 3	Ep	Pennate	332		
Cocconesis sp. 4	Ep	Pennate	207		
Cocconesis sp. 5	Ep	Pennate	8		
Cocconesis sp. 6	Ep	Pennate	228	1366	34.02241594
Coscinodiscus		Centric	12	6	0.149439601
Dinophysis	P	Dinoflagellate	12	6	0.149439601
Diploneis	Ec	Pennate	2	1	0.0249066
Entomeneis	Ec	Centric	37	20	0.498132005
Fallacia	Ec	Pennate	10	5	0.124533001
Frustulia	Ep	Pennate	29	16	0.398505604
Lyrella lyra	El	Pennate	44	23	0.572851806
Mastogloia	Ep,Ec	Pennate	40	22	0.547945205
Melosira sp. 1	Eb	Centric	20		
Melosira sp. 2	Eb	Centric	12	17	0.423412204
Navicula tripunctata	Ec	Pennate	64		
Navicula sp. 1	Ec	Pennate	155		
Navicula sp. 5	Ec	Pennate	148		
Navicula sp. 6	Ec	Pennate	165		
Navicula sp7	Ec	Pennate	8		
Navicula sp. 8	Ec	Pennate	79	619	15.41718555
Nitzschia sp. 1	Ec,P	Pennate	315		
Nitzschia sp. 2	Ec,P	Pennate	248		
Nitzschia sp. 3	Ec,P	Pennate	92		
Nitzschia sp. 4	Ec,P	Pennate	54		
Nitzschia sp. 5	Ec,P	Pennate	21		
Nitzschia sp. 6	Ec,P	Pennate	426	1156	28.79202989
Petrodictyon	Ec	Pennate	92	48	1.195516812
Pinnularia	Ec	Pennate	110	56	1.394769614
Plagiodiscus	Ec	Pennate	24	12	0.298879203
Pleurosigma	Ec	Pennate	4	2	0.0498132
Rhaphoneis sp 1	Ec	Pennate	95		
Rhaphoneis	Ec	Pennate	23	118	2.938978829
Sellaphora	Ec	Pennate	64	32	0.797011208
Staurophora	Ec	Pennate	10	5	0.124533001
Striatella unipunctata	Ep	Pennate	82	41	1.02117061
Synedra sp 1	Ec	Pennate	23		
Synedra sp2	Ec	Pennate	22		
Synedra sp 3	Ec	Pennate	30	75	1.867995019
Trachyneis	Ec	Pennate	36	19	0.473225405
Triceratium		Centric	4	2	0.0498132
Total			2771	4015	

Appendix 5.3: Survival of *K. scalarina* at Smithton

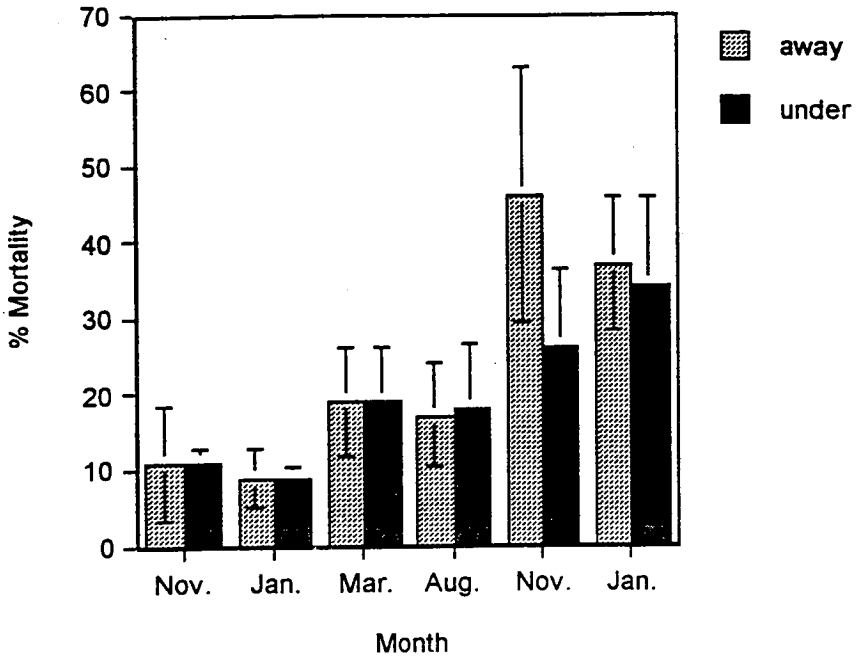

Figure 1: Survival of *K. scalarina* under and away from oyster racks at Duck Bay, Smithton, over the experimental period (mean±S.E. n=4 cages).

Appendix 5.4: Mortality of *K. scalarina*

Table 1: Mortality of *K. scalarina* in pilot trials at various sites around Tasmania.

Site	% Mortality (Under)	S.E.	% Mortality (Away)	S.E.
Pittwater [a]	22.4	1.0	22.2	2.0
Coles Bay [b]	11	1.6	10.7	1.2
Smithton [c]	11	1.9	11	7.55

[a] Trials established 13/12/94, 114.36 clams/m^2.
[b] Trials established 29/11/94, 114.36 clams/m^2.
[c] Trials established 5/06/95, 118.08 clams/m^2.