INFLUENCES OF FOOD AVAILABILITY AND ABIOTIC FACTORS ON GROWTH AND SURVIVAL OF THE LION'S PAW SCALLOP Nodipecten nodosus (LINNAEUS, 1758) FROM A SUBTROPICAL ENVIRONMENT

GUILHERME SABINO RUPP

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by

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Abstract

The lion's paw scallop *Nodipecten* (=*Lyropecten*) *nodosus* is the largest native pectinid in Brazil and has great potential for aquaculture. Cultivation of postlarvae, which is initially carried out in land-based facilities and subsequently in the sea, is an essential step in scallop husbandry, usually involving high costs and high mortalities. The influence of food availability on postlarval growth and survival early after metamorphosis, have been controversial. After deployment in the sea-based culture, postlarvae are subject to uncontrolled environmental variables, which in the subtropical study region display an unusual pattern of variation. The importance of food availability and abiotic environmental factors on growth, survival and byssal attachment of postlarval *N. nodosus* at its southern distribution limit was examined using laboratory and field approaches, aiming to resolve some of the existing controversies and to provide biological information to facilitate the development of scallop farming in Brazil.

The presence of epiflora on the spat collectors enhanced settlement, but not growth. Availability of suspended algae begins to support growth 3–5 days after settlement (230–250 μm shell height), but larval energy reserves were sufficient to meet metabolic demand during the period in which suspension feeding is not functional, with no indications of food deprivation-induced mortality.

After the postlarvae were deployed in the sea, temperature was the major environmental factor affecting growth, with food availability having a negligible influence. Scallops deployed in the sea-based nursery at a size of 0.5 mm (2 weeks post-

set), grew faster and had similar percentage retrievals than those deployed 2–3 weeks later and retrieved simultaneously from the sea.

Sub-surface intrusions of cold and phytoplankton-rich waters from South Atlantic Central Water influenced a near-shore (< 0.5 km) site in late summer–early autumn, resulting in oscillating temperatures and high PIM, reducing postlarval growth. Such a phenomenon was not observed in late autumn–early winter.

N. nodosus is eurythermal but stenohaline tropical species, adults being less tolerant of high temperature and low salinity than spat. Survival was maximized at salinities above 29 psu and at temperatures between 15 and 28°C. At ambient salinity, the maximum rate of byssal attachment occurred at 19–27°C and 23–27°C for spat and juveniles, respectively. These are the suggested temperatures most favourable for growth.

There is a large potential for growing postlarval and juvenile *N. nodosus* in coastal areas of southern Brazil, where growth and retrievals can be maximised by adjusting the size and season of deployment, culture depth, and by selecting an adequate site.

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List of Abbreviations and Symbols

µm	micrometer
A	autumn
ANOVA	analysis of variance
ca.	circa
cm	centimetre
CW	coastal water
df	degrees of freedom
DGR	daily growth rate
E	epiflora-covered collectors
F	"F" statistic
FSW	filtered seawater
g	gram
h	hours
H	high cell concentration treatment
HCl	hydrochloric acid
km	kilometre
L	low cell concentration treatment
Lat.	latitude
LBN	land-based nursery
LC	lethal concentration
LCMM	Laboratory for the Culture of Marine Molluscs
Long.	longitude
LT	lethal temperature
mg	milligram
mL	millilitre
mm	millimetre
n	number of cases or observations

NC	nursery culture experiment
NE	northeast
NTU	nephelometric turbidity units
NW	northwest
°C	degrees centigrades
P	probability level
PIM	particulate inorganic matter
POM	particulate organic matter
psu	practical salinity unit
r^2	coefficient of determination
S	south
SACW	South Atlantic Central Water
SAW	sub-Antarctic water
SBB	South Brazil Bight
SBN	sea-based nursery
SC	Santa Catarina State, Brazil
SCUBA	self contained underwater breathing apparatus
sd	standard deviation
SD	seasonal deployment experiment
se	standard error
SH	shell height
SL	shell length
S_p	spring
S_u	summer
Temp.	temperature
TPM	total particulate matter
Turb.	turbidity
TW	tropical water
U	unfed treatment
UV	ultra-violet light

UFSC	Federal University of Santa Catarina
W	winter

CHAPTER I

General Introduction

I.1. Rationale

The lion's paw scallop *Nodipecten* (=*Lyropecten*) *nodosus* is the largest pectinid in Brazilian waters (Rios 1994), having great potential for aquaculture development. Propagation in hatcheries is the only means to supply juveniles for bivalve farming in circumstances where wild spat[1] collection is not feasible (Bourne et al. 1989), as is the case for the lion's paw scallop in Brazil. Owing to the low-density and highly dispersed adult populations, partial and asynchronous reproduction, and low natural seed collection, hatchery production is the only source of *N. nodosus* juveniles for aquaculture (Rupp 1994, Manzoni et al. 1996, Uriarte et al. 2001). Whereas there are biological and technological bases for successful larval production (Rupp 1994, 1997, Rupp and Poli 1994, Rupp et al. 1997, Rupp et al. 2000a,b, Bem et al. 2001a,b, De la Roche et al. 2002, Thompson et al. 2003), less is known about the factors influencing growth and survival of benthic life stages of *N. nodosus*, especially during postlarval culture, a critical period in scallop husbandry (Bourne and Hodgson 1991). High survivorship and growth are essential for the establishment of any successful aquaculture activity, and require detailed information about the environmental needs of different life history stages. This research provides much of the biological information, essential for the potential of scallop cultivation in Brazil to be realized.

[1] The term "spat" is colloquially used in aquaculture to refer to the life stage from metamorphosis until a few millimeters in body size (Walne 1979). In the present study it is used interchangeably with postlarvae.

I.2. Biology, Ecology and Aquaculture of *Nodipecten nodosus*

Nodipecten (=*Lyropecten*) *nodosus* (Fig. I.1) is a subtidal, non-estuarine bivalve with an inter-tropical distribution in the western Atlantic ocean, inhabiting shallow waters in the Caribbean (south of the Greater Antilles, the Virgin Islands, eastern Antilles, eastern Central America south of the Yucatan Peninsula, eastern Panama to Colombia and Venezuela) and discontinuously along the Brazilian coast (Smith 1991) as far south as Santa Catarina State (Rupp 1994). Controversy persists in the literature with regard to the taxonomy of the genera *Nodipecten* and *Lyropecten*. Smith (1991) distinguishes both genera and characterises the different species, *Lyropecten* being an extinct genus in the Atlantic Ocean. In the western Atlantic two holocenic species of *Nodipecten* are found, *N. fragosus,* which inhabits warm temperate waters from the Gulf of Mexico to Cape Hatteras (North Carolina) in depths from 15 to 82 m, and *N. nodosus*, which is found from the Caribbean southwards. The present study follows the taxonomy according to Smith (1991), also in agreement with Waller (pers. comm., Smithsonian Institution) and Peña (2001). In southern Brazil, *N. nodosus* occurs in depths from 6 to 30 m, mainly at the interfaces between rocky and sandy bottoms, particularly inside small rocky caves and crevices, or on sandy or calcareous patches adjacent to rocks (Rupp, pers. obs.).

The lion's paw scallop is a functional simultaneous hermaphrodite. Gametes are released into the water and fertilization and embryonic development occur externally. The planktonic trochophore larva is formed 10–12 h after fertilization. The veliger larva, also referred to as a straight-hinge or D-shaped larva, appears after 22–24 h at a shell length of about 100 μm (shell height 76 μm) (Rupp 1994). Larval development takes 14–

23 days, depending on diet and water temperature (Rupp 1997). At the end of larval life, at a shell length of about 200–210 μm (shell height 185–190 μm), settlement and metamorphosis occur if a suitable substrate is provided. A variety of substrates can be used in aquaculture settings, including synthetic monofilaments, polyethylene screens, and polycarbonate blades.

Several chemical and biological stimuli induce settlement and metamorphosis of marine molluscs (Hadfield 1977), including scallops (Hodgson and Bourne 1988, Parsons et al. 1993). During the complex process of metamorphosis, the velum disappears and is replaced by the postlarval ctenidium as the organ for feeding and respiration (Bayne 1965, Sastry 1965, Hodgson and Burke 1988). During this period of intense morphological and physiological changes, survival is usually very low in hatcheries (Castagna and Duggan 1971, Bourne et al. 1989, O' Foighil et al. 1990, Bourne and Hodgson 1991, Abarca 2001, Uriarte et al. 2001) and difficult to estimate in natural populations, but presumably also very low (Orensanz et al. 1991). Limited feeding capability immediately after metamorphosis with the exhaustion of endogenous energy reserves has been proposed to explain such mortalities (O'Foighil et al. 1990, Whyte et al. 1992), and this view is revised in Chapter II.

The intermediate step between the controlled hatchery situation and growout in the sea is known as nursery culture (Claus 1981). Unlike other bivalves that strongly attach to the substrate, scallop postlarvae are very fragile, weakly attached with a small byssus, and are able to alternate periods of attachment and crawling on the collectors, making it difficult to manipulate for experimental and aquaculture purposes. After settlement on monofilament collectors or other substrates, scallop postlarvae are usually

cultured in a land-based nursery until they attain a size of approximately 1 mm shell height, when they can be deployed to the sea-based nursery, or further maintained in the land-based nursery until they grow larger (Bourne and Hodgson 1991). The sea-based approach consists of transferring spat collectors directly to the sea inside nylon mesh bags or in mesh-lined plastic trays, or at a larger size, postlarvae can be placed directly inside pearl nets (Bourne and Hodgson 1991, Uriarte et al. 2001). After deployment to the sea, postlarval scallops are subject to the natural variation of environmental factors, and in temperate and boreal regions seasonality significantly affects their growth and survival (Bourne and Hodgson 1991, Grecian et al. 2000). In some tropical regions, on the other hand, the natural variation of environmental factors is much narrower and in the Gulf of Cariaco, Venezuela, for example, it does not influence growth and survival of juvenile scallops (Lodeiros and Himmelman 1994, 2000). It remains unclear whether in a subtropical environment seasonal signals are strong enough to cause significant differences in growth and survival of scallops.

I.3. Environmental Variability in the Study Region

The study area (Fig. I.2) is a subtropical coastal site near the high latitudinal distribution limit of *N. nodosus* in the Southern Hemisphere (Lat. 27° 50' S) characterized as a warm-temperate marine biogeographic province, that is also the limit of distribution of several tropical species (Briggs 1995). The southern Brazilian coast is seasonally influenced by different water masses. The oceanographic structure of this region is quite complex, but macro-scale oceanographic patterns have been identified (Emilson 1961,

Matsuura 1986, Castro and Miranda 1998, Sunyé and Servain 1998, Borzone et al. 1999). As a general model, it can be inferred from these studies that during summer to mid-autumn neritic waters off Santa Catarina State are influenced by the warm and oligitrophic waters of the Brazil Current (tropical waters), which penetrates over the continental shelf. During winter, the region is mainly influenced by coastal advection of cold, relatively low salinity and phytoplankton-rich waters of sub-Antarctic origin (SAW). Furthermore, from October–November to April, intrusions of cold and nutrient-rich South Atlantic Central Water (SACW) into the bottom layers over the continental shelf create a strong stratification in the water column, influencing the inner shelf region. This event leads to the formation of a strong thermocline, creating phytoplankton maxima below the thermocline (Brandini 1990, Castro and Miranda 1998, Borzone et al. 1999). Therefore, in neritic waters of the inner continental shelf in southern Brazil, food availability and temperature tend to vary in opposite directions, which is a different pattern from those described for some temperate and boreal regions, where primary production is usually decreased during winter and increased during warmer periods, so that it is difficult to decouple the effects of both factors on scallop growth (Bricelj and Shumway 1991, Orensanz et al. 1991). In some boreal regions, however, when phytoplankton blooms occur while temperature is still low, growth of scallops (*Chlamys islandica*) is not inhibited by low temperature (Wallace and Reinsnes 1985, Thorarinsdóttir 1994). Prior to the present study, it was uncertain if the pattern of variation of environmental factors reported for neritic waters of the inner continental shelf significantly affected near-shore aquaculture sites in southern Brazil. Whereas several studies have focused on growth of juvenile and adult scallops in temperate and boreal

regions (MacDonald and Thompson 1985, Wallace and Reinsnes 1985, Andersen and Naas 1993, Thorarinsdóttir 1994, Kleinman et al. 1996, Chauvaud et al. 1998, Grecian et al. 2000), as well as in the tropics (Lodeiros et al. 1998, Lodeiros and Himmelman 2000), none has addressed the effects of environmental factors on growth and survival of postlarval scallops (0.5–5 mm) in a subtropical environment. It is postulated in the present investigation that if the temporal and spatial pattern in the properties of inner shelf waters in southern Brazil influences a coastal aquaculture site, then there would be significant effects on growth, survival and byssal synthesis of scallops under culture conditions (Chapters III, IV and V).

I.4. Objectives and Scope of the Study

The main objective of the present study is to examine the influences of food availability and abiotic environmental factors on growth and survival of post-metamorphic life stages of *Nodipecten nodosus*. This investigation consists of four studies focusing mainly on critical periods in which high mortalities often occur, using laboratory and field approaches.

Chapter II examines the effects of food deprivation and the importance of different food sources immediately after metamorphosis, a period during which the feeding capability of scallops is uncertain and low survival has been reported in hatcheries. Chapter III focuses mainly on the influences of temporal variation of several abiotic and biotic environmental factors on growth and survival of postlarvae deployed in a subtropical marine environment, in which temperature and food availability may vary

seasonally in opposite directions. This study is also innovative in the approach of deploying scallops smaller than 1 mm and assessing the environmental influences. Growth and survival of postlarvae deployed in the sea at different sizes are also compared. Chapter IV provides the first data on the influence of sub-surface cold and phytoplankton-rich water intrusions on a coastal aquaculture site in southern Brazil, and reveals important consequences of growing postlarval scallops at different depths. In addition, the influence of stocking density is examined. Chapter V describes the lethal and sublethal responses of different size scallops to temperature and salinity changes. This study was carried out given the high spatial and temporal variability recorded for these factors in the previous chapters, and further explained the observed growth and survival responses recorded in the sea, providing significant ecological, aquaculture, and biogeographic information about *N. nodosus*. In Chapter VI the general conclusions of this investigation are presented, and the implications of these findings for the enhancement of aquaculture of the lion's paw scallop are discussed.

The specific objectives are as follows:

1. To examine the influence of food deprivation and different food sources on growth and survival of *N. nodosus* immediately after metamorphosis (0.2–0.4 mm shell height) (Chapter II).

2. To determine the size at which availability of exogenous food begins to support growth and survival of scallops after metamorphosis (Chapter II).

3. To determine the influence of seasonal variation in food availability and abiotic environmental factors on growth and percentage retrieval of postlarval scallops deployed in the sea-based nursery (Chapter III).

4. To compare growth rates and percentage retrievals of postlarvae deployed at different sizes in the sea-based nursery, determining the size at which growth becomes enhanced under field conditions (Chapter III).

5. To assess growth and percentage retrieval of postlarvae deployed in the sea-based nursery over consecutive samplings (Chapter III).

6. To examine the influences of environmental factors associated with depth, on growth and percentage retrieval of postlarvae (Chapter IV).

7. To investigate the effects of stocking density on growth and percentage retrieval of postlarvae (Chapter IV).

8. To determine the combined influence of temperature and salinity on survivorship and byssal attachment of three size-classes *N. nodosus* (Chapter V).

Figure I.1. Left valve (superior) of *Nodipecten nodosus* (Linnaeus, 1758). Shell height (dorso-ventral) = 126 mm; shell length (antero-posterior) = 121 mm.

Figure I.2. Location of the study sites off Porto Belo, Santa Catarina State (SC), Brazil.

1 - João da Cunha Island (Chapter III), 2 - Porto Belo Point (Chapter IV).

CHAPTER II

Influence of Food Supply on Post-Metamorphic Growth and Survival of Hatchery Produced *Nodipecten nodosus*

II.1. Introduction

Bivalve molluscs undergo important morphological and physiological changes upon completion of larval development, when the planktonic environment is abandoned and benthic life is assumed (Burke 1983). Metamorphosis is an energetically demanding process, and after the loss of the larval velum there is a cessation of filter-feeding capability until the postlarval ctenidia become functional (Bayne 1965, Sastry 1965, Hodgson and Burke 1988). During metamorphosis, the biochemical reserves utilised can be neutral lipids (Holland and Spencer 1973), proteins (Rodriguez et al. 1990), both proteins and lipids (Whyte et al. 1992, Lu et al. 1999), or proteins, lipids and carbohydrates (Farias et al. 1998, Videla et al. 1998), although the energetic contribution of the carbohydrates is minor. Post-metamorphic shell growth of bivalves relies initially on endogenous reserves accumulated during larval life, and subsequent growth and survival are believed to depend upon efficient acquisition of exogenous food before endogenous energy reserves are depleted (Whyte et al. 1992).

While suspension feeding begins early in post-metamorphic oysters (Reid et al. 1992) which rapidly develop functional gills within a few hours after attachment (Baker and Mann 1994), in pectinids it is not clear when suspension-feeding is reestablished after metamorphosis. The anatomy of the gills of adult scallops differs from that of other groups of bivalves due to the highly reduced latero-frontal cirri (Beninger 1991, Beninger and Le Pennec 1991), suggesting that scallops are not as efficient as other lamellibranchs in capturing small particles (< 5–7 μm) (Møhlenberg and Riisgard 1978, Palmer and Williams 1980). In addition, it is not clear if on postlarval stages, the efficiency in

capturing small particles is also reduced. Gills of postlarval *Pecten maximus* at a shell height of 0.25–0.9 mm are believed to be partly functional (Beninger et al. 1994). The development of gills of postlarval *Placopecten magellanicus* is more protracted than in other bivalves, so that suspension feeding is probably inefficient in spat less than 1–2 mm in shell height (Veniot et al. 2003). According to Reid et al. (1992), suspension feeding in *Patinopecten yessoensis* starts one week after metamorphosis at a size of 400 μm, when the supra- and infrabranchial chambers are formed. Some bivalves also utilise a transitional phase of pedal feeding, which allows detritus and benthic diatoms to be ingested, bridging the nutritional gap until suspension feeding becomes effective (Reid et al. 1992). Pedal feeding involves collection of epiflora by protrusion of the ciliated foot, which is then withdraw into the mantle cavity, where benthic particles adhering to the cilia are transferred to the palps and then carried to the mouth (Reid et al. 1992). Pedal feeding is an important feeding mode for up to 12 weeks after metamorphosis in the geoduck *Panope abrupta* (King 1986). It has also been documented in the scallop *Patinopecten yessoensis* at sizes < 350 μm, but in larger spat, its nutritional contribution is not clear (O'Foighil et al. 1990, Reid et al. 1992). The uptake of dissolved organic matter (DOM) is also an alternative mode of nutrition, for bivalve larvae and spat, but its energetic contribution is considered low (Manahan 1983).

The period from metamorphosis to a size of 0.5 mm is critical for successful production of scallops in hatchery/nursery culture, where high mortalities have been recorded for *Nodipecten nodosus* at the Laboratory for the Culture of Marine Molluscs, Santa Catarina, Brazil (pers. obs.), as well as for other scallops (Castagna and Duggan 1971, Bourne et al. 1989, O'Foighil et al. 1990, Bourne and Hodgson 1991, Abarca 2001,

Uriarte et al. 2001). Nevertheless, few studies have focused on the influence of food on growth and survival of scallops within this size range (O'Foighil et al. 1990, Nicolas and Robert 2001). The causes of high mortality of postlarval scallops in hatcheries remain unclear. It has been suggested that depletion of endogenous reserves before exogenous food intake can meet the energetic demands of growth and metabolism may explain these mortalities (O'Foighil et al. 1990, Whyte et al. 1992).

The body size at which suspension feeding becomes an important mechanism supporting growth during early postlarval life of *Nodipecten nodosus* is not known. Neither is it clear to what extent growth relies exclusively on endogenous food reserves, nor is it known whether pedal feeding is an important mechanism of food intake during early postlarval life.

The lion's paw scallop *Nodipecten nodosus* has great potential as an aquaculture species in Brazil, and due to insufficient wild spat collection (Rupp 1994), hatchery production of juveniles is the only source of spat for aquaculture development. Therefore, optimisation of growth and survival during hatchery/nursery production will be essential for the establishment of an aquaculture industry. Considering the uncertainty about the feeding capabilities of early postlarval scallops, the objectives of the present study were to examine the influence of food availability on growth and survival of *N. nodosus* immediately after metamorphosis (0.2–0.4 mm shell height), and to determine the size at which availability of exogenous food begins to support growth and survival.

Postlarval growth and survival of *N. nodosus* were determined in two experiments when presence or absence of epiflora and suspended food at different concentrations were tested. It was hypothesised that if early postlarvae were not able to acquire food by

suspension feeding due to delayed anatomic development of the gills, then growth would depend on endogenous food reserves or on food uptake by pedal feeding. If growth depended solely on endogenous reserves, then it would be identical in the presence or absence of exogenous food sources. Conversely, if postlarvae benefited nutritionally from bacteria and benthic microalgae by pedal feeding, then growth would be higher in the treatment with substrates covered by a biofilm, compared with the unfed treatment. Furthermore, if endogenous energy was depleted in scallops deprived of food, a significant mortality should occur. After the gills become functional, growth would be higher in postlarvae fed with algae. Additionally, postlarval growth, ingestion and survival were determined at two algal concentrations.

II.2. Materials and Methods

II.2.1. General procedures

II.2.1.1. Larval rearing

Larval rearing and postlarval culture experiments were undertaken at the Laboratory for the Culture of Marine Molluscs - Federal University of Santa Catarina (LCMM - UFSC), in Florianópolis, Santa Catarina State, Brazil. Broodstock (shell height 11–12 cm) were collected in the wild by SCUBA diving and maintained in lantern nets off João da Cunha island (Porto Belo, SC) until transfer to the laboratory 14 days before the scallops were induced to spawn. Scallops were maintained in tanks with flowing seawater at a temperature of 17–18°C, and fed a mixture of cultured microalgae (*Isochrysis* sp. (clone T-iso), *Chaetoceros calcitrans*, *C. muelleri*) at final concentrations of 4–6x10^4 cells mL^{-1}. Such conditions produce successful spawnings, resulting in large number of oocytes (Rupp et al. 1997). To induce spawning, 8 to 10 scallops were first immersed for 1 h in UV-irradiated filtered seawater (UV-FSW) with a high concentration (ca. 5x10^5 cells mL^{-1}) of *Isochrysis* sp. (clone T-iso). They were then placed in flowing UV-FSW and the water temperature was increased from 18 to 26°C over 3 hours (Rupp 1994). As soon as gametes started to be released, individual scallops were separated in different containers. *N. nodosus* is a functional hermaphrodite and the spawning scallops were frequently transferred to a new container to avoid mixing the different gametes, therefore minimising self-fertilisation. Fertilisation was carried out using sperm and eggs released by different

individuals (pooled from several individuals). Two batches of larvae were raised using scallop hatchery techniques (Rupp 1994, Uriarte et al. 2001) in 2000-L polyethylene tanks at densities starting at 10 larvae mL^{-1} and ending at 1–2 larvae mL^{-1}. Seawater was filtered (1 μm) and UV-irradiated, salinity was 33 psu and temperature was maintained at 23–24°C. Water was changed daily and a mix (1:2:1) of *Isochrysis* sp. (clone T-iso), *Chaetoceros calcitrans*, and *C. muelleri* (formerly *C. gracilis*, CHAGRA – Provasoli-Guillard CCMP Laboratory) in final concentrations of $1x10^4$ to $3x10^4$ cells mL^{-1} was supplied daily. Algae were cultured in 100-L plastic bags in Guillard "F/2" – modified media, under fluorescent light. Bacterial proliferation was controlled by addition of chloramphenicol (1 mg L^{-1}) to the larval cultures.

II.2.1.2. Settlement

When larvae (pediveligers) displayed morphological and behavioural characteristics indicating competence (conspicuous eyespot, well developed foot, aggregation in clumps, forming mucous strands, and crawling behaviour), and attained a mean shell length of ca. 200 μm (shell height 185–190 μm), they were considered competent to undergo metamorphosis. Pediveligers were then retained on a 140-μm-mesh Nytex screen, sub-sampled, counted in a Sedgwick-Rafter chamber using a microscope, and then transferred to 100-L tanks (experimental treatments) at a stocking density of 1 larva mL^{-1}. Water was changed daily and no antibiotic was used during the experiments. The collectors used for larval settlement were polyethylene screens (mesh size 3-mm),

which in previous experiments resulted in high settlement of spat and were convenient to manipulate for experimental purposes. The collectors were cut into rectangles (25 X 50 cm) and the edges of the longer sides were tied together, so that they assumed a cylindrical shape. During water changes, postlarval scallops in the bottom of setting tanks were collected and observed under a dissecting microscope. When present, these assemblages were composed mainly of dead scallops and were removed.

II.2.2. Experiment 1

In experiment 1 four treatments were carried out in duplicate 100-L tanks. Treatments consisted of (a) no algae supplied, (b) no algae supplied but the collectors had previously been immersed in coarse-filtered seawater (sand filter ca. 100 μm) for 8 days to allow growth of an epiflora of bacteria and benthic microalgae, (c) a mixed algal diet consisting of *Isochrysis* sp. (clone T-iso) and *Chaetoceros muelleri* (1:1) at a final concentration of $4x10^4$ cells mL^{-1}, supplied daily after the water change, and (d) the same volume of algal culture supplied in treatment "c" but with a cell concentration reduced by 90 % by gravity filtration using cellulose paper filter. This procedure resulted in a final algal concentration of $4–5x10^3$ cells mL^{-1} (mean $4.7 x10^3$ cells mL^{-1}) while keeping the volume of the culture and concentrations of dissolved nutrients the same as in treatment "c". Preliminary observations indicated that algal cells were intact after filtration. Algal cells were counted with a Coulter counter model Z1. Algal cultures in the exponential growth phase were supplied daily to treatments (c) and (d) at volumes of ca. 0.3–0.4 L of each species. Treatments (a) (b), (c) and (d) will hereafter be referred to as unfed (U),

epiflora (E), high algal concentration (H), and low algal concentration (L), respectively. Swimming larvae were retained during water changes and returned to the experimental units until day 3 post-settlement, when they were removed, in order to reduce size variability of settled postlarvae. In order to determine shell height (distance from the umbo to the ventral margin of the shell) samples were taken of setting larvae and of spat at 3 days (1 collector/tank), 5 days (3 collectors/tank) and 9 days (3 collectors/tank) post-settlement, when postlarvae were detached from the collectors. Both sides of the collectors were then thoroughly brushed with repeated gentle movements inside plastic trays filled with filtered seawater. This procedure was repeated for four times, until no more spat were retrieved from the collectors. Detached spat were then screened, separated from debris, and preserved in 4 % buffered formaldehyde for further counting and measurements. Shell height, rather than tissue weight was used as a growth parameter in the present experiment, since post-metamorphic scallops display allometric relationship between shell height and tissue weight, growth in size being much faster than tissue weight (Lu and Blake 1996).

Water temperature was maintained at 23°C (sd ± 0.5 °C) and salinity at 33 psu. Sampling to determine larval survival was carried out 5 and 9 days post-settlement (4 collectors/tank each day). Postlarval samples were preserved in 4 % buffered formaldehyde in seawater. Shell heights were measured with a calibrated ocular micrometer using a binocular microscope.

II.2.3. Experiment 2

In experiment 2, treatments consisted of spat fed daily at concentrations equal to those in treatments (H) and (L), as previously described for experiment 1. Each treatment was carried out in duplicate 200-L tanks. General procedures were the same as for experiment 1, but extended until 16 days post-settlement. In this experiment, addition of algal diets started 3 days after settlement. Water temperature was maintained at 25°C (sd ± 0.5) and salinity at 33 psu. Postlarval shell height (2 collectors/tank each day) and survival (6 collectors/tank) were determined on days 3, 10, and 16 post-set. In addition, shell height (maximum dorso-ventral dimension perpendicular to the hinge) and shell length (maximum antero-posterior dimension, parallel to the hinge) were measured (n = 120) to determine the relationship between shell dimensions.

II.2.3.1. Gut pigments

In order to determine ingestion of microalgae by the scallops, the pigment content in the guts of larvae and postlarvae was determined by the gut fluorescence technique (Yentsch and Menzel 1963), which is frequently used to determine pigment content in meso- and macrozooplankton (> 200 μm) (Bamstedt et al. 2000). Larvae (n=100–200) were sampled at settlement and at day 1. Postlarvae were sampled at 5, 10 and 16 days post-settlement, when they were detached from the collectors, counted, and thoroughly rinsed in FSW while retained in a 140-μm mesh screen (Nalgene filter). Pigments were

extracted on 90% acetone at 4°C for 24 h. Fluorescence was determined with a Turner™ digital fluorometer (TD 10-AU), with readings taken before and after acidification with 10% HCl. The concentrations of chlorophyll-*a* and phaeopigments (mainly phaeophytin and phaeophorbide) were calculated following Bamstedt et al. (2000). Since ingested chlorophyll-*a* is rapidly degraded and converted into phaeopigments (Dagg and Wyman 1983), the total pigment content in the guts of postlarval scallops was calculated as the sum of chlorophyll-*a* and phaeopigments.

II.2.4. Data analyses

Data were analysed with the SPSS statistical package (version 10). For experiment 1, nested ANOVAs ($\alpha = 0.05$) were first carried out to determine the significance of differences in postlarval shell height among collectors within a tank, and among tanks within a treatment, which were found to be not significant. One-way ANOVAs were then carried out after pooling the tank and collector data within a given treatment, with treatment as the main factor. Residuals were checked graphically for normality and homoscedasticity (Sokal and Rohlf 1995), and the assumptions for ANOVA were not violated. When significant differences were detected among means ($\alpha=0.05$), a *post hoc* Tukey-B test was performed. In Experiment 2, independent samples Student "t" tests were carried out to determine differences between treatments. The number of postlarvae attached to the collectors was compared among treatments by one-way ANOVA and independent samples Student "t" test for experiments 1 and 2, respectively. Linear regression was used to describe the relationship between postlarval shell height and length.

II.3. Results

II.3.1. Experiment 1

II.3.1.1. Growth

The mean shell height of setting pediveligers was 187 μm (se = 0.8) (shell length 200.8 μm). Three days after settlement, mean shell height for all treatments ranged from 197.2 to 199.1 μm and there were no significant differences among treatments (one-way ANOVA, F = 0.038; df = 3,236; P = 0.99) (Fig. II.1). Five days after settlement, there were significant differences in post-larval shell height among treatments (one-way ANOVA, F = 28.68; df = 3,716; P < 0.001). Postlarvae fed at low algal concentration (L) attained a mean shell height of 247.2 μm (se = 2.2), which was significantly greater than in the other treatments. Nine days after settlement, there were also significant differences in shell height among treatments (one-way ANOVA, F = 155.3; df = 3,716; P < 0.001). Postlarvae fed at low algal concentration (L) attained a mean shell height of 333.6 μm (se = 4.85), which was again significantly greater than for postlarvae exposed to the other treatments (Tukey-B). Furthermore, postlarvae cultured at high ration (H) (mean shell height 284.5 μm, se = 3.35) attained a significantly greater shell height than those in the treatments unfed (U) (mean shell height 246.1 μm, se = 2.61) and epiflora (E) (mean shell height 241.8 μm, se = 2.27) (Tukey-B). Postlarvae in the treatments (E) and (U) displayed similar shell heights (Tukey-B). Daily growth rates from day 3 to day 9 were 22.61, 14.49, 7.43 and 8.15 μm d^{-1} for the treatments L, H, B and U, respectively.

II.3.1.2. Survival

The mean number of postlarvae attached to the collectors 5 days after settlement ranged from 166 (se = 46) in the low food concentration (L) treatment to 369 (se = 92) in the epiflora treatment (E) (Fig. II.2). There were significant differences among treatments (one-way ANOVA, F = 6.71; df = 3, 28; P = 0.001). Collectors covered by an epiflora (E) had significantly more scallops than the other treatments, which were statistically similar to each other (Tukey-B). On average, 78 to 120 % more postlarvae settled on the epiflora-covered collectors than on those in the other treatments. On day 9, there were also significantly more postlarvae on the collectors in the epiflora treatment. Overall, there was a decrease of about 26.8 % in the number of spat attached to the collectors from day 5 to 9, and the percent loss of spat from the collectors did not differ among treatments (one-way ANOVA, F = 0.57; df = 3,4; P = 0.67).

II.3.2. Experiment 2

II.3.2.1. Growth

Setting larvae had a mean shell height of 190.7 μm (se = 0.8) (shell length 202.6 μm). Three days after settlement, mean postlarval shell height was 205.0 μm (se = 1.67) and 208.1 μm (se = 1.97) for the treatments of low algal concentration (L) and high algal

concentration (H) respectively, which were statistically similar ("t" test, t = -1.42; df = 1,238; P = 0.255) (Fig. II.3). On the other hand, ten days after settlement postlarvae from treatment (L) had attained a significantly greater shell height (mean = 320.2 μm, se = 5.29) than those from treatment (H) (mean = 295.0 μm, se = 4.07) ("t" test, t = -3.76; df = 1,238; P < 0.001). After 16 days, mean shell heights were 401.08 μm (se = 7.24) and 403.17 μm (se = 7.44) for treatments (L) and (H) respectively, which were statistically similar ("t" test, t = 0.2; df = 1,238; P = 0.84). Daily growth rates from days 3 to 10 were 16.02 and 12.85 μm d^{-1}, respectively for treatments (L) and (H), and from days 10 to 16, 13.48 and 18.03 μm d^{-1}, respectively.

The relationship between shell height (SH) and length (SL) of postlarval *N. nodosus* followed the linear equation **SH= 0.8322 SL + 13.482** (n = 120, r^2 = 0.96) (Fig. II.4), which was highly significant (ANOVA, F = 2488; df = 1,119; P < 0.0001). Such equation is provided as a useful means of comparison of shell dimension with other studies in which sizes are presented as shell length.

II.3.2.2. Survival

The mean numbers of postlarvae attached per collector in treatment H were 397.6, 237.5 and 176.3 for days 3, 10 and 16, respectively (Fig. II.5). In treatment L, the mean numbers of postlarvae attached per collector were 482.5, 447.1 and 445.4 for days 3, 10 and 16, respectively. While there was no statistically significant difference in the number of spat/collector between treatments at day 3, in subsequent days the number of post-larvae

in treatment L was significantly higher than in treatment H. In treatment L, loss of post-larvae from day 3 to 16 was 7 % percent, while in treatment H the loss was 55%.

II.3.2.3. Gut pigments

Total pigments in the gut of larvae decreased from 108.7 ng ind $^{-1}$ to 48 ng ind $^{-1}$ after 24 hours in the absence of suspended food, since algae were not supplied until 3 days post-settlement (Fig. II.6). Five days after settlement, however, postlarvae had a higher pigment content in the gut than in the previous sample, both in treatment H (143.3 ng ind^{-1}) and in treatment L (170.48), which were similar (t test, t = -1.39; df = 1,6; P = 0.1). Ten days after settlement, postlarvae in treatment H displayed a slightly higher gut pigment content (316.11 ng ind^{-1}) than those in the treatment L (212.42 ng ind^{-1}) (t test, t = 1.99; df = 1,6; P = 0.048). Subsequently, at 16 days post-set, the pigment content in postlarvae from treatment H (1307.6)was significantly higher in treatment L (462.5) (t test, t = 4.54; df = 1,5; P = 0.003).

II.4. Discussion and Conclusions

This study focused on shell growth of *Nodipecten nodosus* shortly after settlement. While settlement in bivalves involves a behavioural component of exploratory activity of the substrate in which larvae may alternate periods of crawling and swimming, metamorphosis is an irreversible developmental process of morphological and physiological changes (Chia 1977, Burke 1983). In scallops, larval settlement on artificial collectors and subsequent metamorphosis can extend from hours to several days (Sastry 1979). For example, 50 % of *Pecten maximus* larvae undergo settlement and metamorphosis within a 2-week period (Nicolas and Robert 2001). In the present study, the remaining unsettled larvae were removed from the experimental units 72 h from initiation of the experiment, so that variability in postlarval size was minimised. Scallops displaying conspicuous dissoconch shell were considered to have accomplished metamorphosis.

Growth in juvenile bivalves occurs when energy acquisition exceeds metabolic demand to sustain basic physiological processes, since there is no energy allocation to reproduction. In scallops, active suspension feeding on phytoplankton is the major form of food acquisition (Bricelj and Shumway 1991). During postlarval life, bivalves exhibit fast shell growth rates, which are not accompanied by a proportional increase in biomass. This growth pattern may be a protective adaptation of all bivalves, to reduce susceptibility to predation (Holland and Spencer 1973, Whyte et al. 1992, Lu and Blake 1996). In the present study, therefore, it was assumed that shell growth of postlarval scallops is maximised after the onset of acquisition and utilisation of exogenous food.

There was a significant increase in postlarval shell height (10–13 μm) from settlement to 3 days post-set in all treatments from experiment 1, regardless of the availability of exogenous food. The presence of a biofilm, suspended algae, or dissolved organic matter associated with the algal cultures did not result in faster shell growth than in the unfed control up to 3 days. These results demonstrate that shell growth of *Nodipecten nodosus* immediately after metamorphosis is not dependent on exogenous food, but fuelled by endogenous reserves accumulated during larval life. This was also demonstrated in both treatments of experiment 2, when algae were not supplied for the first 72 hours. Although the complete nutritional requirements of *Nodipecten nodosus* larvae remain unknown, the mixed diet consisting of the flagellate *Isochrysis* sp. (clone T-iso) and the diatoms *Chaetoceros calcitrans* and *C. muelleri* (=*C. gracilis*) used during larval culture is widely used in scallop hatcheries, supporting significant growth and providing a balanced biochemical composition for larvae (Bourne et al. 1989, Farias 2001, Uriarte et al. 2001). In this study, *N. nodosus* pediveligers accumulated sufficient energy reserves to accomplish metamorphosis in the absence of exogenous food. In a study by Lu et al. (1999) *Argopecten irradians* was able to complete metamorphosis when deprived of food, and energy losses accounted for 57.9 % of the total organic reserves. *Ostrea chilensis* pediveligers catabolised 65.5 % of their energy reserves (Videla et al. 1998).

Five days after settlement, growth of postlarvae was significantly higher at low algal concentration (ca. 4.7 $x10^3$ cells mL^{-1}) than in the other treatments. This indicates that postlarval *N. nodosus* at a shell height between 200 and 250 μm (days 3–5) can already capture and utilise phytoplankton as a food source, suggesting that the gills are already capable of capturing particles. Furthermore, 9 days after settlement, postlarvae

were larger when fed at low algal concentration than at high concentration ($4x10^4$ cells mL^{-1}). Algal cell concentrations fed to postlarval scallops in hatcheries usually range from $2.0–2.5x10^4$ cells mL^{-1} (Bourne et al. 1989), $3.5x10^4$ cells mL^{-1} (Dabinett et al. 1999) to $8.0–10x10^4$cells mL^{-1} (examples in Uriarte et al. 2001). The present results indicate that the feeding requirement of *N. nodosus* immediately after metamorphosis is very low, and that high algal concentrations are deleterious to growth for postlarvae smaller than ca. 350 μm. High algal concentration inhibits filtration in bivalve larvae (Gerdes 1983, Sprung 1984, Riisgard 1988). In juveniles and adults the gills are completely functional, and feeding activity can be controlled at high particle concentrations by discontinuous feeding behaviour, reduction of clearance rate, or increasing pseudofaeces production (Bricelj and Shumway 1991). However, none of these mechanisms has been demonstrated in postlarval scallops smaller than 0.5 mm in shell height, in which the gills are not fully developed (Beninger et al. 1994, Veniot et al. 2003). The present study suggests that high algal concentrations may physiologically stress the scallops, preventing them from fully exploiting their growth potential. Riisgard (1991) postulated that low growth rates of bivalves recorded in laboratories may partly be due to unnaturally high algal concentrations, which lead to sub-optimal conditions (valve closure, reduced metabolism, and reduced biosynthesis). Two other studies on postlarval scallops reported an optimum thresholds for growth at low concentrations: $1.0–2.0\ x10^4$ cells mL^{-1} for *Argopecten irradians* (Lu and Blake 1996), and $0.7–1.7x10^4$ cells mL^{-1} for *Pecten maximus* larger than 0.5 mm (Nicolas and Robert 2001).

Nine days after settlement, postlarvae from the epiflora and unfed treatments had reached the same size, and were significantly smaller than fed postlarvae. Surfaces

immersed in seawater are rapidly colonised by bacteria, and subsequently by diatoms and protozoans (Corpe 1970). Thus, the provision of biofilm-colonised substrates provided no nutritional benefit for *N. nodosus* for up to 9 days after settlement. These results suggest that pedal feeding, if indeed it does occur, makes a negligible contribution to growth of *N. nodosus*. It is also possible that the algae growing on the collectors were too large to be ingested by postlarval scallops, or had no nutritional value. Microalgal assemblages on the collectors were dominated by the cosmopolitan non-planktonic chain-forming diatom *Melosira* sp. (Hasle and Syvertsen 1997), which had an individual cell size of ca. 25–30 μm but formed large chains (> 300 μm). Although *Melosira* is an abundant diatom in the gut contents of adult scallops (*Argopecten irradians*) (Davis and Marshall 1961) and *Placopecten magellanicus* (Shumway et al. 1987) it was probably not ingested by postlarval *N. nodosus* either due to the large size of the chains, or absence of pedal feeding behaviour. Within pectinids, pedal feeding has been reported only in *Patinopecten yessoensis* as a vestigial behaviour, and its nutritional contribution is much less than suspended algae and limited to the first week after settlement (O'Foighil et al. 1990, Reid et al. 1992). If pedal feeding is a general mechanism in scallops, the suitability of different species of epibenthic algae that could be induced to grow on the collectors remains an interesting area for future investigations, which could contribute to optimisation of postlarval growth.

Although the presence of an epiflora on the collectors did not enhance growth of postlarval *N. nodosus,* it significantly increased settlement success, leading to 78–120 % more postlarvae on the fouled collectors. Increased settlement induced by the presence of a bacterial film has been demonstrated for some bivalves, including the scallops

Patinopecten yessoensis (O'Foighil et al 1990), *Chlamys hastata* (Hodgson and Bourne 1988) and *Placopecten magellanicus* (Parsons et al. 1993), although in *Pecten maximus* a certain bacterial film did not influence settlement (Trittar et al. 1992). The effects of biofilms on settlement of *N. nodosus* have not been previously studied, and the present results have direct implications for the optimisation of hatchery production. The immersion of the spat collectors in seawater to allow the formation of a bacterial film is therefore a recommended hatchery practice.

As the water used in the experiments passed through a series of filters with decreasing pore size from 60 to 1 μm, the concentration of food particles in the water was negligible, as confirmed by particle counts carried out with the Coulter counter. Furthermore, water was exchanged daily, and tanks were covered to prevent entry of light, thus minimising uncontrolled algal growth. There was an increment in shell height of 55–60 μm in the unfed and epiflora treatments, which was probably sustained by endogenous reserves. Furthermore, mortality in these treatments did not differ from the fed group, suggesting no significant effect of food availability on survivorship during this period. Although survival beyond 9 days post-set was not followed, it is clear that the energy accumulated during the larval phase was sufficient to supply energy for maintenance for at least 9 days after settlement. Considering that acquisition and utilisation of suspended food began 3 to 5 days after metamorphosis, the energy reserves accumulated during the larval period were sufficient to meet the demands of metabolism and growth of *N. nodosus* during the period in which there was no suspension feeding. In contrast to the rock scallop (*Crassodoma gigantea*), in which pediveligers had high mortality during 4 days of starvation (Whyte et al. 1992), *N. nodosus* not only survived, but also

metamorphosed and displayed limited shell growth in the absence of algal food for at least 9 days post-settlement.

According to Whyte et al. (1992) the fatty acid profile of postlarval scallops *Crassodoma gigantea* indicate food assimilation at 25 days after metamorphosis, but their calculations suggest that before this, the acquisition of exogenous energy is not sufficient to meet the metabolic demand for growth, although earlier postlarvae were not analysed. Postlarval mortality of *C. gigantea* was explained by insufficient acquisition of exogenous food soon after metamorphosis to meet the energetic demands of shell growth, which was postulated to be unimpeded by total energy depletion. The results of the present study, however, clearly indicate that shell growth of *N. nodosus* postlarvae is inhibited by the absence of suspended food compared to fed postlarvae, suggesting that energy acquisition from exogenous sources is a limiting factor for shell growth, and endogenous energy is not indiscriminately allocated to growth. Restrained growth in the absence of food may be a strategy to prolong survival under unfavourable conditions. If postlarval growth had been unimpeded until all endogenous energy was depleted, then mortality, not reduction in growth, would have been observed in the unfed treatment. The present results give no indication of mortality induced by deprivation of suspended food for up to 9 days post-settlement, and food acquisition started well before endogenous energy was depleted. It is possible that the tropical scallop *N. nodosus* develops more rapidly than temperate species, so the that gills become functional earlier, being capable of capturing suspended particles at a smaller body size (200–250 μm shell height) than *Patinopecten yessoensis*, in which effective suspension feeding begins at 400 μm (Reid et al. 1992) or *Crassodoma gigantea*, two cold water species. Indeed, Barré et al. (2002), comparing morphological

development of *Placopecten magellanicus* and *Argopecten irradians*, concluded that key events in gill development occurred at a smaller size in *A. irradians*, indicating inter-specific variability in gill ontogeny within pectinids.

Manahan (1983) demonstrated that filtrates from algal cultures (*Thalassiosira pseudonana*) contained large concentrations of dissolved free amino acids, which could be taken up by bivalves. In the present study, the same volume of algal culture media was added for both treatments of high and low food concentrations, but reducing the algal cell density by means of filtration in the latter treatment. This approach ensured that bacteria and concentrations of dissolved nutrients in the algal culture media, as well as exuded dissolved polymers and free amino-acids released by microalgae, which could have nutritional value for bivalves, were similar in both high and low food concentration treatments. Therefore, the only factor differing between treatments was the concentration of algal cells supplied, and any differential contribution of DOM among the treatments in which food was supplied was eliminated.

In experiment 2, after 10 days from settlement, postlarvae cultured at low algal concentration had shell heights significantly larger than those in the other treatments, consistent with experiment 1. However, sixteen days after settlement the shell heights of postlarval scallops were similar between treatments. The increase in the gut pigment content from settlement to day 5 post-set further confirms that at day 5 scallops were already able to ingest phytoplankton. Furthermore, at day 10 the pigment concentration was slightly higher in the scallops cultured at high algal concentration. As growth rates from day 3 to 10 were higher in the scallops cultured at low concentration, postlarvae utilized food more efficiently at low concentrations, as previously discussed. The present

results suggest that there was an increase in food uptake around day 10 at ca. 300 μm shell height, but this increase in ingestion was not immediately reflected in growth, but in the subsequent period, when postlarvae cultured at high algal concentration displayed higher growth rates than those cultured at the low concentration. The sharp increase in the gut pigment of postlarvae from the high concentration treatment from day 10 to 16, suggests that between 300–400 μm shell height there is a significant increase in feeding activity, which is probably associated with a critical stage in the gill development, as described for *Placopecten magellanicus* (Veniot et al. 2003) and *Pecten maximus* (Beninger et al. 1994). Postlarvae cultured at low algal concentration also displayed an increase in pigment content, but less than those at high cell concentration. At this point, the availability of food probably became limiting in the low food treatment and ingestion of algae was therefore lower, but not resulting in immediate differences in shell height among treatments. Two non-exclusive hypotheses can be proposed to explain this result. First, since there is a lag between food acquisition, digestion, assimilation and the shell growth response, differences in shell heights between treatments would only be apparent after day 16, and faster growth rates were indeed displayed by postlarvae at the high food treatment from days 10 to 16. Second, absorption efficiency tends to decrease at high food concentration, as demonstrated for other bivalves (Thompson and Bayne 1974, Griffiths and King 1979, Navarro and Winter 1982, Yukihira et al. 1998). Thus, as the capacity of the digestive gland is exceeded, more organic material is rejected in faeces. In this manner, even at lower food concentration postlarval *N. nodosus* could maximise absorption of ingested food and display significant growth, despite lower algal ingestion. The present results suggest that food levels usually supplied to postlarval scallops in

hatcheries are higher than the demand, and the high cell concentrations supplied may lead to reduced feeding rate and/or lower absorption efficiency, therefore, resulting in a significant waste of cultured microalgae. The present study sets the stage for further studies on the effect of cell concentration on the feeding physiology of scallop postlarvae, in order to maximise growth potential soon after settlement. Feeding early postlarvae at an optimum cell concentration of a suitable algal species could increase growth, significantly reduce the time to transfer to the sea-based nursery, and therefore, reduce production costs.

Survivorship significantly differed between low and high food treatments in experiment 2, higher mortality being recorded at the higher food level. It is unlikely that the high cell concentration directly caused scallop mortality, but an excess of microalgae may have led to settlement of cells to the bottom of the tank, where they may have stimulated bacterial proliferation and reduced water quality. Nicolas and Robert (2001), also found the lowest mortality in postlarval *Pecten maximus* fed at the lowest food concentration. High bacterial concentration and accumulation of ectometabolites are detrimental to larval and postlarval bivalves (Loosanoff and Davis 1963, Elston and Leibovitz 1980, Prieur et al. 1990). Likewise, larval pectinids are highly susceptible to bacterial infections in hatcheries (Freites et al. 1993, Riquelme et al. 1995, Nicolas et al. 1996, Sainz et al. 1999), being more vulnerable to vibriosis than are larval oysters and penshells (Luna-Gonzales et al. 2002). The relatively immature immune system in larval pectinids compared with other bivalves may explain such differential susceptibility to pathogens (Luna-Gonzales et al. 2002). Furthermore, differences in the immune system of adult scallops have been reported. Granular hemocytes, the cells more actively

involved in the immune defence in oysters and mussels (Cheng 1981, Hine 1999), have not been detected in *N. nodosus* (Vargas-Albores and Barraco 2001) nor in other scallops (Hine 1999). In a mytilid (*Mytilus galloprovincialis*), antimicrobial peptides, mytilina and defensina are synthesized during and after metamorphosis, respectively (Mitta et al. 2000). Although there is a lack of immunology studies of the early life stages of scallops, it is possible that early post-metamorphic scallops have a delayed development of the immune system compared with other bivalves, and are therefore more susceptible to bacterial infection in hatcheries.

In conclusion, the present study demonstrated that the presence of epiflora on the collectors significantly increased settlement of the lion's paw scallop, but did not enhance postlarval growth. Furthermore, settlement, metamorphosis and early postlarval growth were accomplished in the absence of suspended food, but 3–5 days after settlement, the availability of algae was an important factor determining growth of *N. nodosus*, and a low concentration (ca. 4.7 $x10^3$ cells mL^{-1}) of *Isochrysis* sp. (clone T-iso) and *Chaetoceros muelleri* was more advantageous to growth and survival than 4 $x10^4$ cells mL^{-1}. The absence of exogenous food does not limit survival for at least 9 days after settlement, since ingestion of exogenous food started well before that. On the contrary, at high food concentration (4 $x10^4$ cells mL^{-1}) mortality increased. Depletion of endogenous reserves before initiation of suspension feeding did not account for postlarval mortality. Alternatively, an immature immune system in early postlarval scallops, and therefore high susceptibility to disease, may explain the high postlarval mortality often reported in scallop hatcheries.

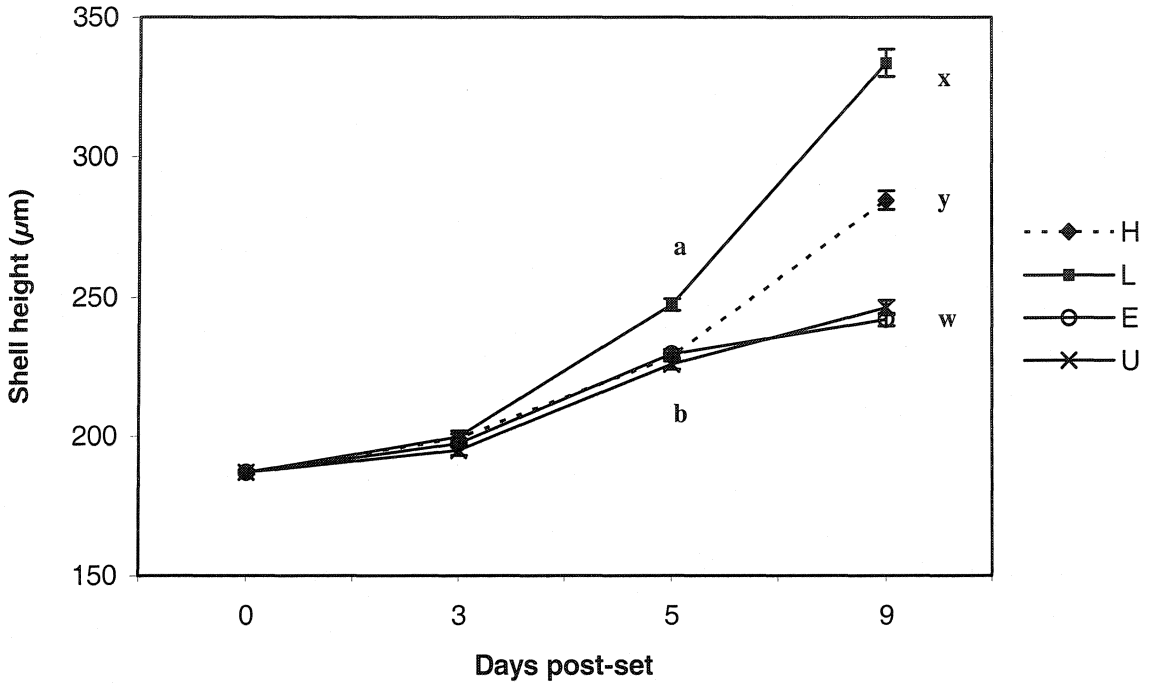


Figure II.1. Shell heights (μm) of setting pediveligers (day = 0) and postlarvae 3, 5 and 9 days after settlement, cultured under four treatments (H = high algal concentration, L = low algal concentration, E = collectors covered by epiflora, U = no algae supplied). (mean ± se) (For each day, different letters denote significant differences).

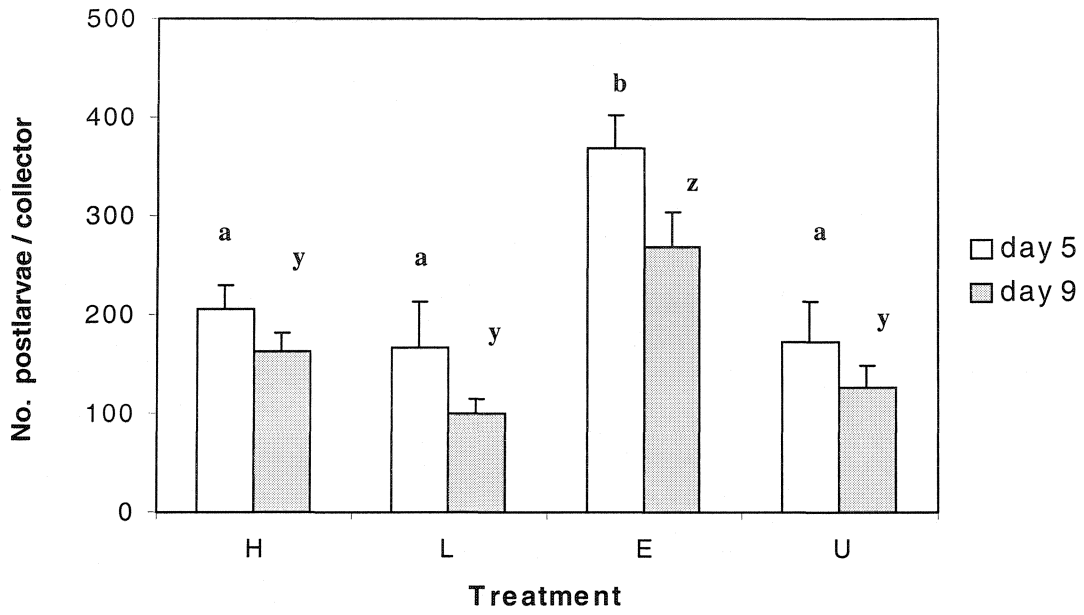


Figure II.2. Number of postlarvae attached to collectors 5 and 9 days after settlement, cultured under four treatments (H = high algal concentration, L = low algal concentration, E = collectors covered by epiflora, U = no algae supplied). (mean + se) (For each sampling date, different letters denote significant differences).

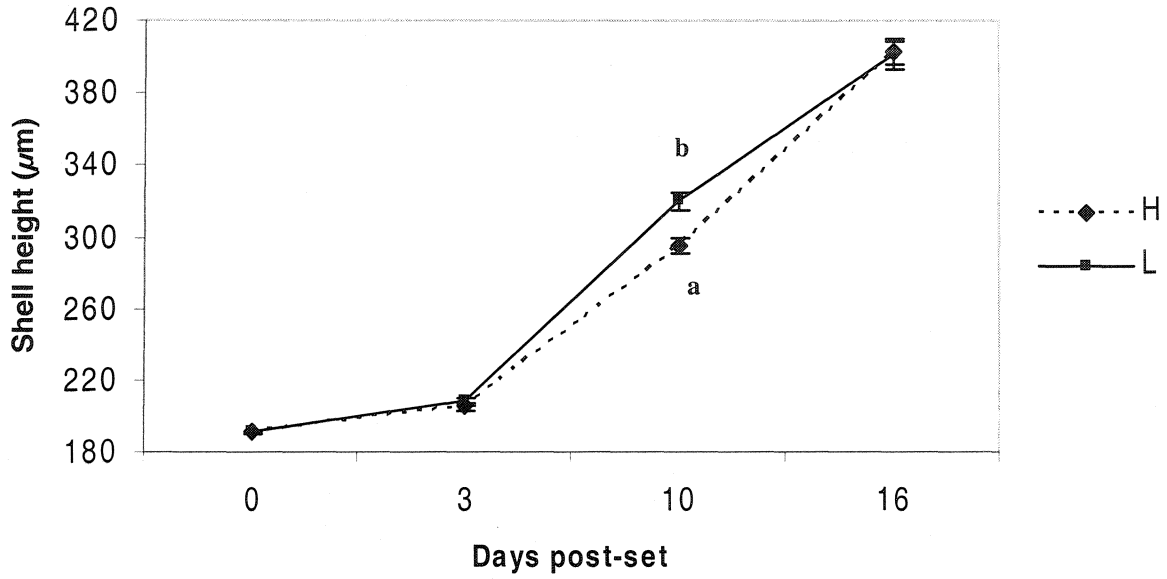


Figure II.3. Shell heights (μm) of setting pediveligers (day = 0) and postlarvae 3, 10 and 16 days after settlement, cultured under two treatments (H = high algal concentration, L = low algal concentration). (mean ± se) (Different letters denote significant differences).

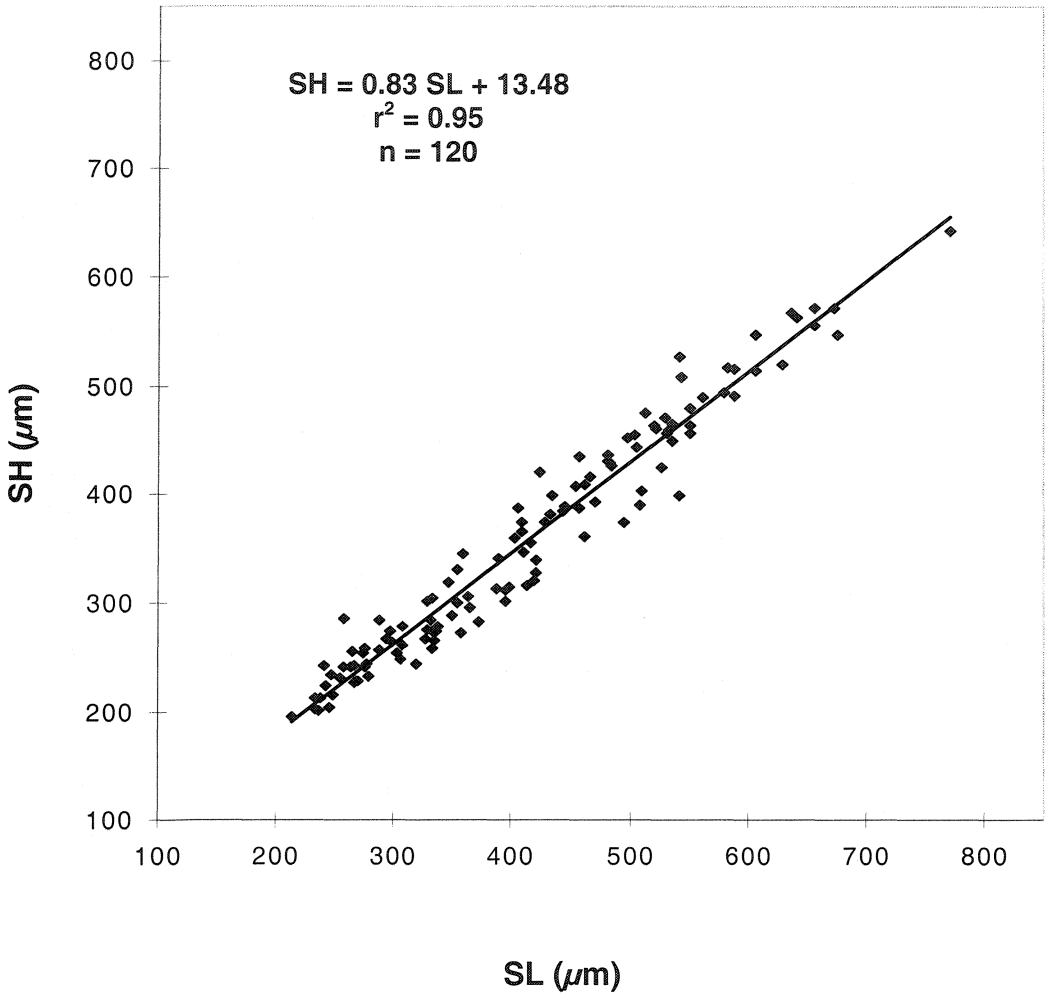


Figure II.4. Relationship between shell height (SH) and shell length (SL) of postlarval *Nodipecten nodosus* ($P < 0.001$).

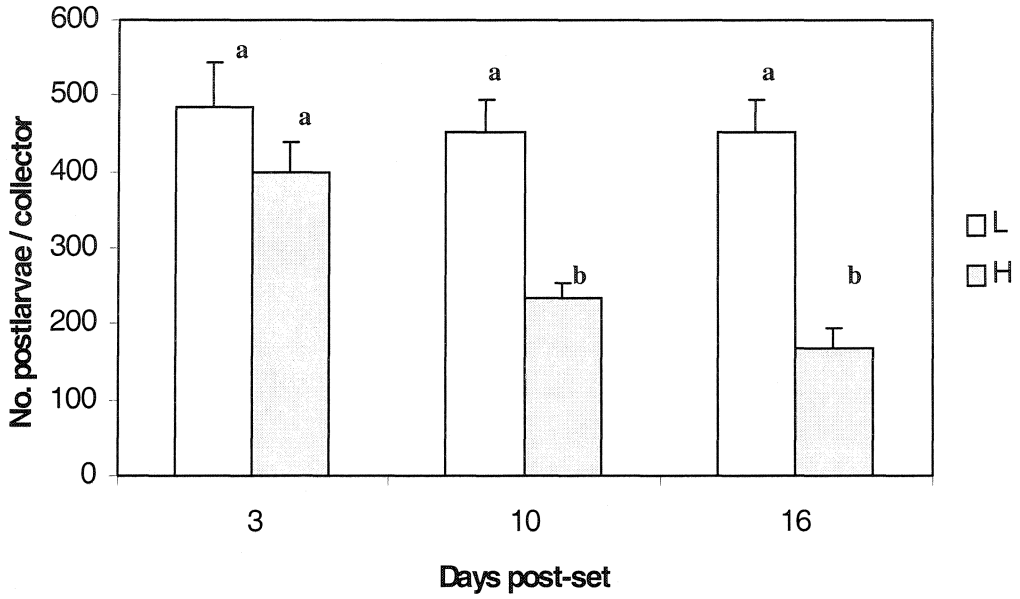


Figure II.5. Number of postlarvae attached to the collectors 3, 10 and 16 days after settlement, cultured under two treatments (H = high algal concentration, L = low algal concentration). (mean + se) (Different letters denote significant differences).

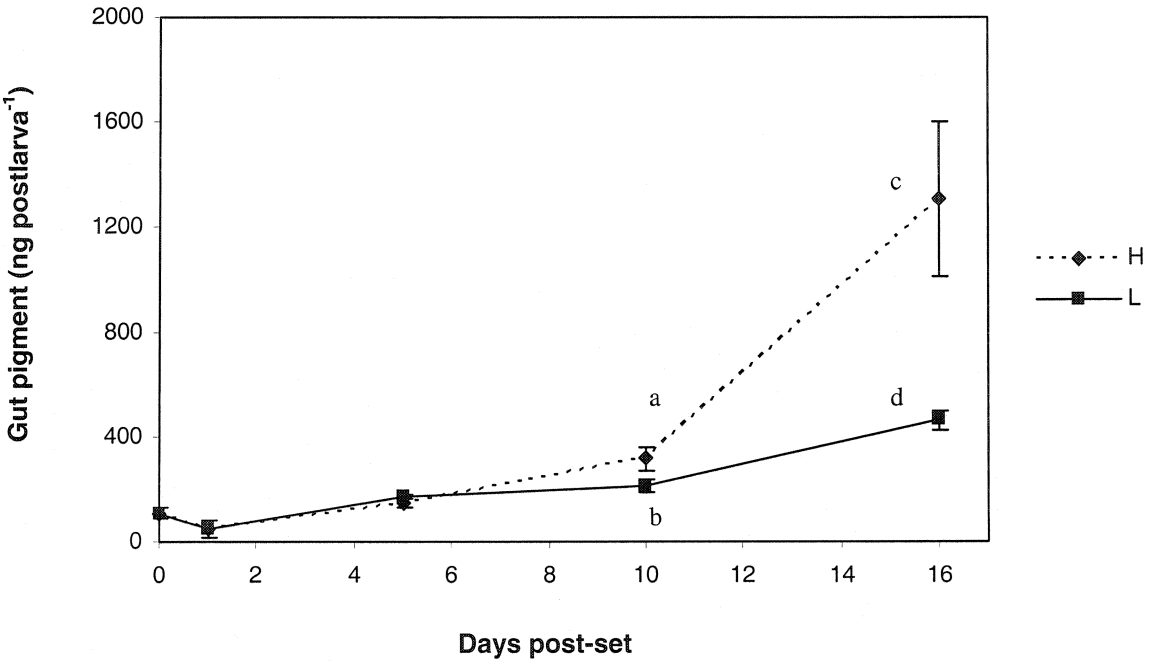


Figure II.6. Pigment content (ng postlarva^{-1}) determined by gut fluorescence of postlarvae cultured under two treatments (H = high algal concentration, L = low algal concentration) after 1, 5, 10 and 16 days post-settlement. (mean ± se) (Different letters denote significant differences).

CHAPTER III

Influences of Environmental Factors, Season and Size at Deployment on Growth and Retrieval of Postlarval *Nodipecten nodosus*

III.1. Introduction

Nodipecten nodosus is a promising aquaculture species in Brazil, and hatchery production is the only feasible approach to supply juveniles (Rupp 1994, Uriarte et al. 2001). The intermediate step between the controlled hatchery situation and growout in the sea is known as nursery culture (Claus 1981). The first phase of nursery includes the period from metamorphosis to approximately 1 mm shell height, when postlarvae are cultured in land-based facilities, usually attached to monofilament collectors or other substrates. Unlike other bivalves that strongly attach to the substrate, scallop postlarvae are extremely fragile and easily detachable from the collectors, being difficult to manipulate. Therefore, culturing them requires nursery strategies different from those used for some other molluscs. The second phase of nursery culture, until juveniles reach about 5–10 mm in shell height, can be either land-based (upwellers, downwellers, re-circulating waters, or flow-through systems), or carried out in the sea (Bourne and Hodgson 1991). The sea-based approach consists of transferring spat collectors inside nylon mesh bags or in mesh-lined plastic trays directly to the sea after several weeks of land-based nursery, when spat are generally larger than 1 mm (Bourne and Hodgson 1991, Uriarte et al. 2001). High losses are reported in both types of nursery culture. In the sea-based nursery, spat may be subject to high mortalities due to environmental factors such as salinity and temperature exceeding the limits of tolerance, smothering, predation or handling (Bourne and Hodgson 1991). In the land-based nursery, high mortalities have been explained by nutritional deficiencies resulting from limited filter-feeding capabilities of early postlarvae (Whyte et al. 1992, Beninger et al. 1994), but see Chapter II. In

addition, intensive culture facilities for molluscs may be subject to infectious disease outbreaks causing high mortalities (Elston 1984).

Growth of spat held in land-based nurseries, after a certain size, is limited by nutritional deficiencies of the cultured microalgae (Bourne et al. 1989, O'Foighil et al. 1990, Coutteau and Sorgeloos 1992, Whyte et al. 1992, Heasman et al. 1996). Furthermore, the increasing quantity of algae required to satisfy the growing demand of a large number of organisms and associated costs, is also a factor limiting the maintenance of bivalves for long periods under artificial conditions (De Pauw 1981, Rhodes et al. 1981), requiring transfer of spat to the sea-based nursery as early as possible. However, deployment of small scallops may be constrained by the small mesh size required to retain them and by losses due to detachment, and this approach has not been well documented in the literature. While nursery culture for temperate and boreal scallops has been studied (O'Foighil et al. 1990, Bourne and Hodgson 1991, Widman and Rhodes 1991, Grecian et al. 2000), there have been fewer studies in subtropical regions (Martinez et al. 1992), and none on nursery culture of *N. nodosus*.

The study area is a subtropical region near the high latitudinal distribution limit of *N. nodosus* in the Southern Hemisphere (Lat. 27° 50' S), which is classified as a warm-temperate marine biogeographic province (Briggs 1995). The oceanographic patterns of the southeastern Brazilian coast have been studied on meso- and large-scales, but no study has so far addressed the effects of abiotic and biotic environmental factors on growth and survival of marine bivalves in this region. According to the general oceanographic model proposed originally by Emilsson (1961), the southeastern Brazilian coast is seasonally influenced by different water masses. Subsequent studies by Matsuura (1986), Brandini

(1990), Castro and Miranda (1998), Sunyé and Servain (1998), and Borzone et al. (1999) have surveyed the neritic region beyond the 20 m isobath, including waters off Santa Catarina State. From these studies it can be inferred that during summer, surface layers of the inner shelf off Santa Catarina State are influenced by Coastal Water (CW - salinity < 35 psu) or Shelf Water (SW - salinity 35–36 psu), with high temperature (20–28°C) and oligotrophic characteristics, whereas further offshore, warm, oligotrophic and high salinity (> 36 psu) Tropical Water (TW) is present. On the contrary during winter (June to September), CW disappears and is replaced mainly by a coastal branch of the Malvinas Current (sub-Antarctic water – SAW), with cold (< 20°C), low salinity (as low as 29 psu) and phytoplankton-rich waters (Sunyé and Servain 1998). As a result, waters off Santa Catarina State are subject to a strong semi-annual thermal cycle in which food availability and temperature tend to vary in an inverse manner. This pattern differs from higher latitudes, where there are also marked seasonal variations in temperature and phytoplankton biomass, but both factors generally co-occur, making it difficult to decouple the effects of food and temperature on bivalve growth (Orensanz et al. 1991). In temperate and boreal regions, the relative importance of temperature and food availability on growth has been controversial. While Vahl (1978), MacDonald and Thompson (1985), Wallace and Reinsnes (1985), and Thorarinsdóttir (1994) found that food availability was the most important factor influencing somatic growth of scallops in the sea, Andersen and Naas (1993), Kleinman et al. (1996), Chauvaud et al. (1998) and Laing (2000) concluded that temperature was the major factor driving growth of scallops. On the other hand, in a tropical region growth of juvenile scallops was not influenced by food variability or temperature (Lodeiros and Himmelman 1994, 2000), but after sexual maturity, periods of

low food abundance and high temperature, which coincided with high settlement of biofouling organisms on shells, were stressful for the scallops (Lodeiros and Himmelman 1996, 2000). It is not clear the extent to which the variability in conditions reported for neritic waters of the inner continental shelf in southern Brazil, influence sheltered aquaculture sites. In addition, it is not known whether growth of bivalves is mainly driven by temperature or food availability in this subtropical environment, nor whether such variability is an important aspect that should be considered in developing aquaculture strategies.

The size at which scallops are transferred to the sea is an important factor for the success of nursery culture, and the trade-off among growth, percentage retrieval and costs of maintaining spat in the sea and in the land-based nursery must be evaluated in order to establish protocols for optimising yields. Furthermore, when deployed in the sea, scallops are exposed to seasonal variation in environmental factors, and an understanding of the influence of temporal variation in food availability and abiotic variables on growth and survival of spat is essential for the establishment of viable culture strategies in southern Brazil. While the sea-based nursery approach has been studied for scallops larger than 1.5 mm (O'Foighil et al. 1990, Bourne and Hodgson 1991, Widman and Rhodes 1991, Grecian et al. 2000, Christophersen and Magnesen 2001), growth and survival in the sea of scallops smaller than 1 mm have only been addressed in one study (Heasman et al. 2002), but environmental influences were not considered. The present study the first to use the approach of assessing the influences of food availability and abiotic factors on scallops deployed in the sea-based nursery at a size of 0.5 mm, as well as in comparing

growth and percentage retrievals of postlarvae deployed at different sizes (0.29 to 1.1 mm) and culturing them simultaneously in the sea and in the laboratory.

The present study was designed to test the hypotheses that (1) seasonal variability in environmental factors significantly influences growth rates of postlarval and juvenile *N. nodosus* in a coastal sheltered subtropical environment, with growth mainly driven by temperature rather than food variation, (2) after a certain size, postlarvae deployed in the sea-based nursery display higher growth rates than those maintained in the land-based nursery, and (3) loss of spat in the sea-based nursery is initially high and then stabilizes. The specific objectives of the present study were: (1) to determine the influence of seasonal variation in food availability and abiotic environmental factors on growth and percentage retrieval of postlarval scallops deployed in the sea-based nursery, (2) to compare growth rates and percentage retrievals of scallops deployed at different sizes as well as maintained simultaneously in the sea-based and land-based nurseries, determining the size at which growth becomes enhanced under field conditions, and (3) to assess growth and percentage retrieval of spat after consecutive samplings.

III.2. Materials and Methods

III.2.1. Study area

Larvicultures and land-based nursery experiments were undertaken at the Laboratory for the Culture of Marine Molluscs (LCMM - UFSC), in Florianópolis, Santa Catarina State, Brazil. Sea-based nursery experiments were carried out on the East side of João da Cunha Island, Porto Belo (Lat. 27° 08' S; Long. 48° 33'W) (Fig. I.2), Santa Catarina State, located 90 km from the laboratory.

III.2.2. Environmental variables

Water samples were taken weekly at the field site with a 2-L Van Dorn sampling bottle and transferred to the laboratory in a cooler containing ice packs. Temperature in the sea was recorded hourly with Stowaway® data loggers and dissolved oxygen was determined *in situ* with a digital dissolved oxygen meter (YSI model 55/12FT). Within two hours, water samples were gently stirred and filtered through a 350-μm-mesh screen to remove larger zooplankton. Total suspended particulate matter was determined gravimetrically after filtering 400 mL seawater on a 24 mm diameter Whatman 934-AH glass microfibre filter (pore size 1.5 μm) and drying for 72 h at 60°C. Particulate organic and inorganic matter were determined by weight loss on ignition at 450°C as described by Parsons and Dadswell (1992). Chlorophyll-*a* concentration was determined with a Turner™ digital fluorometer (TD 10-AU), after filtering triplicate, 100-mL water samples

and extracting chloropigments in 90 % acetone, using standard methods (USEPA Method 445.0). Turbidity was determined by a Lamotte 2020 turbidity meter and salinity by a digital WTW Multiline P4 water quality meter. All variables were determined in triplicate samples.

Rainfall data were obtained from a meteorological station (CLIMERH – Centro Integrado de Meterologia e Recursos Hídricos) located in Itajaí (SC), approximately 35 km NW from the study site. Precipitation was recorded daily and cumulative precipitation was calculated for the periods during which the nursery culture experiments were carried out.

III.2.3. Spat production

Broodstock (shell height 12–14 cm) were collected in the wild by SCUBA diving and maintained in lantern nets at the field site throughout the study. Scallops were transferred to the laboratory 2 to 3 weeks prior to spawning and maintained in tanks with flowing seawater at temperature of 17–18°C, fed a mixture of cultured microalgae (*Isochrysis* sp. (clone T-iso), *Chaetoceros calcitrans*, *C. muelleri*) at final concentrations of 4–6x10^4 cells mL^{-1} (Rupp et al. 1997). Broodstock (8-12 scallops) were induced to spawn and gametes pooled from several scallops were used for fertilization. Five larval cultures were carried out using hatchery techniques described in Chapter II. When larvae (pediveliger) displayed certain morphological and behavioural changes (conspicuous eyespot, protrusion of foot and crawling behaviour), and attained shell lengths of 200–210 μm (shell height 185–190 μm), they were considered competent to undergo settlement and

metamorphosis. Pediveligers were then retained on a 140-μm-mesh Nytex screen, sub-sampled, counted in a Sedgwick-Rafter chamber under a microscope, and then transferred to the experimental nursery tanks. The substrates used for larval settlement were polyethylene screens (Nortene®) with 3-mm mesh which in previous experiments had resulted in high settlement and were convenient to manipulate for experimental purposes. The collectors were cut into rectangles of 25 X 50 cm (mean weight of 31.35 g; se = 1.4 g) and the edges of the longer sides were tied together, so that they assumed a cylindrical shape. The relationship between the number of collectors and the volume of water was similar in all experiments, and the number of eyed pediveligers deployed per tank ranged from 1.2 to 1.9 larvae mL^{-1}. The collectors were thoroughly cleaned with 5 % sodium hypochlorite solution and then rinsed in filtered seawater before use.

Settlement and land-based nursery cultures were carried out in 100 or 300-L fibreglass tanks. Seawater was UV-irradiated, filtered through a 1 μm cartridge filter and salinity was 33–34 psu. Water was changed daily, and diet consisted of a mixture of at least three of the following species: *Isochrysis* sp. (clone T-iso), *Chaetoceros calcitrans*, *C. muelleri* and *Nanochloropsis occulata* in exponential growth phase, at final concentrations ranging from $4x10^4$ cells mL^{-1} after larval settlement to 8 $x10^4$ cells mL^{-1} prior to final deployment in the field. To reduce the risk of bacterial outbreaks during experiments, a bacteriostatic agent (chloramphenicol – 12.5 mg L^{-1}) was added to the microalgal cultures 1 h before feeding the scallops. The final concentration of chloramphenicol in the experimental tanks was lower than 0.5 mg L^{-1}, after food was

supplied. In the laboratory, water temperatures were maintained at 24–25°C for experiments 1 and 3, and 23–24°C for the other experiments.

III.2.4. Transfer to the sea-based nursery

For every spat deployment in the sea-based nursery, collectors were taken from a setting tank and placed inside spat bags (3 collectors/bag; 3 bags). This procedure was repeated for each of the setting tanks (3 replicates). Spat bags consisted of 0.7 mm-mesh (diagonal) nylon bags with one end sealed by a Velcro strip to allow opening and closure. The total number of collectors and spat bags varied among nursery culture experiments, depending on the number of deployments and retrievals, but triplicate spat bags were retrieved at any given sampling.

Prior to each deployment, 3–4 collectors were sampled per tank in order to determine the initial number of postlarvae. Collectors were thoroughly brushed with repeated gentle movements inside plastic trays filled with filtered seawater. Detached spat were then screened, separated from debris, and preserved in 4 % buffered formaldehyde for further counting and measurements

The nylon spat bags to be transferred to the field were placed inside plastic bags filled with filtered seawater which were tightly closed and transported to the field site inside Styrofoam containers. Transfer time from experimental tanks to the sea was about 3 h for all nursery culture experiments. Emersion time of the collectors was less than 1 minute, at transfer from the experimental tanks to the transport bags and at deployment in

the sea. Water temperature during transfer remained within 2°C of the temperature in the experimental culture tanks. For the winter and early-spring deployments, the seawater temperature in the sea-based nursery site was lower than in the land-based facilities, so the water in the experimental tanks was cooled overnight (Δ 2°C), in order to minimise thermal shock to postlarvae and reduce possible spat detachment upon deployment in the sea at a lower temperature (Bourne and Hodgson 1991).

At the sea-based nursery, the spat bags were tied to a surface long-line 2 m below the surface by SCUBA diving at a 5 m deep site. During the immersion period, the outside of each spat bag was cleaned weekly by gentle brushing to prevent fouling and sediment build up, so that water flow was not be impeded. Similar collectors without spat were deployed inside spat bags during three of the nursery experiments in order to determine natural settlement, which resulted in no wild spat of *N. nodosus* settled on the collectors. Therefore, the influence of natural settlement in the experiments was ruled out. For retrievals, spat bags were taken from the long-line by SCUBA diving, placed in plastic bags filled with site seawater, tightly closed, placed inside Styrofoam containers and returned to the laboratory for further analysis.

III.2.5. Experimental approach

III.2.5.1. Seasonal comparison

The influence of seasonal variability of environmental factors on daily growth rates and percentage retrieval of scallops (objective 1) was determined in five seasonal deployments (SD) in the sea-based nursery, carried out in: late winter–early spring-00 (W-S_p), late spring-00–early summer-01 (S_p-S_u), late summer–early autumn-01 (S_u-A), late autumn–early winter-01 (A-W), and late winter–early spring-01 (W-S_p). Postlarvae were deployed in the sea-based nursery 14 to 17 days after settlement at about 0.5 mm shell height and retrieved 30–33 days later (Table III.1).

III.2.5.2. Comparison of land-based and sea-based nursery

The comparison of growth rates and percentage retrievals between post-metamorphic scallops in the land-based nursery and those in the sea-based nursery (objective 2) was carried out in nursery culture experiments (NC) in late winter–early spring-00 (NC-1) and in late summer–early autumn-01 (NC-2) (Table III.2). Postlarvae were deployed in the sea two weeks after settlement (shell height ca. 0.5 mm), while a corresponding group was simultaneously maintained in the laboratory for an identical period. A second group of postlarvae were deployed in the sea 10–11 days after the first deployment (24–25 days post-set; shell height ca. 0.8–0.9 mm), and a third group was deployed 10–11 days later (five weeks post-set; shell height ca. 1.1 mm). For each

deployment, triplicate spat bags were retrieved from the sea after 10–11 day intervals. The final retrieval from all three groups deployed was carried out 10 days after the final deployment (i.e., 30–33 days after the first deployment; 44–47 days post-set).

The influence of spat size at deployment was also studied in late winter–early spring-01 (NC-3), when final shell height and percentage retrievals of postlarvae from the sea-based nursery were compared among three groups deployed at different initial sizes (mean shell heights 0.29, 0.54 and 0.72 mm) after 7, 17 and 27 days in the laboratory.

Land-based nursery cultures of postlarvae were undertaken under conditions described in *Spat production* (III.2.3). Sampling for determination of shell height and number of spat attached per collector was carried out on the same dates the collectors were transferred to, or retrieved from the sea-based culture. In addition, the number of spat detached from the collectors during transport was determined simultaneously in three of the nursery culture experiments (NC-2, 3 and SD-4).

III.2.5.3. Consecutive samplings from the sea-based nursery

Growth and percenaget retrievals of postlarval scallops in consecutive samplings (objective 3) were determined in nursery cultures simultaneously with the seasonal deployments (SD) 3 and 4 carried out in late summer–early autumn-01 and in late autumn–early winter-01 (Table III.3). Postlarvae were deployed in the sea-based nursery two weeks after settlement and retrieved at intervals of 10–11 days until 53 days post-deployment.

III.2.5.4. Growth rate

After the spat collectors were retrieved from the sea-based nursery, spat were detached and shell heights were measured with an ocular micrometer (± 0.01 mm) using a compound or a dissecting microscope, depending on spat size. Daily growth rates (DGR) were calculated for scallops in each replicate bag by subtracting the initial mean shell height at deployment (SH_i), from the shell height at the end of the experimental period (SH_f) and dividing by the period of time (in days) the spat were maintained in the sea-based or in the land-based nursery [$DGR = (SH_f - SH_i)\, d^{-1}$] (n = 30; when fewer spat were retrieved, all were measured). Shell height, rather than tissue weight was used as a growth parameter in the present experiment, since post-metamorphic scallops display allometric relationship between shell height and tissue weight, growth in size being much faster than growth in tissue weight (Lu and Blake 1996).

III.2.5.5. Percentage retrieval

Retrieval of spat from the sea-based nursery is defined as the number of live scallops remaining in a spat bag after mortality and/or losses due to detachment and escape through the nets. The number of spat retrieved was determined by counting the total number of postlarvae per collector for a given sampling date. Percentage retrieval was determined in relation to the initial mean number of spat settled on the collector for a given deployment. In the land-based nursery, percentage survival refers to the number of

live scallops present after a given period in relation to the initial number of postlarvae settled on the collectors.

III.2.6. Statistical analyses

Data were analysed with the SPSS statistical package (version 10). One-way ANOVA was undertaken to determine the differences between environmental factors among the experiments carried out at different seasons, as well as for differences in DGR and shell height of scallops, at a significance level of 0.05. Residuals were checked graphically for normality and homoscedasticity. If assumptions for ANOVA were violated, variables were log 10 transformed (Sokal and Rohlf 1995) and residuals re-examined. Since turbidity data violated the assumptions of ANOVA before and after transformation, the non-parametric Kruskal-Wallis test was used in this case. When significant differences were detected among means (α= 0.05), a *post hoc* Tukey B test was performed. Spearman correlation analysis was used to determine significant correlations among environmental variables and between mean daily growth rates and environmental variables (significance at $P < 0.05$). Environmental variables that were significantly different among seasons and independent (temperature, salinity, turbidity, TPM) were used to build a multiple linear regression model, using the enter method, to identify the factors best explaining variability in growth. The comparison of daily growth rates between spat maintained in the land- and sea-based nurseries was performed with two-way ANOVA followed by an independent sample Student "t" test to compare DGR between experiments.

III.3. Results

III.3.1. Seasonal comparison

III.3.1.1. Environmental variables

There were significant differences in environmental factors among the nursery culture experiments carried out in different seasons (Fig. III.1). Temperature was significantly higher in spring-00–summer-01 (S_p-S_u) and summer–autumn-01 (S_u-A), when maximum values reached 28.4 and 28.6°C and means were 25.6 and 26.1°C, respectively (Fig. III.1A). Temperature for winter–spring-00 (W-S_p-00), autumn–winter 01 (A-W) and winter-spring-01 (W-S_p-01) averaged 17.7, 20.4 and 20.8°C, respectively, being significantly lower in W-S_p-00, when minimum values reached 16.1°C. Salinity was significantly higher in S_p-S_u averaging 33.7 psu, whereas in W-S_p-00 and W-S_p-01 salinities were significantly lower, averaging 31.3, 30.3 psu, respectively (Fig. III.1B), with minimum values of 29.4 and 26.7 psu, respectively. Maximum concentrations of chlorophyll-*a* were recorded during W-S_p-00 (2.75 μg L^{-1}) and W-S_p-01 (3.02 μg L^{-1}), when mean concentrations were 1.41 and 1.7, mg L^{-1} respectively (Fig. III.1C). Mean concentrations of chlorophyll-*a* in S_p-S_u, S_u-A and A-W were 0.77, 0.67, and 0.94 μg L^{-1}, respectively, with maximum values reaching 1.19, 1.68 and 1.78 μg L^{-1}, respectively. However, differences in chlorophyll-*a* among nursery cultures were not statistically significant (P = 0.056). Total suspended particulate matter (TPM) in W-S_p-00, Sp-Su and

W-S_p-01 averaged 4.36, 6.73 and 6.53 mg L^{-1}, respectively (Fig. III.1D), being significantly higher than TPM recorded in A-W (1.70 mg L^{-1}). TPM in S_u-A (2.13 mg L^{-1}) was similar to W-S_p-00. Particulate inorganic matter (PIM) and particulate organic matter (POM) followed the same pattern as TPM (Fig. III.1E). Mean PIM in W-S_p-00, S_p-S_u and W-S_p-01 (3.33, 5.22 and 5.38 mg L^{-1}, respectively) was significantly higher than in A-W (1.29 mg L^{-1}), but mean PIM in nursery cultures W-S_p-00 and S_u-A was similar (1.66 mg L^{-1}). Mean POM in nursery cultures W-S_p-00, S_p-S_u and W-S_p-01 (1.03, 1.51 and 1.15 mg L^{-1}, respectively) was significantly higher than in A-W (0.41 mg L^{-1}), but mean POM in nursery cultures 3 (0.48 mg L^{-1}), 1 and 5 were similar. Mean percent PIM ranged from 73.4 to 81.7 % throughout the study, and the ratio PIM:POM averaged from 3.1 to 4.5 with no significant differences among seasons (Figs. III.1F and G). Percent oxygen saturation, ranging from 88.7 to 95.1 %, was also similar among seasons. Results of the ANOVA comparing environmental factors among nursery cultures are shown in Table III.4. Mean turbidity in W-Sp-01 (4.48 NTU) was significantly higher than in the other nursery cultures (0.77 to 1.63 NTU) (Fig. III.1H). Cumulative precipitation at a nearby station during W-Sp-01 was 440.4 mm, whereas during the other nursery cultures it ranged from 119.7 to 157.1 mm (Fig. III.2).

Spearman correlation analysis (Table III.5) indicated that temperature was significantly correlated with salinity ($P = 0.023$). Chlorophyll-*a* was positively correlated with POM ($P = 0.006$), PIM ($P = 0.013$), TPM ($P = 0.007$) and turbidity ($P = 0.003$), and was negatively correlated with salinity ($P = 0.017$).

III.3.1.2. Growth rate

Postlarval scallops deployed to the sea-based nursery at various times of the year approximately two weeks after settlement (14 to 17 days) had a mean shell height ranging from 0.48 to 0.53 mm. Final shell heights, determined 30 to 33 days after deployment, were 2.03, 5.34, 4.96, 4.52 and 2.88 mm, respectively, for the five consecutive seasonal deployments (W-S_p-00, S_p-S_u, S_u-A, A-W and W-S_p-01) (Fig. III.3), and significant differences in DGR were recorded ($F = 42.0$; $df = 4,10$; $P < 0.001$). Spat deployed in W-S_p-00 displayed significantly lower DGR (0.045 mm d^{-1}) than spat deployed at all other seasons. Spat deployed in S_p-S_u (0.152 mm d^{-1}), S_u-A (0.149 mm d^{-1}) and A-W (0.130 mm d^{-1}) had significantly higher DGR than those deployed in W-S_p-01 (0.078 mm d^{-1}).

Spearman correlation analysis, indicated significant positive correlations between DGR and temperature ($P < 0.001$) and between DGR and salinity ($P < 0.001$). Negative correlations were found between DGR and chlorophyll-*a* ($P < 0.001$), and DGR and turbidity ($P = 0.0024$).

The relationships between DGR and environmental factors were examined by multiple linear regression. Temperature was the main factor affecting growth, explaining 69 % of the observed variation in DGR ($F = 28.33$; $df = 1,13$; $P < 0.001$). A model including temperature and turbidity explained 78.2 % of the variability in DGR ($F = 21.48$; $df = 2,12$; $P < 0.001$). The addition of salinity and TPM to the model explained 90.3 % of the observed variability in DGR ($F = 23.39$; $df = 4,10$; $P < 0.001$). The multiple regression equation of growth rate and environmental factors is expressed as: **DGR = 65.69 + 0.46 Temp – 2.37 Sal – 1.97 Turb + 0.88 TPM,** where, DGR = daily growth

rate, Temp = temperature, Sal = salinity, Turb = turbidity and TPM = total particulate matter.

III.3.1.3. Percentage retrieval

The percentage retrievals of *Nodipecten nodosus* deployed at the sea-based nursery in different seasons at an average initial shell height of approximately 500 μm and retrieved after 30 to 33 days of suspended culture were, 3.36, 6.04, 15.24, 42.8, and 1.45 % for nursery cultures deployed in W-S_p-00, S_p-S_u, S_u-A, A-W and W-S_p-01, respectively (Fig. III.4).

III.3.2. Comparison of land-based and sea-based nursery

III.3.2.1. Three spat deployments at different sizes with simultaneous culture in sea- and land-based nurseries

Two weeks after settlement, mean shell heights of postlarval scallops were 0.534 mm and 0.505 mm, respectively for nursery cultures NC-1 (late winter–early spring-00) and NC-2 (late summer–early autumn-01), when the comparison of scallops in the land- and sea-based nurseries began. Growth of postlarvae in the sea was faster than in the land-based nursery. After 20–22 days of deployment (34–36 days post-set), scallops maintained in the sea attained a mean shell height of 1.61 mm in NC-1 and 2.95 mm in NC-2, whereas those maintained in the land-based nursery had a mean shell height of 1.13

mm and 1.14 mm, respectively. The resulting DGR of postlarvae held in the sea-based nursery was 0.045 mm day^{-1} in NC-1 and 0.122 mm day^{-1} in NC-2, both being significantly higher ($F = 93.2$; $df = 1,8$; $P < 0.001$) than the DGR displayed by spat maintained in the land-based nursery (0.027 and 0.032 mm day^{-1}) (Fig. III.5). There were also significant differences in DGR between NC-1 and NC-2 ($F = 52.1$; $df = 1,8$; $P < 0.001$), and a significant interaction between ambient (sea-based or land-based) and nursery culture ($F = 41.04$; $df = 1,8$; $P < 0.001$). This interaction was due to the significant differences in DGR of postlarvae maintained in the sea ("t" test, $t = -7.24$; $df = 1,4$; $P = 0.002$), since the DGR of postlarvae maintained in the land-based nursery was statistically similar between NC-1 and NC-2 ("t" test, $t = -1.19$; $df = 1,4$; $P = 0.3$).

On the other hand, in both nursery cultures, percentage retrievals after 20–22 days of deployment (34–36 days post-set) were lower for spat collectors maintained in the sea than for those maintained simultaneously in the land-based nursery (Fig. III.6). In NC-1, percentage retrieval from the sea-based nursery was 5.78 %, whereas from the land-based nursery survival was 14.30 %. In NC-2, percentage retrieval from the sea-based nursery was 23.21 % whereas percentage survival from the land-based nursery was 42.79 %. Postlarvae maintained for an additional 10–11 days in the land-based nursery and deployed in the sea at 0.8–0.9 mm (24–25 days post-set) showed similar percentage retrieval to the collectors deployed earlier, i.e., 4.20 % and 21.22 % for NC-1 and NC-2, respectively, when scallops were retrieved simultaneously with the other groups (Fig. III.7). However, a different result was obtained when the postlarvae maintained longer in the land-based nursery for both NC-1 and NC-2 (34–36 days post-set) were deployed in the sea at a size of 1.1 mm, and retrieved 10 days later (i.e., 44–47 days post-set),

simultaneously with postlarvae deployed earlier at a size of 0.5 mm (deployment 1, 14 days post-set) and 0.7–0.8 mm (deployment 2, 24 and 25 days post-set). In this case, percentage retrievals were similar for the collectors held at different periods in land-based nursery, for both NC-1 and 2 (Fig. III.7), but postlarvae deployed earlier to the sea were significantly larger ($F = 17.37$; $df = 2,178$; $P < 0.001$ for NC-1 and $F = 181.1$; $df = 2,238$; $P < 0.001$ for NC-2) than those maintained for longer periods in the land-based nursery (Fig. III.8).

III.3.2.2. Three spat deployments at different sizes with one final retrieval

A comparison of postlarvae transferred at different sizes to the sea-based nursery after 7, 17 and 27 days in land-based culture was carried out in NC-3 (deployed in winter–spring-01). Mean shell heights at deployment were 0.29, 0.54 and 0.72 mm, respectively, for the three consecutive deployments. At retrieval, carried out 47 days after settlement (40, 30 and 20 days from deployment, respectively, for deployments 1 to 3), spat had mean shell heights of 3.05, 2.88 and 2.48 mm, respectively (Fig. III.8). Differences in shell heights were significant among groups ($F = 10.8$; $df = 2,203$; $P < 0.001$), shell heights of postlarvae from deployments 1 and 2 being statistically greater than those from deployment 3 (Tukey B test). The percentage retrievals from the sea-based nursery in NC-3, in relation to the number of spat initially settled in the collectors, were 2.7, 0.82 and 0.99 %, respectively, for the three consecutive deployments (Fig. III.7).

III.3.2.3. Detachment during transport

The mean number of postlarvae detached during transport to the sea-based nursery, decreased as size at deployment increased (Fig. III.9). In NC-2, the mean numbers of spat detached from collectors during transport were 46, 9.3 and 1.5, for postlarvae deployed at mean sizes of 0.50, 0.89 and 1.14 mm, respectively. In SD-4, when postlarvae were deployed at 0.50 mm, the mean number of postlarvae detached was 52.8. In NC-3, the mean numbers of spat detached during transport were 369, 74.4 and 27.4, respectively, for postlarvae deployed at mean sizes of 0.29, 0.54, and 0.72 mm. For postlarvae maintained in the land-based nursery for approximately 2 weeks, and deployed to the sea-based nursery at approximately 0.5 mm, the percentage detachment was 41, 7.1 and 7.4 % for NC-2, 3, and SD-4, respectively.

III.3.3. Consecutive samplings from the sea-based nursery

Postlarvae deployed in late summer–early autumn-01 (S_u-A) and late autumn–early-winter-01 (A-W) had an initial size of 0.50 mm. After 30–31 days (retrieval 3) there were minimal differences in shell height (means 4.9 and 4.5 mm, respectively) (Fig. III.10). However, 11 days later postlarvae deployed in S_u-A were significantly larger than those deployed in A-W, displaying mean shell heights of 7.6 mm and 5.7 mm respectively, which were significantly different ("t" test, $t = 5.2$; $df = 1, 192$; $P < 0.01$). Furthermore, at the final retrieval (53 days in the sea-based nursery) the difference in shell height between

both groups increased, postlarvae reaching 9.1 mm in S_u-A and 6.6 mm in NC-2, indicating a significant reduction in postlarval growth in A-W.

The percentage retrieval of spat in five consecutive samplings ranged from 15.24 to 26.0 % in S_u-A and 40.9 to 55.7 %, in A-W (Fig. III.11). Losses in both groups were higher between deployment and the first retrieval (10–11 days after deployment). Subsequently, percentage retrievals tended to stabilise for up to 53 days after deployment.

III.4. Discussion and Conclusions

III.4.1. Seasonal comparison

III.4.1.1. Environmental variables

This is the first study carried out in Brazil to determine the variability of several environmental factors in a sheltered coastal area, and to relate this variation to growth of scallops. The mean values recorded in the present study for temperature, salinity and chlorophyll-*a* in the sheltered coastal site off Porto Belo, were within the range reported for inner shelf surface waters off Santa Catarina by previous studies in similar seasons (Matsuura 1986, Brandini 1990, Castro and Miranda 1998, Sunyé and Servain 1998, Borzone et al. 1999). The clear seasonal variation in temperature indicated that this coastal aquaculture site at latitude 27° S was influenced by warming and cooling of the open ocean. The influence of Coastal Water (CW) (temperature > 20°C; salinity 29–35 psu, Sunyé and Servain 1998) was evident during most of the year, but during late winter – early spring-00, the site was also influenced by sub-Antarctic water (SAW) (temperature < 20°C; salinity 29–35 psu, Sunyé and Servain 1998). In the experiment carried out in winter–early spring-01 the mean temperature was 20.8°C, and periods of temperature < 20°C were recorded in July-August, before the beginning of the experiment, indicating an inter-annual variation in the period of influence of SAW. The negative correlation between temperature and salinity at the study site agrees with the observations from the previous studies in the inner continental shelf. Periods of lower salinities coincided with

lower temperatures during the presence of the SAW, which is phytoplankton-rich and influenced by estuarine waters from Rio de la Plata and Lagoa dos Patos, whereas higher salinities occurred when oligotrophic waters of tropical origins were influencing the shelf region (Brandini 1990, Sunyé and Servain 1998). However, differences in salinity between the coastal site and expected values for the inner shelf were occasionally recorded. Low salinity events (< 29 psu) at the study site indicated a significant influence of freshwater runoff, since these events were preceded by periods of precipitation. The significant negative correlation between salinity and chlorophyll-*a* also reflects the influence of SAW in winter–spring-00, when temperatures were below 20°C. However, the coincidence of some peaks of chlorophyll-*a* with low salinity events but temperature > 20°C other than in winter spring, suggests that the input of nutrients from freshwater runoff could also stimulate phytoplankton production. Although chlorophyll-*a* was variable within a given seasonal deployment period, maximum concentrations were recorded in the winter of both years simultaneously with lower temperatures, and minimum values recorded during summer–autumn experiments, when water temperatures were higher. However, due to the high variability within a given seasonal deployment, probably due to inputs of nutrients from continental runoff, mean concentrations of chlorophyll-*a* were not significantly different among seasons, as it could be expected in the neritic waters of the inner shelf (Brandini 1990). Another important oceanographic feature in southern Brazil is the sub-surface intrusion of cold and phytoplankton rich water (South Atlantic Central water - SACW) which reaches the inner continental shelf during summer (Brandini 1990, Borzone et al. 1999). The effects of temporal and spatial

variation in SACW intrusions on growth and percentage retrieval of postlarval scallops in a coastal aquaculture site is examined in detail in Chapter IV.

The variation of water temperature (12.5°C) among different seasons recorded in the present study is comparable to the annual variation of temperature characteristic of some scallop habitats in temperate and boreal regions, such as Passamaquoddy Bay, Canada (11.4°C) (Parsons and Dadswell 1992), and western Norway (10°C) (Christophersen and Magnesen 2001). The levels of chlorophyll-*a* in southern Brazil (0.28–3.02 μg L^{-1}) are also comparable with values recorded for temperate-boreal scallop habitats, e.g. Sunnyside and Colinet (Newfoundland, Canada) (0.2–5.5 μg L^{-1}) (Macdonald and Thompson 1985), and for west Iceland (0.2–3.8 μg L^{-1}) (Thorarinsdóttir 1994). However, the peak chlorophyll-*a* levels recorded in these studies were associated with the spring phytoplankton bloom, whereas during winter chlorophyll-*a* values were lower. In contrast, in southern Brazil, there was no indication of phytoplankton blooms, and variability on a short temporal scale (ca. weeks) was of similar magnitude to seasonal variation.

No published information is available on suspended particulate material (seston) concentrations in coastal areas of southern Brazil, except for a study simultaneous to this one (Suplicy et al. 2003). TPM in the present study ranged from 0.95 to 10.8 mg L^{-1}, displaying greater variability than those recorded for coastal non-estuarine areas in temperate-boreal regions where scallops are found, such as Bellevue (2–6 mg L^{-1}) (Thompson 1984) (Newfoundland, Canada) and Trømso (Norway) (5–12 mg L^{-1}) (Vahl 1980). However, values for POM in southern Brazil (0.2–2.37 mg L^{-1}) were lower than

those for temperate-boreal areas reported in the above studies (1–4 mg L^{-1}). Furthermore, seston levels in high latitudes are generally higher during spring-summer than in winter, while in southern Brazil there is no such seasonal pattern for TPM. Differences in TPM recorded in southern Brazil are possibly caused by wind-driven resuspension and/or freshwater run-off.

III.4.1.2. Daily growth rate and percentage retrieval

Significant differences in environmental conditions were detected among the seasonal deployments, influencing DGR of postlarval scallops. Daily growth rate in December-00–January-01 (S_p-S_u) was 3.4 fold higher than in August–September-00 (W-S_p-00) and 1.9 fold higher than in August–September-01 (W-S_p-01), whereas no difference in DGR was recorded among deployments carried out in mid-March to mid-April-01 (S_u-A) and in June–July-01 (A-W). The influence of environmental factors on DGR was determined for postlarvae deployed to the sea-based nursery at an initial shell height of 0.48 to 0.53 mm. Over a large size range, DGR in scallops decreases with increasing in shell height (Parsons et al. 1993b, Lu and Blake 1996). However, in the present study the difference in initial shell height among the different nursery cultures was minimal, allowing comparison of DGR in different nursery cultures. Temperature was the major factor influencing DGR in the sea-based nursery, as shown by the significant correlation of temperature and DGR and the high contribution of temperature to the total variance in the multiple regression model. When mean temperature was 17.6°C in W-S_p-00 DGR was significantly lower than in the other experiments, in which mean

temperatures were ≥ 20°C. The significant correlation between DGR and salinity also suggests an important effect of freshwater runoff. Although there were no significant differences in temperature between A-W and W-S_p-01 differences in DGR were significant, which can be explained by salinity and turbidity. In W-S_p-01 there was a period of high precipitation and freshwater runoff (see Fig. III.3), resulting in an episode of low salinity (26.7 psu), possibly stressing the scallops and inhibiting growth (see chapter V). Another consequence of rainfall was the increased turbidity of the seawater during W-S_p-01, which may also have affected growth, since scallops are vulnerable to high turbidity (Brand 1991, Orensanz et al. 1991). In the present study turbidity was determined with a turbidity meter, which measures the attenuation of light by dissolved substances and suspended particles in the seawater. Dissolved organic compounds associated with continental runoff generally have a strong absorbance of light (Kitchen et al. 1982). Therefore, the presence of dissolved compounds associated with continental runoff in W-S_p-01 may explain turbidity being higher in W-S_p-01 than in S_u-A, but TPM being similar in both periods.

Daily growth rates were similar in the nursery cultures carried out in S_p-S_u (December-00 to early January-01), S_u-A (mid-March to mid-April-01) and A-W (June to early-July-01), when seawater temperatures were > 20°C, indicating that there is a wide range of temperatures (20–27°C) for which growth is not significantly affected by temperature. Conversely, growth was depressed below 19°C, in agreement with results from Chapter V, in which the rate of byssal attachment of spat was higher at temperatures from 19 to 27°C, and significantly depressed at temperatures near the lower limits recorded in southern Brazil.

Growth of bivalves in seasonal environments is influenced by the interaction of several factors, particularly food supply and temperature (Bayne and Newell 1983, MacDonald and Thompson 1985, Parsons and Dadswell 1992, Côté et al. 1993, Grant 1996). Scallops, like other active suspension feeding bivalves, rely on phytoplankton and suspended detritus as their food source (Bricelj and Shumway 1991). Suspended material (seston) includes a variety of organic particles (POM) of different qualitative value and inorganic matter (PIM) with little or no nutritional value (Bricelj and Shumway 1991). Phytoplankton biomass, as indicated by chlorophyll-*a*, and seston indices such as ratio PIM:POM or percent PIM, have been used as indicators of food availability for scallops (Broom and Mason 1978, Wallace and Reinsnes 1985, Thorarinsdóttir 1994). The influence of chlorophyll-*a* on scallop growth was negligible in the present study, given the unusual negative correlation between chlorophyll-*a* and DGR. Food supply, as expressed by concentrations of chlorophyll-*a*, was not, therefore, a factor limiting growth in this subtropical environment. Chlorophyll-*a* was significantly correlated with PIM and turbidity, as well as negatively correlated with salinity, all of which could have stressed the scallops, reducing shell growth. The effects of PIM, turbidity and salinity were probably responsible for the inverse correlation between DGR and chlorophyll-*a*. Furthermore, the negative correlation between temperature and chlorophyll-*a* probably contributed to the observed relationship. Shell growth of juvenile *Pecten maximus* was also mainly regulated by water temperature and salinity, rather than food availability (Chauvaud et al. 1998). The food supply for cultured bivalves is also largely influenced by water flow, concentration, size and quality of food particles (Grant 1996). It is likely that in the present study, water flow was similar between experiments at different seasons,

since all deployments were carried out in the same site and depth, having a duration of approximately one month. Scallops were, therefore, exposed to a complete lunar cycle and consequently similar tidal and water flow conditions. Although it was not possible to identify the phytoplankton species in the present study, pectinids are capable of ingesting algal cells of a wide size range, from 2–950 μm (examples in Bricelj and Shumway 1991). It is possible that there is a seasonal variation in phytoplankton composition in the study area, but there are no indications that during the periods of lowest growth (late winter–early spring), the phytoplankton species present would not support scallop growth. According to Soares (1983) the phytoplankton composition near the study region in late winter–early spring was dominated by diatoms, (*Skeletonema costatum*, *Thalassiosira* spp., *Chaetoceros* spp., *Nitzchia* spp., among others), followed by phytoflagellates, which are among the suitable algal species used as food for bivalves (Webb and Chu 1983, Duerr et al. 1998).

The ratio PIM:POM is an important factor affecting growth of bivalves. The amount of PIM in seawater dilutes POM, reducing the nutritional value of seston for scallops (Vahl 1980, Wallace and Reinsnes 1985, Bricelj and Shumway 1991, Emerson et al. 1994). For example, Wallace and Reinsnes (1985) recorded a reduction in growth rate of *Chlamys islandica*, when PIM:POM reached a critical value of 3.5, i.e., when PIM represent ca. 78 % of the seston. Although certain bivalves, including the scallop *Placopecten magellanicus*, can selectively ingest organic particles compensating for dilution of food quality (Widdows et al. 1979, Bricelj and Malouf 1984, MacDonald and Ward 1994), at high concentration of low quality seston *P. magellanicus* lack this ability (Bacon et al. 1998), suggesting that scallops are less suited than other bivalves to

compensate for organic dilution. The percent PIM in the present study averaged 74.4 to 81.7, (PIM:POM range 3.5–4.5) which is close to the critical value inhibiting growth in *C. islandica*. However, since percent PIM did not differ among the seasonal deployments, it did not account for differences in DGR.

Whereas the influences of food availability on growth of adult bivalves have been extensively studied, less is known about the effects of food availability on early postlarvae. Postlarval scallops undergo major anatomical changes during metamorphosis, and development of feeding structures is more prolonged than in other bivalves (Veniot et al. 2003). The gills of *Pecten maximus* only become fully functional at a shell length of 4 mm (Beninger et al. 1994). However, as demonstrated in Chapter II, *N. nodosus* postlarvae at 0.25–0.30 shell height can already ingest suspended algae. Furthermore, at a size of 0.5 mm *Argopecten irradians* can actively capture and ingest the microalga *Isochrysis galbana* (Lu and Blake 1997a), and Shumway et al. (1997) demonstrated that three species of scallops, ranging from 0.8 to 1.16 mm, can clear unialgal diets and natural phytoplankton assemblages. In the present study *N. nodosus* deployed in the sea at a size of 0.5 mm was already feeding on phytoplankton, thus chlorophyll-*a* and seston measurements were appropriate parameters to estimate food availability. In this coastal subtropical environment, growth of postlarval scallops is not food limited, and the present results suggest that variability in abiotic factors, such as temperature, salinity and turbidity, override any influence of food availability on growth.

In the present study, growth of postlarval scallops was evaluated in terms of shell height, since logistical constraints prevented the determination of dry tissue weight, and postlarvae were too small to allow removal of soft tissues. In postlarval bivalves,

including scallops, all energy available is allocated to maintenance and growth, and postlarvae normally undergo fast shell growth, which is not accompanied by a proportional increase in biomass (Lu and Blake 1996). Furthermore, from an aquaculture perspective, size, not weight, is the parameter used to select the culture gear.

Percentage retrievals varied greatly among the five nursery culture experiments carried out in different seasons (range 1.45 to 42.8 %). It is difficult, however, to establish a direct seasonal correlation, owing to uncontrollable factors such as possible predator settlement, or detachment of spat resulting from wave action on the long-line, which were not evaluated. Nevertheless, the variations in retrievals among nursery cultures can be explained by observed variations in environmental factors. Percentage retrievals of scallops are determined by mortality and/or byssal detachment with subsequent loss of spat through the bag mesh. Mortality of bivalves in the sea are attributed to biotic factors such as low food availability and predation (Kennedy 1996), or to siltation (examples in Bricelj and Shumway 1991), or to values of abiotic factors such as temperature and salinity that exceed the tolerance limits (Kinne 1970a). The limits of tolerance for these factors have been studied for *N. nodosus* (Chapter V), and were not exceeded during the present study. It is likely that sublethal changes in temperature and salinity affected byssus synthesis (see Chapter V) and contributed to detachment of spat and subsequent loss. A low percentage retrieval obtained in W-S_p-00 (3.33 %) was associated with lower temperature, and in W-S_p-01 (1.45 %) with low salinity and high turbidity. The degree of byssus attachment has been proposed as an indicator of unfavourable environmental conditions, and a low rate of byssus attachment has been related to sub-optimal salinities and temperatures (Paul 1980a,b, Chapter V). Heasman et al. (1996) also found that byssus

attachment is higher at temperatures supporting the highest growth rates of *Pecten fumatus*, and significantly reduced at low temperature. In the present study, low temperature in W-S_p-00 and a low salinity event in W-S_p-01 may have stressed the scallops, suppressing byssal synthesis and inducing spat detachment, as suggested by the result obtained in Chapter V. Furthermore, in W-S_p-01 fine sediments accumulated in the collectors (pers. obs.) as a result of higher turbidity, which may also have contributed to spat detachment. Few studies have reported survival or retrieval rates of postlarval scallops under culture conditions in the sea. Survival from setting larvae to commercial size can be as low as 0.5 % owing to high losses during the nursery stage, in which survival is often less than 5 % (Bourne and Hodgson 1991). O'Foighil et al. (1990) reported that high mortalities of post-metamorphic *Patinopecten yessoensis* occur before spat reach 400 μm in shell height, with a second period of high mortality at 600–1000 μm, indicating that yields of 6 % from setting larvae to juveniles > 1 mm are exceptional. Uriarte et al. (2001) reported yields of 3 to 15 % from pediveliger to 1–2 mm postlarva. Lu and Blake (1997b) recorded percentage survival of 17.8 to 22.6 % for *Argopecten irradians* from 0.5 mm to 10 mm when cultured in spat bags. Yields of *A. purpuratus* postlarvae in commercial operations in Chile vary from 2 to 6 %, when deployed on spat collectors in the sea-based nursery a few weeks after settlement (Abarca 2001), as in the present study. Heasman et al. (2002), using a similar approach to the present study, recorded percentage retrievals of 5–41 % for *Pecten fumatus* spat deployed in the sea-based nursery at sizes from 0.35 to 1 mm and retrieved after 1 month, as in the present study. Percentage retrievals for *N. nodosus* deployed at 0.5 mm and retrieved after approximately one month (6.04–42.8 % in the seasonal deployments S_p-S_u, S_u-A and A-

W, when environmental conditions were more favourable), were within the range reported in the above studies. Higher percentage retrievals (70–80 %) were obtained when postlarvae were deployed in the sea-based nursery on a sub-surface long-line (Chapter V).

III.4.2. Comparison of land-based and sea-based nursery

III.4.2.1. Three spat deployments at different sizes with simultaneous culture in sea- and land-based nurseries

Postlarvae grown in the sea-based nursery attained higher shell heights than those cultured simultaneously in the land-based nursery. In the late winter-early spring-00 deployment (NC-1), DGR was 1.6 fold higher in the sea than in the land-based nursery, and in late summer – early autumn-01 deployment (NC-2) DGR in the sea was 3.8 fold higher than in the land-based nursery. Differences in growth rates in the sea between nursery cultures were mainly correlated with water temperature (mean 17.4°C in NC-1 and 26.1°C in NC-2). The possibility of endogenous factors or disease causing a reduction in growth rates of postlarvae maintained in land-based culture can be ruled out, since the spat were subsequently transferred to the sea, where postlarval growth rates increased in both experiments. In NC-1, although temperature was higher in the laboratory (24°C), growth was higher in the sea. This suggests that in the land-based nursery, growth was nutritionally limited, despite favourable temperatures. It is unlikely that food quantity was a limiting factor in the laboratory, because algal concentrations were monitored daily before and after water changes, and suspended algae always remained in the tanks before

the water changes. It is more likely that the mixture of algal species supplied (*Isochrysis* sp. (clone T-iso), *Chaetoceros calcitrans*, and *Nanochloropsis occulata* in NC-1, and *Isochrysis* sp. (clone T-iso), *C. calcitrans* and *C. muelleri* in NC-2), which are widely used in hatcheries as food for bivalves, did not fulfil the nutritional requirements of postlarval scallops. Furthermore, according to the results from Chapter II, the algal concentration supplied in the land-based nursery, although within the range normally used in scallop nurseries, may have been sub-optimal for growth. Bourne and Hodgson (1991) also found that growth of *Patinopecten yessoensis* spat was higher in systems supplied with natural phytoplankton assemblages, and Heasman et al. (1996) demonstrated that growth of *Pecten fumatus* in the hatchery/nursery ceased 6 weeks after settlement, when mean shell heights were 2–4 mm. Martinez et al. (1992) indicated that *Argopecten purpuratus* grown in the sea attained greater shell heights after 6 weeks of deployment than spat maintained in laboratory, and this was related to lower levels of docosahexaenoic acid (DHA) in scallops held in the laboratory. In the present study, differences in growth rates between sea-based and land-based experiments were clearly related to nutrition, and slow growth in the land-based nursery in relation to the sea became evident as early as 2 weeks after settlement. In summary, the earlier deployment resulted in spat significantly larger than those deployed later, when retrieved simultaneously about two months post-set.

Few studies have reported growth rates of postlarval scallops within the size range (0.5–3 mm) used in the present study. Lu and Blake (1996) reported growth rates of 20 to 50 $\mu m\ day^{-1}$ for 0.5 mm *Argopecten irradians* fed different concentrations of *Isochrysis galbana* in the laboratory. The rock scallop *Crassodoma gigantea* 0.6 to 2 mm in shell height, had growth rates varying from 8.1 to 26.5 $\mu m\ day^{-1}$ when fed different algal diets

in a land-based nursery (calculated from Bourne and Hodgson, 1991). Postlarval *Chlamys asperrimus* displayed growth rates of 14 μm day^{-1} in the laboratory (Rose and Dix 1984). Under field conditions, Parsons et al. (1993b) recorded DGR of *Placopecten magellanicus* (shell height 0.3 to 1.5 mm) ranging from 30 to 60 μm day^{-1}. *A. gibbus* within the same size range displayed growth rates in the sea of 41 to 69 μm day^{-1} (calculated from Allen 1979). Daily growth rates of *N. nodosus* in the laboratory (27–32 μm day^{-1}) from the present study are consistent with published data for scallops, but growth rates in the sea (45–122 μm day^{-1}) were higher than literature values for other scallops. Thus, there remains a large scope for improvement in the composition of algal diets for postlarval scallops, so that their full growth potential can be exploited in land-based aquaculture facilities.

Spat collectors maintained for ca. 5 weeks post-set in the land-based nursery initially yielded higher percentage retrievals of spat than the collectors deployed earlier at the sea-based nursery (ca. 2 and 3 weeks post-set) (Fig. III.6). Furthermore, when spat were deployed in the sea approximately 5 weeks post-set, and retrieved 10–11 days later (44–47 days post-set) simultaneously to the collectors deployed earlier, the percentage retrievals were similar (see Fig. III.7). Thus, although losses in the sea-based nursery were initially higher, soon after the collectors maintained longer in the land-based nursery were transferred to the sea, percentage retrievals were similar. In addition, spat deployed earlier had a greater mean shell height than those deployed later (Fig. III.8). Therefore, it was more advantageous to deploy postlarvae to the sea-based nursery at ca. 0.5 mm (2 weeks post-set) than at ca. 1.1 mm after 5 weeks in the land-based nursery.

III.4.2.2. Three spat deployments at different sizes with one final retrieval

Since there was no advantage in maintaining spat in the land-based nursery for longer than about 2 weeks after settlement (shell height ca. 0.5 mm), as demonstrated in NC-1 and 2, a deployment of spat one-week post-set (shell height ca. 0.29 mm) was tested in NC-3. The results showed that postlarvae deployed one week after settlement attained a final shell height similar to those deployed 17 days post-set (Fig III.8). Deploying scallops at 27 days post-set (mean shell height 0.72 mm), on the other hand, resulted in smaller shell heights than spat deployed earlier, when retrieved simultaneously. Therefore, when deployed at 0.29 mm spat displayed similar growth rates to those maintained in the laboratory until 17 days post-set, but after that, spat transferred to the sea-based nursery grew faster than those maintained in the land-based nursery. These results demonstrate that postlarval growth becomes hindered in the laboratory at a shell height of about 0.5 mm, or after approximately two weeks post-set, compared with spat transferred to the sea. It is possible that postlarvae smaller than 0.5 mm could not benefit from more favourable nutritional conditions from natural phytoplankton assemblages in the sea.

III.4.2.3. Detachment during transport

The number of postlarvae detached during transport decreased as spat size increased. Detachment was greatest in postlarvae from collectors deployed one week after settlement (0.29 mm shell height), and was significantly less when spat were deployed around two weeks from metamorphosis (ca. 0.5 mm). Considering the mesh opening of the spat bags (0.7 mm diagonal), a lower escape of larger spat would be expected, but spat at 0.29 and 0.5 mm shell height were smaller than the mesh opening and would equally escape if detached from the collectors. Thus, it is possible that one week after metamorphosis spat are not as strongly attached as after two weeks. There was less detachment in subsequent deployments, but the number of postlarvae in the collectors deployed later was lower due to mortality in the laboratory. The use of a smaller mesh to avoid loss would not be practical, since it would quickly clog due to sediments and fouling. Percentage retrievals could possibly be increased if transport conditions were optimised. The distance from the land-based nursery required a 2.5 h journey in a truck, followed by 0.5 h in a boat, before the collectors were deployed in the sea. In situations where the hatchery is close to the sea-based nursery, detachment during transport could possibly be significantly lower. The method used to transport the collectors could also be improved, (e.g., racks inside the transport boxes to reduce agitation among the collectors) to reduce detachment.

III.4.3. Consecutive samplings from the sea-based nursery

Growth of spat deployed in late summer–early autumn-01 (S_u-A) and late autumn–early winter-01 (A-W), and retrieved consecutively, was similar until 30–31 days after deployment, when spat attained ca. 5 mm shell height. At this size, scallops could be handled and detached from the collectors for transfer to intermediate culture in pearl-nets. Subsequently, however, there was a significant reduction in growth in the A-W deployment, which can be attributed to a decrease in temperature. While in S_u-A the mean temperature remained around 24°C, in A-W it dropped from 21.3 to 18.4°C. This is in agreement with results obtained in the seasonal deployment carried out in W-S_p-00, when significantly lower growth rates were associated with temperatures below 19°C. Fifty-three days after deployment (67 days post-set), scallops attained mean shell heights of 9.1 and 6.5 mm in S_u-A and A-W, respectively, further evidencing that lower temperatures significantly reduced growth.

While initial losses of spat were higher in the first 10 days (from the deployment to the first sampling), losses tended to stabilise in subsequent retrievals from both experiments, suggesting that the major source of postlarval loss was detachment early after deployment. The data also suggest that predation was negligible, since there must have been a lag period before potential predators could settle on the collectors, undergo metamorphosis and reach a size at which they could prey on the scallops. Therefore, if predation had been a significant factor causing mortality, there would have been a continued decrease in the number of scallops retrieved over time. In the present study, spat bags were deployed on a surface long-line, vulnerable to the effects of wave action,

which may have induced detachment of spat in the initial period post-deployment. Although the nursery site was sheltered and normally protected from the swell, exceptionally strong winds could have occasionally induced wave action. Deployment of spat on a sub-surface long-line, unaffected by wave action was examined in Chapter IV.

III.4.4. Conclusions and implications for aquaculture strategies in southern Brazil

Temporal variability of environmental factors, as reported in other studies of neritic waters of the inner continental shelf in southern Brazil (Matsuura 1986, Brandini 1990, Castro and Miranda 1998, Sunyé and Servain 1998, Borzone et al. 1999) significantly affected the coastal sheltered aquaculture site at Porto Belo, which was also influenced by continental run-off during severe rainfall. Such variability significantly affected growth rates of postlarval and juvenile scallops cultured in the sea-based nursery, and is therefore an important consideration in establishing aquaculture sites and practices. Water temperature was the major seasonal factor affecting growth, whereas food abundance as expressed by concentrations of chlorophyll-*a* had a negligible effect. Periods of lower temperatures (< 19°C) and lower salinities (< 29 psu) also reduced postlarval growth, and possibly increased losses, by inhibiting byssus attachment, causing detachment and escape through the mesh of the nursery enclosures. In southern Brazil there is a wide window of opportunity for deploying postlarval scallops in the sea-based nursery, with growth being maximised during most of the year, except for a short time in winter when temperature decreases below 20°C, and during periods of high rainfall, if the site is affected by freshwater runoff. Growth rates of scallops transferred to the sea-based

nursery at ca. 0.5 mm shell height were significantly higher than in scallops maintained in the land-based nursery. As a result, when scallops were deployed in the sea at 0.5 mm, the time to reach ca. 5 mm (a size at which spat could be detached from the collectors and transferred to pearl-nets) was approximately 6 weeks since settlement, whereas when the postlarvae were deployed at 1.1 mm an additional 2–3 weeks would be required to reach the same size in periods of favourable environmental conditions. Percentage retrieval of postlarvae transferred to the sea-based nursery 2 weeks post-set (ca. 0.5 mm) was initially lower than survival of those maintained in the laboratory for approximately 5 weeks post-set (ca. 1.1 mm). However, 10 days after postlarvae maintained longer in the laboratory were deployed in the sea, percentage retrievals were similar to that for spat deployed earlier, which attained larger size. Considering the high cost of maintaining a large number of postlarval scallops in a land-based nursery for long periods, transfer of postlarval *N. nodosus* to the field nursery at a size of about 0.5 mm, may be economically advantageous when the seawater temperature is above 20°C. Although the loss of spat was initially higher in the sea, improvements in transport method and a selection of a deeper nursery site unaffected by freshwater runoff, together with the use of a sub-surface long-line, could greatly increase percentage retrievals of spat from the sea-based nursery.

Table III.1. Summary of the seasonal deployments (SD) to the sea-based nursery undertaken from August 2000 to September 2001.

Seasonal deployment	Deployment	Date of deployment	Age at deployment (days post-set)	Retrieval days post-deployment (No. spat bags = 3)
SD-1	Winter/Spring	24-Aug-00	14	33
SD-2	Spring/Summer	1-Dec-00	16	32
SD-3	Summer/Autumn	17-Mar-01	14	30
SD-4	Autumn/Winter	4-Jun-01	14	31
SD-5	Winter/Spring	10-Sep-01	17	30

Table III.2. Summary of experiments comparing sea- (SBN) and land-based nurseries (LBN) indicating the dates of deployment, days in the LBN and days in the SBN at different retrievals. (number of spat bags retrieved/sampling = 3; * n=2).

Nursery culture (NC)	Deployment	Date of deployment	Days in LBN (days post-set)	Shell height at deployment (mm)	Days in SBN Retrieval 1 (days post-depl.)	Days in SBN Retrieval 2 (days post-depl.)	Days in SBN Retrieval 3 (days post-depl.)
	1	24-Aug-00	14	0.53	11	22	33
NC-1	2	4-Sep-00	25	0.80	11	22	
	3	15-Sep-00	36	1.13	11		
	1	17-Mar-01	14	0.50	10	20	30
NC-2	2	27-Mar-01	24	0.90	10	20	
	3	6-Apr-01	34	1.14	10		
	1	31-Aug-01	7	0.29	40		
NC-3	2	10-Sep-01	17	0.54	30		
	3	20-Sep-01	27	0.72	20 *		

Table III.3. Summary of sea-based nursery cultures in which growth and percentage retrieval of spat were compared in consecutive samplings. (number of spat bags retrieved/sampling = 3).

Date of deployment	Season	Days post-settlement	Retrieval 1 (days post-depl.)	Retrieval 2 (days post-depl.)	Retrieval 3 (days post-depl.)	Retrieval 4 (days post-depl.)	Retrieval 5 (days post-depl.)
17-Mar-01	Summer/Autumn	14	10	20	30	41	53
4-Jun-01	Autumn/Winter	14	11	21	31	42	53

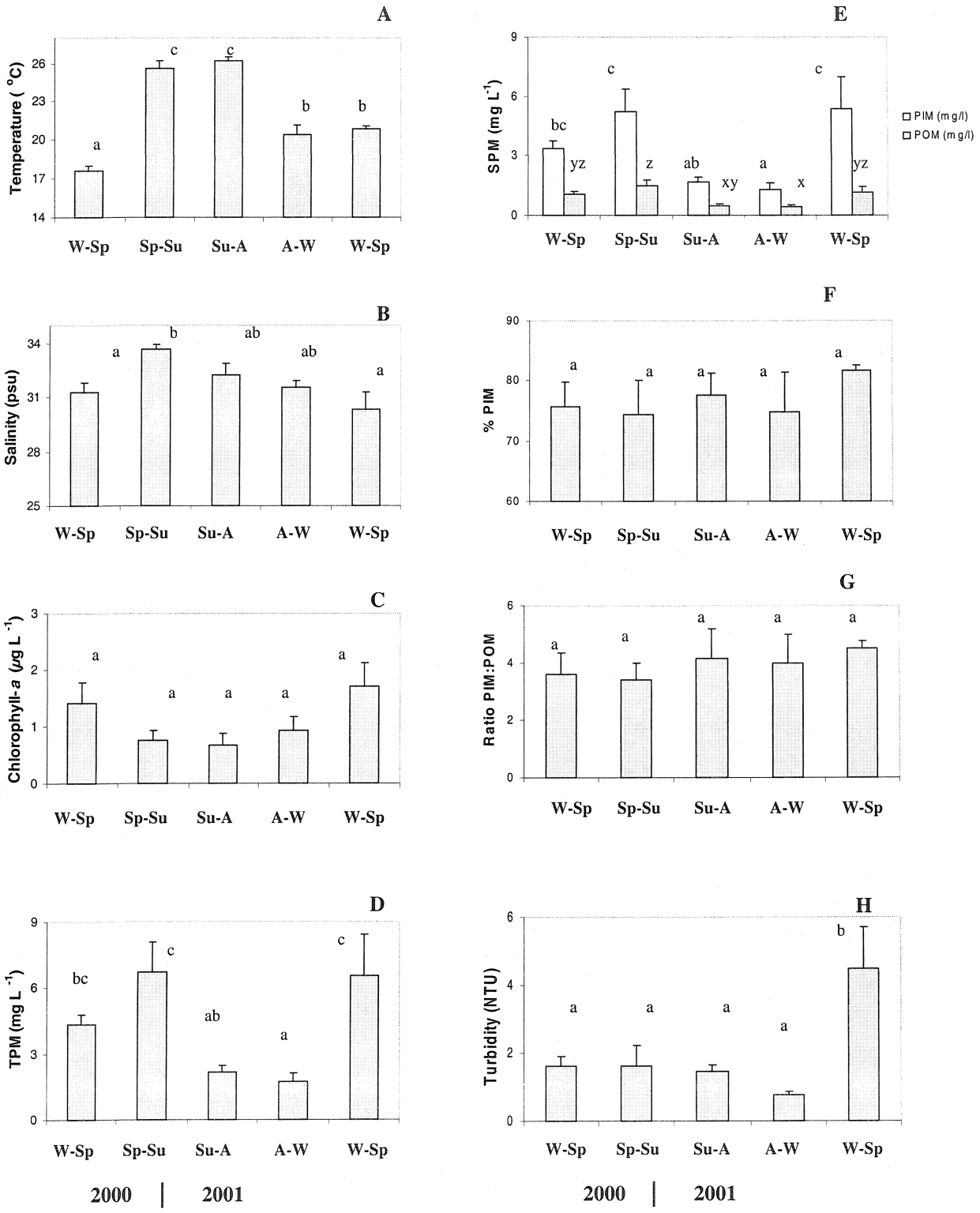
Table III.4. One-way ANOVA for environmental factors surveyed during five sea-based nursery experiments off Porto Belo, SC, Brazil, from August 2000 to October 2001.

Environmental Factor	df	F	P
Temperature	4, 22	52.74	< 0.001
Salinity	4, 22	4.83	0.006
Chlorophyll-*a*	4, 22	2.72	0.056
TPM	4, 22	7.59	0.001
PIM	4, 22	5.97	0.002
POM	4, 22	7.27	0.001
% O_2	4, 22	0.900	0.481
Ratio PIM:POM	4, 22	0.384	0.866
% PIM	4, 22	0.440	0.778

Table III.5. Spearman correlation coefficients among environmental variables surveyed during five sea-based nursery experiments off Porto Belo, SC, Brazil, from August 2000 to October 2001. $P < 0.05$ (*), $P < 0.01$ (**).

	Temperature	Salinity (n=27)	Chlorophyll-*a* (n=27)	TPM (n=27)	PIM (n=27)	POM (n=27)	% PIM (n=27)	PIM:POM (n=27)	% O2 (n=27)	Turbidity (n=27)
Temperature	–	0.436 *	-0.330	-0.094	-0.093	-0.046	-0.122	-0.130	0.186	0.040
Salinity		–	-0.455 *	0.118	0.115	0.280	-0.180	-0.170	-0.272	-0.289
Chlorophyll-*a*			–	0.510 **	0.472 *	0.516 **	-0.096	-0.092	-0.150	0.544 **
TPM				–	0.990 **	0.872 **	0.142	0.140	-0.234	0.445 *
PIM					–	0.808 **	0.250	0.248	-0.283	0.426 *
POM						–	-0.284	-0.288	-0.044	0.363
						–	27	27	27	27
% PIM							–	0.998 **	-0.437 *	0.082
PIM:POM								–	-0.449 *	0.085
% O_2									–	-0.125
Turbidity										–

Figure III.1. Values for environmental variables in five sea-based nursery cultures off Porto Belo, SC, Brazil, undertaken in different seasons: late winter–early spring 2000 (W-Sp); late spring 2000–early summer 2001 (Sp-Su); late summer–early autumn 2001 (Su-A); late autumn–early winter 2001 (A-W); late winter–early spring 2001. **A** - temperature; **B** - salinity; **C** - chlorophyll-*a*; **D** - TPM; **E** - PIM and POM; **F** - %PIM; **G** - ratio PIM-POM; **H** - turbidity. (mean + se) (common letters denote no significant differences) (A–G - Tukey B test; H - Kruskal-Wallis).


A
Temperature (°C)
26
23
20
17
14
a
c
c
b
b
W-Sp
Sp-Su
Su-A
A-W
W-Sp
B
Salinity (psu)
34
31
28
25
a
b
ab
ab
a
W-Sp
Sp-Su
Su-A
A-W
W-Sp
C
Chlorophyll-a (µg L -1)
3
2
1
0
a
a
a
a
a
W-Sp
Sp-Su
Su-A
A-W
W-Sp
D
TPM (mg L -1)
9
6
3
0
bc
c
ab
a
c
W-Sp
Sp-Su
Su-A
A-W
W-Sp
2000
2001
E
SPM (mg L-1)
9
6
3
0
bc
yz
c
z
ab
xy
a
x
c
yz
W-Sp
Sp-Su
Su-A
A-W
W-Sp
PIM (mg/l)
POM (mg/l)
F
% PIM
90
80
70
60
a
a
a
a
a
W-Sp
Sp-Su
Su-A
A-W
W-Sp
G
Ratio PIM:POM
6
4
2
0
a
a
a
a
a
W-Sp
Sp-Su
Su-A
A-W
W-Sp
H
Turbidity (NTU)
6
4
2
0
a
a
a
a
b
W-Sp
Sp-Su
Su-A
A-W
W-Sp
2000
2001

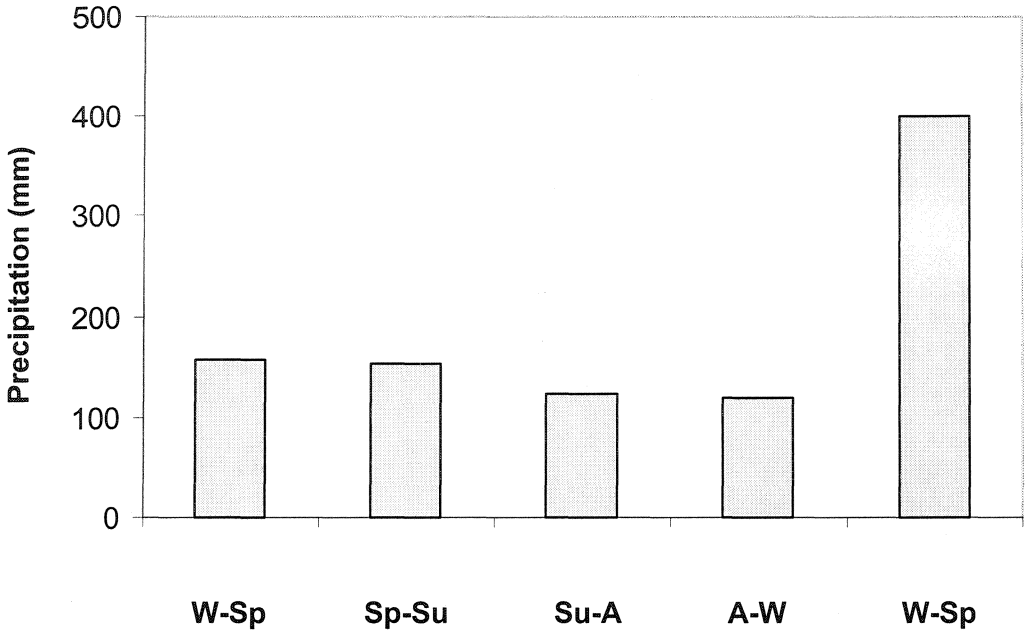


Figure III.2. Cumulative precipitation (mm) at a coastal meteorological station (CLIMERH- Itajaí, SC) during sea-based nursery experiments undertaken in different seasons: late winter–early spring 2000 (W-Sp); late spring 2000–early summer 2001 (Sp-Su); late summer–early autumn 2001 (Su-A); late autumn–early winter 2001 (A-W); late winter–early spring 2001 (W-Sp).

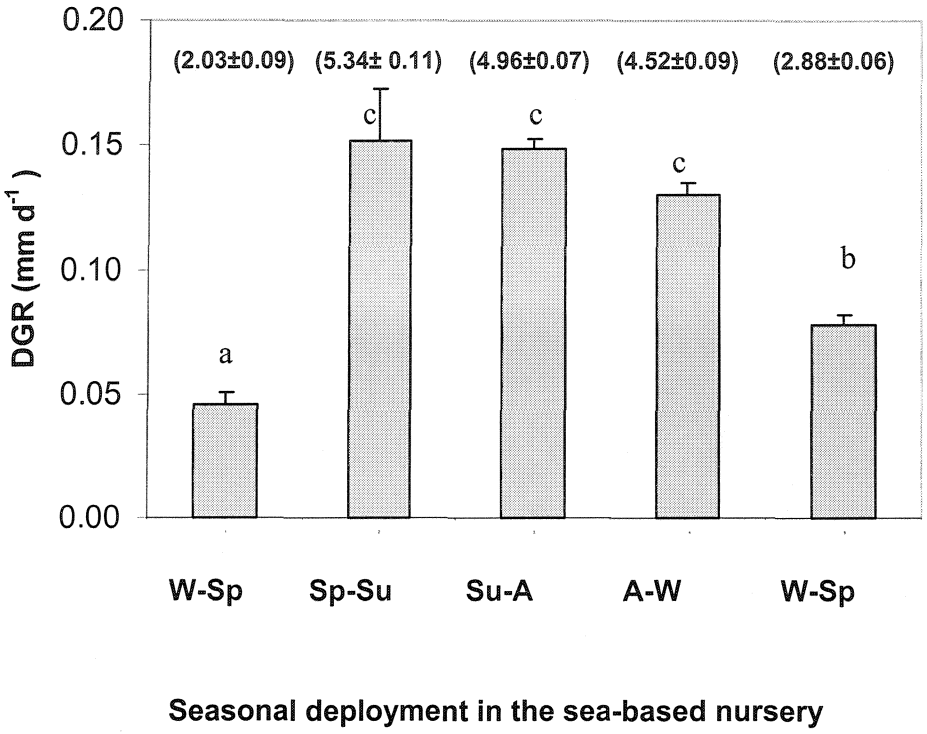


Figure III.3. Daily growth rate (DGR) of *Nodipecten nodosus* spat cultured in the sea-based nursery in different seasons, deployed at an average initial size of about 500 μm and retrieved after one month: late winter–early spring 2000 (W-Sp); late spring 2000–early summer 2001 (Sp-Su); late summer–early autumn 2001 (Su-A); late autumn–early winter 2001 (A-W); late winter–early spring 2001(W-Sp). (mean + se). (Common letters denote no significant difference). Numbers in brackets = final shell height (mm) at retrieval (± se).

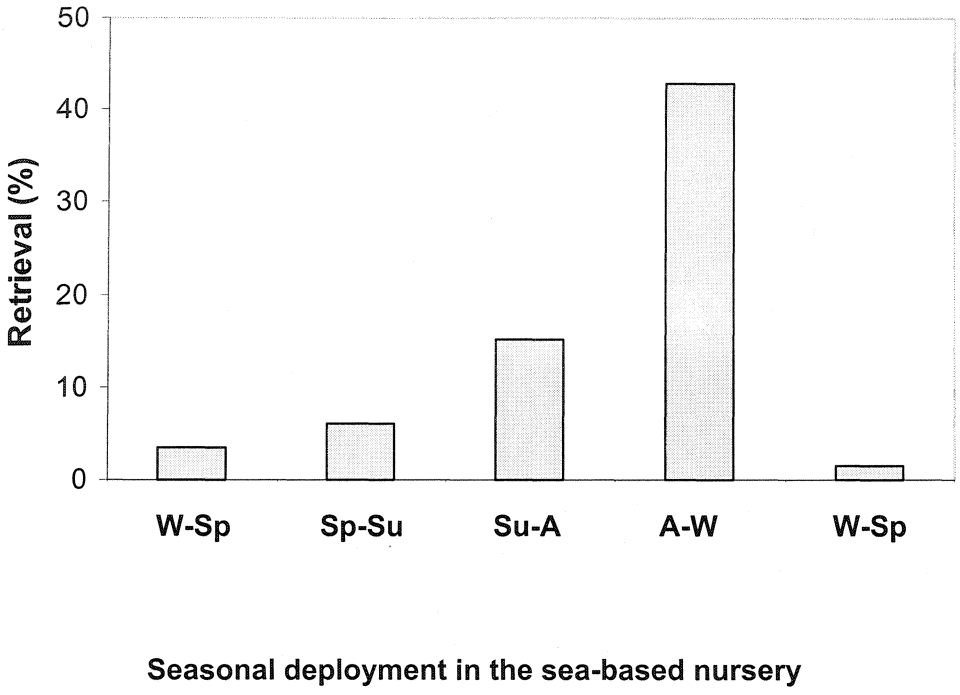


Figure III.4. Percentage retrieval of *Nodipecten nodosus* spat cultured in the sea-based nursery in different seasons, deployed at an average initial size of about 500 μm and retrieved after one month: late winter–early spring 2000 (W-Sp); late spring 2000–early summer 2001 (Sp-Su); late summer–early autumn 2001 (Su-A); late autumn–early winter 2001 (A-W); late winter–early spring 2001 (W-Sp).

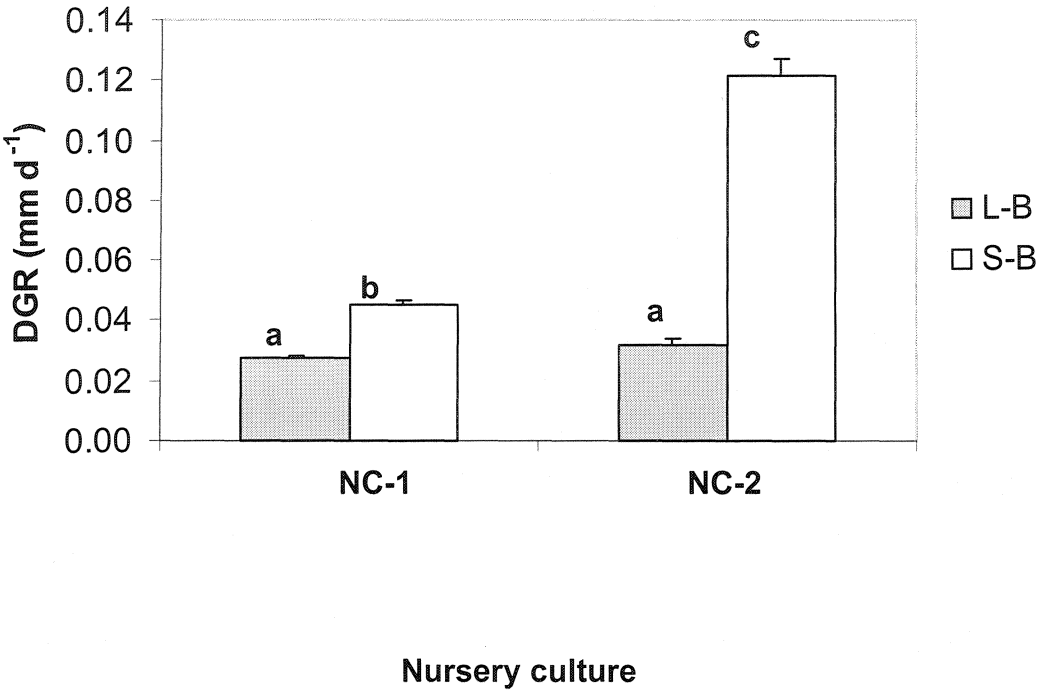


Figure III.5. Daily growth rate (DGR) of *Nodipecten nodosus* spat reared simultaneously in land-based (L-B) and sea-based (S-B) nurseries, deployed at an initial average size of about 500 μm and retrieved after 20–22 days (34–36 days post-set). NC-1 = late winter–early spring 2000, NC-2 = late summer–autumn 2001. (means + se) (Common letters denote no significant difference).

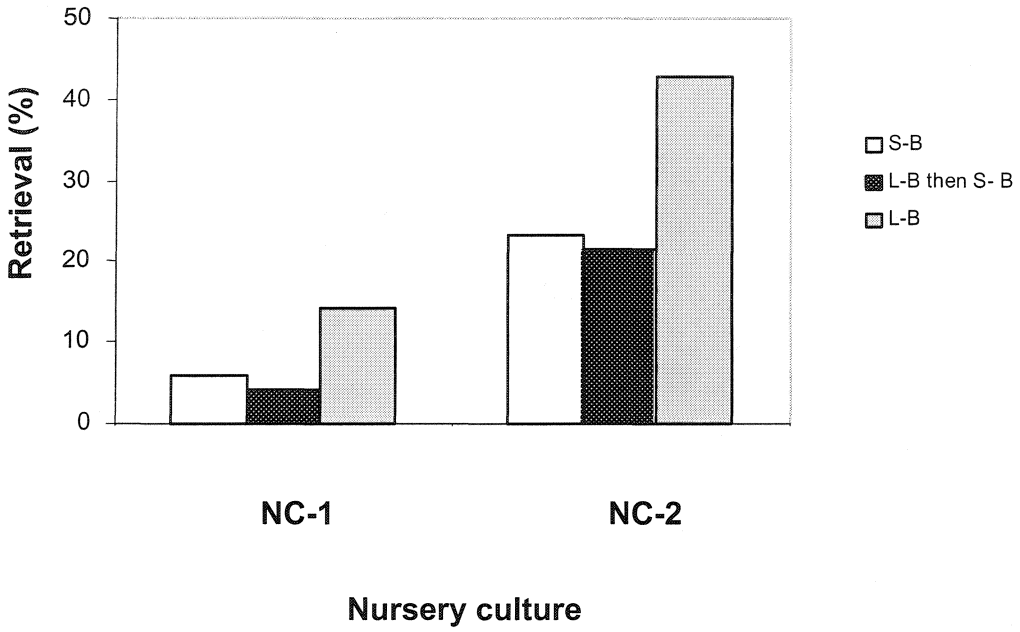


Figure III.6. Percentage retrieval of *Nodipecten nodosus* spat held simultaneously in land-based (L-B) and sea-based nurseries (S-B) for 20–22 days, and of spat transferred to the sea-based nursery after 10–11 days in the land based-nursery (L-B then S-B). NC-1 = late winter–early spring 2000, NC-2 = late summer–autumn 2001.

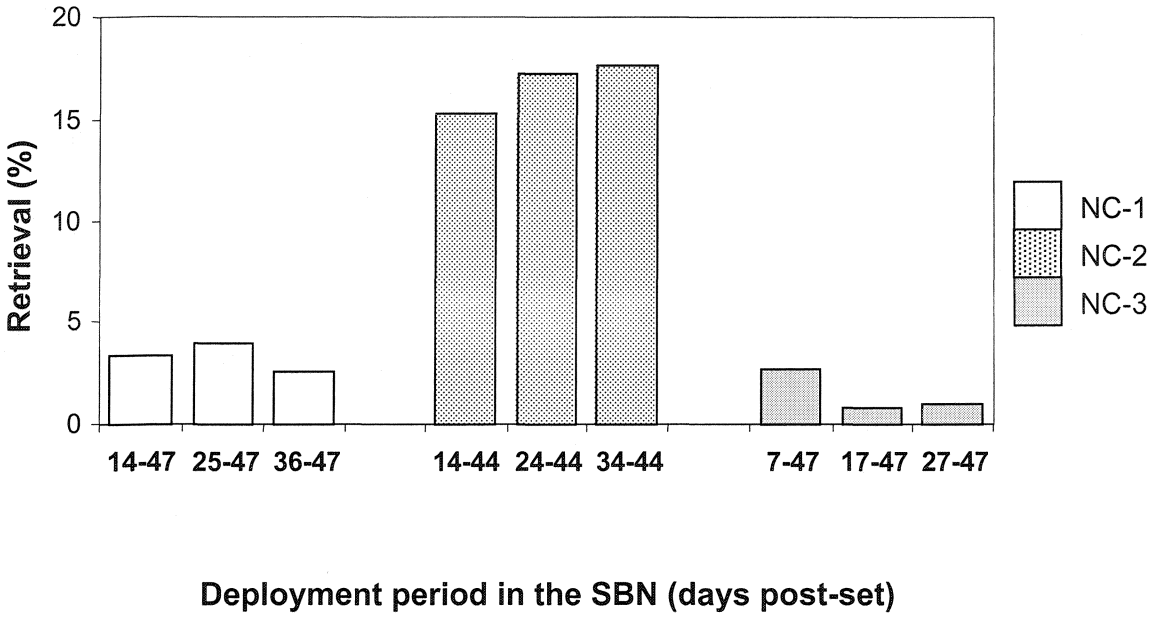


Figure III.7. Percentage retrieval of *Nodipecten nodosus* spat from the sea-based nursery (SBN) after being maintained for different periods in the land-based nursery, and retrieved 44–47 days post-set, for three nursery culture experiments: NC-1 = late winter–early spring 2000, NC-2 = late summer–autumn 2001, NC-3 = late winter–early spring 2001.

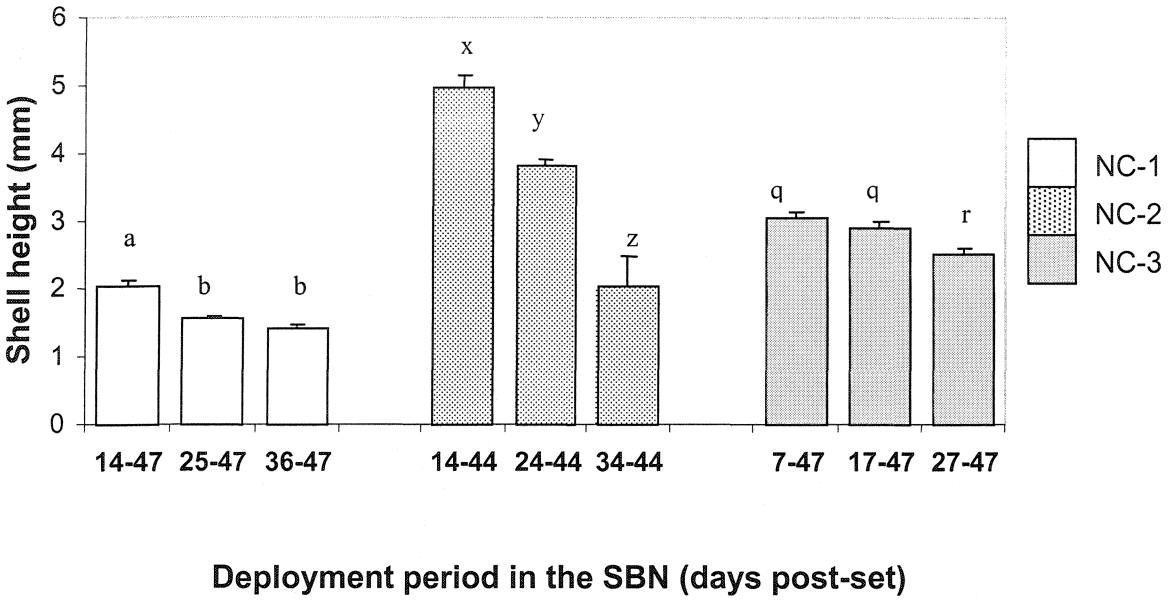


Figure III.8. Final shell height (mm) of *Nodipecten nodosus* spat from the sea-based nursery (SBN) after being maintained for different periods in the land-based nursery, and retrieved 44–47 days post-set, for three nursery culture experiments: NC-1 = late winter–early spring 2000, NC-2 = late summer–autumn 2001, NC-3 = late winter–early spring 2001. (Common letters denote no significant difference between means).

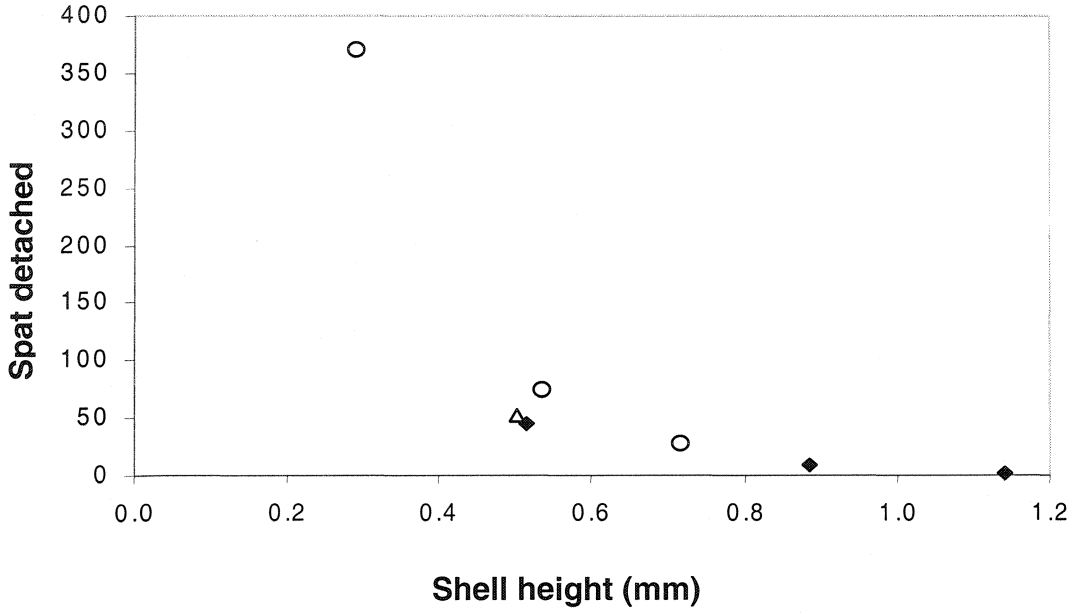


Figure III.9. Number of spat detached from the collectors during transport to the sea-based nursery in relation to mean shell height at deployment, for NC-2 (♦), NC-3 (○) and SD-4 (Δ).

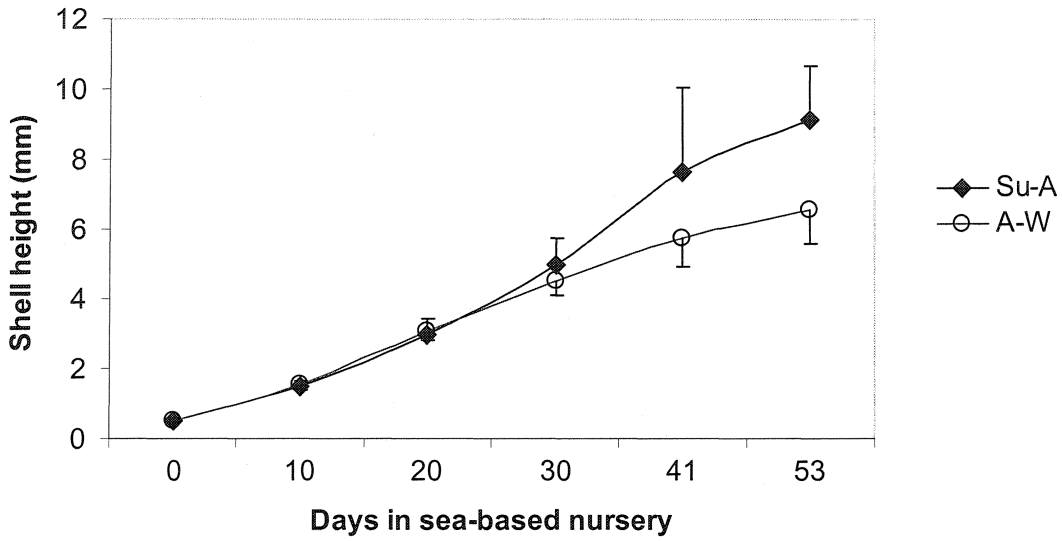


Figure III.10. Mean shell heights (mm) of *Nodipecten nodosus* spat reared in the sea-based nursery sampled at 10–11 day intervals in late summer–early autumn 2001 (Su-A) and late autumn–early winter 2001 (A-W). (mean + or - se).

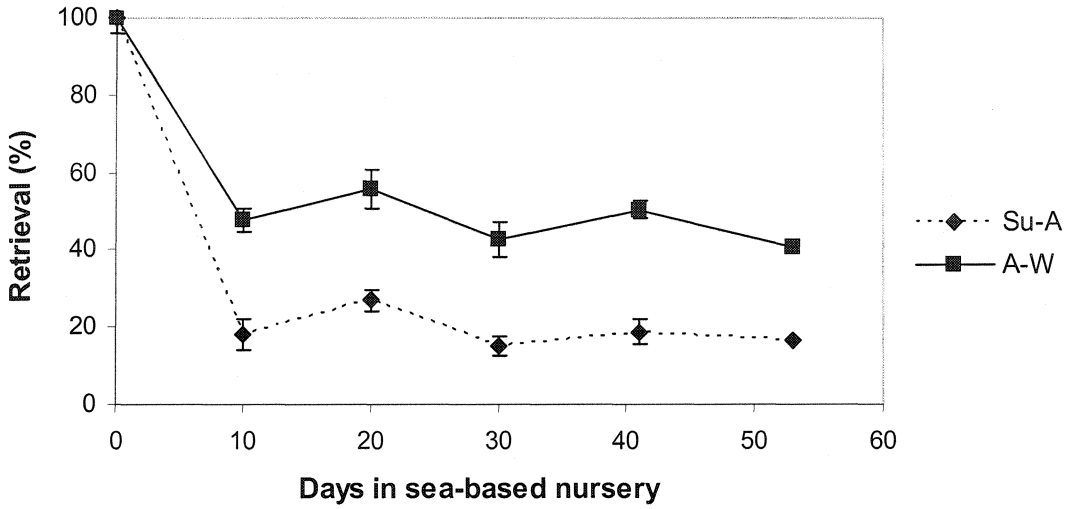


Figure III.11. Percentage retrieval of *Nodipecten nodosus* spat from the sea-based nursery at 10–11 day intervals in late summer–early autumn 2001 (Su-A) and late autumn–early winter 2001 (A-W). (Day 0 = Deployment).

CHAPTER IV

Influences of Depth and Stocking Density on Growth and Retrieval of Postlarval *Nodipecten nodosus*

IV.1. Introduction

Coastal sheltered areas off Santa Catarina State are currently experiencing an intense development of bivalve aquaculture, but the influence of environmental variability on bivalve growth and survival has only recently been addressed (Chapter III). As available embayments continue to be occupied, there will be a need in the near future to expand aquaculture activities to deeper areas farther from shore. It is therefore critical to understand the influence of depth-related variability in environmental factors on growth and survivorship of bivalves in order to establish sustainable culture strategies. As demonstrated in Chapter III, the seasonal temperature variation and a low salinity/high turbidity event associated with a period of heavy rain significantly influenced growth and possibly percentage retrieval of postlarval *Nodipecten nodosus* in sea-based nursery off Porto Belo, a coastal site in southern Brazil. While Chapter III focused on the effects of temporal variability of environmental factors on growth and survival of postlarval scallops, the present chapter focuses mainly on the influence of spatial variability in environmental conditions on performance of postlarvae.

The South Brazil Bight (SBB), is the region of the continental shelf that lies between two prominent capes, Cabo Frio (23° S) and Cabo de Santa Marta (28.5° S) (Castro and Miranda 1998), where the shelf displays a crescent shape, being wide in the centre and narrow at the northern and southern limits. The SBB is one of the most economically and ecologically important regions on the Brazilian coast, but the effects of its oceanographic variability are still poorly understood (Brandini 1990, Campos et al. 1996, Borzone et al. 1999).

An important and poorly understood oceanographic feature influencing the central SBB is the seasonal sub-surface intrusion of cold and phytoplankton-rich South Atlantic Central Water (SACW), which ascend the continental slope from deeper layers (200–500 m), influencing the shelf region (Matsuura 1986, Brandini 1990, Campos et al. 1996, Castro and Miranda 1998, Sunyé and Servain 1998). Except for coastal upwelling at the extreme limits of SBB, SACW has previously been considered to be an event mainly affecting the middle and outer continental shelf, detected at distances greater than 30 km from shore (Castro and Miranda 1998). However, sub-surface intrusions of SACW have been recently documented to influence inner shelf waters up to 15 m in a 38 m deep station during summer (Borzone et al. 1999). This oceanographic feature may have an important influence on growth and reproduction of benthic invertebrates, but to date biological effects of SACW on marine bivalves have not been addressed. Furthermore, it is not known whether SACW reach nearshore (< 1 km) coastal areas off Santa Catarina State where aquaculture activities are now expanding, nor if these sub-surface intrusions could affect growth and survival of cultured bivalves to such a degree as to be an important factor to be considered when establishing aquaculture practices.

Whereas the influence of depth on growth of juvenile and adult bivalves has been extensively studied in temperate and boreal regions (examples in Bricelj and Shumway 1991, Thompson and MacDonald 1991), no studies have focused on the influence of environmental factors related to depth on growth and survival of postlarval scallops in a subtropical environment. While some studies have reported slower growth near the surface (MacDonald and Bourne 1989, Román et al. 1999), growth usually decreases below a critical depth, as environmental conditions such as temperature, food availability

or turbidity often display a vertical gradient, reaching sub-optimal levels in deeper waters (Leighton 1979, MacDonald and Thompson 1985, MacDonald and Bourne 1989, Côté et al. 1993, Emerson et al. 1994, Lodeiros et al. 1998, Grecian et al. 2000, Fréchette and Daigle 2002). In view of the environmental variability characteristic of waters off southern Brazil, it is possible that an unusual situation of high food level and low temperature below a critical depth could influence growth of scallops in suspended culture close to shore.

The stocking density of bivalves within enclosures is an important variable to be considered in aquaculture, due to intraspecific competition for food and space. Density-dependent growth of juvenile and adult scallops cultured in lantern or pearl nets has been well documented (Duggan 1973, Ventilla 1982, Parsons and Dadswell 1992, Côté et al. 1993, Côté et al. 1994, Maeda-Martinez et al. 1997, Román et al. 1999, Grecian et al. 2000) but none of these studies focused on postlarval scallops within the size range examined in the present one, in which they were attached to the collectors by byssal threads.

The main objective of the present study was to investigate the influences of environmental factors associated with depth on growth and percentage retrievals of postlarval *N. nodosus*. Experiments were carried out during a period when it was predicted that SACW could influence the coastal site (experiment 1) (March–April), as well as when no influence of SACW was expected (experiment 2) (June–July). It was hypothesised that a cold and phytoplankton rich water mass originating from SACW intrusions could reach the coastal aquaculture site in late summer, resulting in significant differences in growth of postlarval scallops above and below the thermocline, growth

being slower in the deeper layer. Conversely, in periods of SACW absence, growth should be similar at different depths. It is possible, therefore, that the influence of depth on scallop growth varies on a temporal scale on the southeastern coast of Brazil. A secondary objective was to investigate the effects of stocking density on growth of postlarval scallops.

IV.2. Materials and Methods

IV.2.1. Study area

Experiments were undertaken off Porto Belo Point (Lat. 27° 07' S; Long. 48° 31' W) (Fig. I.2), Santa Catarina State, Brazil. A sub-surface long-line, on which the main line was located at 3 m below the surface, was deployed at a 15 m deep site, at a distance of 150 m from shore. This site was located approximately 2 nautical miles to the NE of João da Cunha Island, where the experiments from Chapter III were carried out.

IV.2.2. Spat production

Experimental production of postlarval *N. nodosus* was undertaken at the Laboratory for the Culture of Marine Molluscs (LCMM-UFSC), Florianópolis, Santa Catarina State, Brazil. Two experimental spat productions were carried out in February and May 2001, using procedures described in Chapter II. When larvae were competent to undergo settlement and metamorphosis, they were retained on a 140-μm-mesh nytex screen, counted and transferred to the experimental tanks. Settlement was carried out in 200-L tanks, stocking pediveligers at densities of 1.2 larvae mL^{-1}. Japanese type Netlon® monofilament collectors of about 1.5 m (averaging 105.2 g, se = 1.2; n=9), commonly used in scallop commercial operations, were used as substrate for settlement. During settlement, seawater (salinity = 33–34 psu) was UV-irradiated and passed through a 1-μm

cartridge filter. Water was changed daily, and feeding consisted of a mixture of *Isochrysis galbana* (T-iso), *Chaetoceros calcitrans* and *C. muelleri* (2:2:1) in exponential growth phase, supplied daily in final concentrations ranging from $4x10^4$ to $5x10^4$ cells mL^{-1}. In the laboratory water temperatures were 24–25°C and 21–22°C for experiments 1 and 2, respectively.

In experiment 1, carried out from mid March to mid April, 2001 (late summer–early autumn), postlarval scallops were transferred to the field after 12 days post-settlement. The Netlon collectors were placed inside 1.5 mm-mesh nylon spat bags (1 collector/bag) and then transferred to the sea, using procedures described in Chapter III. Prior to deployment, four collectors per tank were sampled to estimate the initial number and size of postlarvae. Initial sampling indicated two different densities of spat/collector in different setting tanks, which are hereafter termed high and low densities. Deployment was undertaken by SCUBA diving, spat-bags being tied at depths of 4 and 12 m from the surface to two ropes hanging perpendicularly from the long-line. A 2 kg weight was attached to the bottom of each drop line. Spat-bags (high and low densities) were tied individually to the ropes at 4 and 12 m. In this manner, a factorial design with 2 replicates was used to evaluate the effects of depth and density on growth of postlarval scallops. Control collectors were deployed in the sea simultaneously to the experimental units to monitor wild scallop settlement. All collectors were retrieved 26 days after deployment (38 days post-set), and transferred back to the laboratory, where spat were detached and sampled as described in Chapter III. No spat of *N. nodosus* settled on the control collectors.

In experiment 2, carried out from early June to early July, 2001 (late autumn–early winter), postlarval scallops were transferred to the field 15 days after settlement. The collectors, spat-bags and procedures were similar to experiment 1. As initial sampling indicated that numbers of spat/collector were similar among setting tanks, single spat-bags (1 collector/bag) were tied to triplicate ropes hanging perpendicularly to the long-line at 4 and 12 m depth. Collectors were retrieved 27 days after deployment (42 days post-set) and transferred to the laboratory, where spat were detached and sampled as previously described.

For both experiments, the initial shell height before deployment (maximum dorso-ventral distance perpendicular to the umbo) (n=30) and final shell height at retrieval (n=30 / spat bag) were determined with an ocular micrometer. To estimate the degree of clogging of the spat bags, individual bags from both experiments were thoroughly scrubbed inside plastic containers after retrieval. The sediments and fouling deposited in the containers were collected on pre-weighed 0.5 x 0.5 m cellulose filter papers folded into a funnel shape. The filters were then dried for 72 h at 60°C, cooled in a dessicator and re-weighed to determine the dry weight of the sediments and fouling attached to the external spat bags.

IV.2.3. Environmental variables

Water samples from 4 and 12 m depth were taken weekly at the field site using a 2-L Van Dorn sampling bottle. Samples were transferred to the laboratory in a cooler containing ice packs. Temperatures at both depths were recorded hourly with

Stowaway™ data loggers and dissolved oxygen was determined *in situ* with a digital dissolved oxygen meter (YSI™ model 55/12FT). In the laboratory, water samples were gently stirred and filtered through a 350-μm-mesh screen to remove larger zooplankton. Total suspended particulate matter (TPM), particulate organic matter (POM) and particulate inorganic matter (PIM) were determined gravimetrically after filtering 400 mL seawater samples on a 24 mm diameter Whatman™ 934-AH glass fibre filter (Parsons and Dadswell 1992). Chlorophyll-*a*, as an index of phytoplankton biomass, was determined with a Turner™ digital fluorometer (TD 10-AU) after filtering triplicate, 100 mL water samples (Whatman™ 934-AH) and extracting chloropigments in 90 % acetone, using standard methods (USEPA Method 445.0). Salinity was determined by a digital WTW Multiline P4 water quality meter. All variables were determined in triplicate samples.

IV.2.4. Statistical analyses

Data were analysed with the SPSS statistical package (version 10). Residuals were checked graphically for normality and homoscedasticity (Sokal and Rohlf 1995). A factorial design was used in experiment 1, and a two-way ANOVA ($\alpha = 0.05$) was performed to test for differences in shell heights and percentage retrievals at different depths and densities, using depth and density as fixed factors. In experiment 2, an independent sample "t" test was used to determine the differences in shell height and percentage retrievals between depths.

IV.3. Results

IV.3.1. Growth

In experiment 1, carried out from mid March to mid April 2001, postlarval scallops were deployed in the sea at an initial mean shell height of 0.403 mm. The mean number initially attached per collector was 340 (se = 54.7) and 150 (se = 47.0) for the high and low density treatments, respectively. After 26 days of immersion, the mean shell height of scallops at 4 m was 5.07 mm (se = 0.13) and 5.67 mm (se = 0.16) for collectors with high and low densities, respectively (Fig. IV.1A), whereas at 12 m the corresponding shell heights were 3.28 mm (se = 0.10) and 3.60 mm (se = 0.11). Significant differences in final shell height were found between depths (ANOVA, $F = 232.4$; $df = 1, 236$; $P < 0.001$) and between collectors with high and low densities of scallops (ANOVA, $F = 13.31$; $df = 1, 236$; $P < 0.001$), but the interaction between density and depth was not significant ($P = 0.26$). The resulting daily growth rates (DGR) at 4 m were 0.179 mm day^{-1} and 0.203 mm day^{-1} for scallops grown at high and low densities, respectively, whereas at 12 m DGR was 0.11 mm day^{-1} and 0.123 mm day^{-1}, respectively.

In experiment 2, carried out in from early June to early July 2001, postlarval scallops were deployed in the sea at an initial mean shell height of 0.434 mm, and the initial mean number of spat attached per collector was 401 (se = 95.9). No significant difference in mean shell height was detected between depths ("t"test, $t = 1.93$; $df = 1, 178$; $P = 0.056$). Mean shell height at retrieval (after 27 days) was 4.34 mm (se = 0.25) at

4 m and 4.12 mm (se = 0.15) at 12 m (Fig. IV.1B). DGR was 0.144 and 0.137 mm day^{-1} at 4 and 12 m, respectively.

The dry weight of sediments attached to the spat bags on retrieval was 33.05 g (se = 1.47) and 30.06 g (se = 2.16) for 4 m and 12 m in experiment 1, respectively, and 35.01 g (se = 1.55) and 34.18 (se = 5.12) for 4 m and 12 m in experiment 2, respectively. There was no significant difference in the sediment content between depths in both experiments, eliminating sediment load as a factor influencing growth rates ("t"test, t = 1.5, df = 1,4; P = 0.10 in experiment 1, and t = 0.99, df = 1,6; P = 0.18 in experiment 2).

IV.3.2. Percentage retrievals

In experiment 1, the mean number of spat retrieved from the collectors at 4 m was 247 (se = 42) and 111 (se = 10) for high and low densities, respectively, whereas at 12 m the number of spat per collector was 245 (se = 87) and 122 (se = 2), respectively. The percentage retrievals were similar between depths and densities (ANOVA, F = 0.05; df = 3, 4; P = 0.9), ranging between 72.2 % and 81.3 % (Fig. IV.2A). In experiment 2, the mean number of spat retrieved per collector was 317 (se = 28) and 278 (se = 54) at 4 m and 12 m, respectively. Percentage retrievals were similar between depths ("t"test, t = 0.64; df = 1, 4; P = 0.56) ranging from 69.3 % to 79.05 % (Fig. IV.2B).

IV.3.3. Environmental variables

IV.3.3.1. Temperature

Water temperature in experiment 1 (March–April), recorded hourly, was highly variable (Fig. IV.3A), especially in the first 2–3 weeks of the experiment (March), after which the water column became homogeneous (April). At 12 m temperature variability CV = 10.4 %) was greater than at 4 m CV = 5.3 %) and temperatures were relatively lower. The mean temperature at 4 m was 25.3°C, with a maximum of 28.2°C and a minimum of 19.6°C. At 12 m, mean temperature was 22.8°C, with a maximum of 27.2°C and a minimum of 17.8°C. Furthermore, on several occasions in March temperature at 12 m varied 6–9°C within a 24 h period.

In experiment 2, from early June–early July, temperature was uniform throughout the water column and thus similar at both depths (Fig.IV.3B). The mean temperature was 20.4°C and 20.1°C at 4 and 12 m, respectively. While maximum temperature at 4 m and 12 m was 22.2°C and 21.7°C, respectively, the minimum temperature at both depths was 17.8 °C.

IV.3.3.2. Salinity

There was negligible variation in salinity at 12 m (mean 33.3 psu) throughout experiment 1 (Fig. IV.4A), whereas at 4 m mean salinity was 32.2 psu, and a lower value (29 psu) was recorded on one occasion. In experiment 2, the water column was

homogeneous with mean salinities of 32.1 psu and 32.4 psu at 4 m and 12 m, respectively.

IV.3.3.3. Chlorophyll-a

The concentration of chlorophyll-*a* was highly variable during experiment 1, and there were marked differences between 4 and 12 m (Fig. IV.4B). The mean concentration at 12 m (1.16 $\mu g\ L^{-1}$) was more than twice that at 4 m (0.5 $\mu g\ L^{-1}$). Maximum concentrations at 12 m reached 1.55 and 2.82 $\mu g\ L^{-1}$, which coincided with periods when the difference in temperature between 4 and 12 m was about 6°C. Simultaneously to these samplings, mean concentrations at 4 m were 0.21 $\mu g\ L^{-1}$and 1.15 $\mu g\ L^{-1}$, respectively. In experiment 2, on the other hand, variability of chlorophyll-*a* was relatively small and there were negligible differences between depths. At 4 m the mean chlorophyll-*a* was 0.85 $\mu g\ L^{-1}$, whereas at 12 m it was 0.82 $\mu g\ L^{-1}$.

IV.3.3.4. Seston

Total particulate matter (TPM) was highly variable in experiment 1, and was higher at 12 m (mean 2.17 mg L^{-1}) than at 4 m (mean 1.16 mg L^{-1}) (Fig. IV.5A). On the other hand in experiment 2, there were negligible differences in TPM between 4 m (mean 1.56 mg L^{-1}) and 12 m (mean 1.71 mg L^{-1}), and TPM was higher towards the end of the experiment.

In experiment 1, PIM was higher at 12 m, averaging 1.76 mg L^{-1} , whereas at 4 m mean PIM was 0.69 mg L^{-1} (Fig. IV.5B). POM followed a similar pattern at both depths with a mean of 0.47 mg L^{-1} at 4 m and 0.44 mg L^{-1} at 12 m (Fig. IV.5C). PIM as percentage of TPM was higher at 12 m (mean 81.4 %) than at 4 m (mean 59.6 %) (Fig. IV.6A). In contrast in experiment 2, PIM values were similar at 4 and 12 m, averaging 1.10 mg L^{-1} and 1.14 mg L^{-1}, respectively (Fig. IV.5B). Differences in POM levels between 4 m (mean 0.46 mg L^{-1}) and 12 m (mean 0.57 mg L^{-1}) were negligible (Fig. IV.5C). As a result, mean PIM as a percentage of TPM was 69.7 % and 65.2 % at 4 and 12 m, respectively (Fig. IV.6A).

IV.3.3.5. Dissolved oxygen

In experiment 1, dissolved oxygen was higher at 4 m, where percent saturation ranged from 82.8 to 92.1 % (mean 91.1 %) (Fig. IV.6B), whereas at 12 m (mean 75.3 %) minimum levels reached 61.9 % and 48.8 %. These dissolved oxygen minima coincided with periods when temperature at 12 m was about 6°C lower than at 4 m. In experiment 2, mean percent oxygen saturation was 90.82 % and 84.50 % at 4 m and 12 m, respectively.

IV.4. Discussion and Conclusions

IV.4.1. Growth

Differences in growth of postlarval *Nodipecten nodosus* were recorded between 4 and 12 m in experiment 1, during late summer–early autumn. Scallops attained a larger size near the surface, where growth rate was approximately 1.6 times higher than at 12 m. Conversely, in experiment 2, during late autumn–early winter, growth of scallops was similar at both depths. Whereas similar growth rates in experiment 2 could be explained by the homogeneous conditions throughout the water column, the differential growth recorded in experiment 1 can be attributed to differences in environmental factors between depths.

This is the first study to report the influence of a sub-surface intrusion of cold water with higher phytoplankton biomass in a coastal aquaculture area in southern Brazil, and to demonstrate its significant effect on bivalve farming. Although food quantity, as expressed by concentration of chlorophyll-*a*, was higher at 12 m in experiment 1, growth was lower. Differences in mean temperature between depths were too small (2.5 °C) to account for the observed differences in growth. However, the higher temperature variation on a short time scale recorded at 12 m, together with the longer exposure period to lower temperatures, may have stressed the scallops, thus limiting growth. In addition, while at 4 m temperature dropped below 20°C for only 4 h, and at 12 m this happened for at least 78 h, a period which growth rates may have been reduced. Growth in bivalves is an integrated response to food availability and temperature (Bayne and Newell 1983,

MacDonald and Thompson 1985, Bricelj and Shumway 1991, Parsons and Dadswell 1992, Côté et al. 1993). No studies on bivalve growth have considered the effects of temperature variation on such a short time scale as in the present study (ca. semi-daily). Pilditch and Grant (1999) studied the effect of food availability and temperature variation in 8 d cycles on growth and metabolism of juvenile scallops *Placopecten magellanicus* under laboratory conditions. Temperature variation in such cycles, had a minimal effect on growth, but scallops did not acclimate to the temperature cycles, displaying metabolic rates tightly coupled with water temperature, which is also in agreement with results obtained by Shumway et al. (1988) who found that metabolic rates of *P. magellanicus* follow the seasonal and laboratory acclimation temperatures. The scallop *P. magellanicus* in Newfoundland also displayed feeding and metabolic rates significantly correlated with environmental temperatures, but also with food availability (MacDonald and Thompson 1986). In mussels (*Mytilus edulis*), on the contrary, compensatory acclimation to temperature changes have been demonstrated (Widdows 1976). A strong dependence of physiological rates on environmental temperature in scallops suggests that the periods of lower temperature may have depressed physiological rates in *N. nodosus*, contributing to the observed reduction growth during periods of lower temperature.

PIM as percentage of TPM is an important environmental factor affecting growth of bivalves. The amount of PIM in seawater dilutes POM, reducing the nutritional value of seston for scallops (Vahl 1980, Wallace and Reinsnes 1985, Bricelj and Shumway 1991, Emerson et al. 1994). For example, when PIM reaches a critical value of about 80 % of TPM, *Chlamys islandica* cannot absorb POM, resulting in a reduction of feeding rate, thus reducing or stopping growth (Vahl 1980). Although certain bivalves (including

some scallops) can selectively ingest organic particles to compensate for dilution of food quality (Widdows et al. 1979, Bricelj and Malouf 1984, MacDonald and Ward 1994), at high concentration of low quality seston *P. magellanicus* lack this ability (Bacon et al. 1998). In experiment 1, percent PIM was markedly higher at 12 m than at 4 m, averaging 81.4 % and reaching a maximum of 90.5 %. Thus, the benefits of higher food abundance, as expressed by levels of chlorophyll-*a*, were probably offset by the negative effect of a higher inorganic content of seston, resulting in significantly lower growth rates at 12 m. It was therefore difficult to decouple the effects of temperature and inorganic content of the seston in determining which was the major factor affecting growth, since high PIM was associated with temperature oscillations in experiment 1. It is more likely, however, that an interaction of both variables contributed to the slower growth recorded at 12 m. In general, POM concentrations were similar between depths, but on one occasion an unusual situation arose in which POM was higher at 4 m, but chlorophyll-*a* was higher at 12 m. This coincided with a low salinity event at 4 m, probably caused by continental runoff due to rainfall (as also reported in Chapter III), which may have exported terrigenous organic matter.

The food supply for cultured bivalves is also largely influenced by water flow and the concentration, size and quality of food particles (Grant 1996). It is unlikely that water flow have differed between 4 and 12 m in the present study causing differences in growth, because such differences would have also been observed in experiment 2, in which growth was similar between depths. Food size and quality for bivalves is dependent on the phytoplankton species composition, which was not possible to evaluate in the present study. The possibility that phytoplankton composition differed between depths in

experiment 1 cannot, therefore, be ruled out and requires further investigation. Although several studies have surveyed phytoplankton composition in coastal and oceanic regions of southern Brazil (citations in Soares 1983), none focused on the composition of sub-surface intrusions of SACW. Nevertheless, from an aquaculture perspective, it is significant that during summer–early autumn the coastal culture site is under the influence of a thermocline oscillation caused by the proximity of SACW, and under these circumstances nutritional conditions and thermal variation are less favourable for growth of postlarval scallops.

Brandini et al. (2000) proposed that if bivalves were grown offshore in depths influenced by SACW, growth would be maximised due to higher food abundance as expressed by levels of chlorophyll-*a*. The present study suggests that growing scallops below the thermocline during the SACW intrusion may not necessarily be beneficial, since other concurrent factors may neutralise the benefits of enhanced food levels. It is possible, however, that the influence of SACW on growth may vary according to the species under consideration. *Nodipecten nodosus* occurs in shallow tropical waters (Smith 1991) and the study area is at its southern limit of distribution in the Southern Hemisphere, where minimum temperatures approach values that reduce growth rates (Chapters III and V). Furthermore, scallops display low tolerance to high PIM (examples in Bricelj and Shumway 1991). Therefore, if *N. nodosus* is to be cultured in open areas of southern Brazil in the future, it is suggested that growth would be enhanced if the culture nets were suspended in waters above the influence of SACW. Conversely, other species, such as the Pacific oyster *Crassostrea gigas* and the mussel *Perna perna*, which are currently cultured in embayments in southern Brazil, may grow well if cultured under

SACW influence. Unlike scallops, intertidal bivalves are usually more tolerant to temperature variability and high PIM levels (Bayne 1976, Newell and Langdon 1996, Shumway 1996). The influence of environmental instability associated with SACW intrusions on bivalve growth requires further examination before aquaculture expands further to deeper waters off southern Brazil.

In the present study, the postlarval scallops cultured at a higher stocking density (340/bag) displayed a slight but statistically significant reduction in growth compared with those grown at lower density (150/bag) at both depths in experiment 1. Grecian et al. (2000), however, found no difference in growth of *Placopecten magellanicus* at stocking densities of 2600 and 5200 spat/bag, but the surface area of the spat bags and the mesh opening were larger than those used in the present study, so that a direct comparison is difficult. A negative relationship between growth and stocking density inside culture nets has been described for other scallops (Duggan 1973, Ventilla 1982, Parsons and Dadswell 1992, Côté et al. 1993, Côté et al. 1994, Maeda-Martinez et al. 1997, Román et al. 1999). Parsons and Dadswell (1992) postulated that at higher stocking density intra-specific competition for food reduces its availability per individual, limiting growth. Higher densities also lead to frequent contact among organisms, which may induce irritation, retraction of the mantle or valve closure, resulting in less feeding, and may also cause shell breakage along the edges (Côté et al. 1993). Within the range of densities tested, the reduction in growth at higher density was slight and it would be more cost-effective to grow postlarval *N. nodosus* at the higher density. This strategy, however, is only appropriate if the spat bags are retrieved after an immersion period of about 26–30 d, as in the present study, when scallops at 4 m in experiment 1 reached a mean size (> 5

mm) at which they could be transferred to pearl nets. On the other hand, if the spat bags are to be immersed for longer periods before spat are detached and transferred to pearl nets, it is likely that the density dependence of growth would increase as scallops attain larger sizes.

A comparison of daily growth rates (DGR) can be made between the high density treatment in experiment 1 (350 scallops/bag; se = 54.7) and experiment 2 at both depths (401 scallops/bag; se = 95.9), in which stocking densities were equivalent. While the lowest DGR was recorded in experiment 1 at 12 m (0.11 mm d^{-1}), and the highest at 4 m (0.179 mm d^{-1}), DGR in experiment 2 was 0.144 and 0.137 mm d^{-1}, respectively, at 4 and 12 m, which were statistically similar. Lower growth rates at 12 m in experiment 1 can be explained by differences in environmental factors between depths as previously noted. Differences in DGR between 4 m in experiment 1 and both depths in experiment 2 can be explained by seasonal differences in temperature. Chlorophyll-*a*, seston, salinity and oxygen were similar at 4 m in experiment 1 and at both depths in experiment 2, and therefore cannot account for observed differences in growth. Temperature, on the other hand, was significantly lower in experiment 2 (mean 20.4°C and 20.1°C at 4 and 12 m, respectively) than experiment 1 at 4 m (mean 25.6°C). This difference probably accounted for differences in growth between seasons, in accordance with the results from Chapter III, which showed that variation in temperature, rather than food availability, explained seasonal differences in DGR of postlarval scallops.

IV.4.2. Percentage retrievals

Percentage retrievals of postlarval *N. nodosus* were similar between depths and experiments, and ranged from 69.3 % to 81.3 %. These results are much higher than those obtained in Chapter III (1.5–42.8 %) when spat were deployed in the sea at the same size as in this study, but spat bags were suspended from a surface long-line. Percentage retrievals of *N. nodosus* were also higher than those reported in Heasman et al. (2002) (25.4 %) for *Pecten fumatus*, when postlarval scallops at a shell height of 0.5 mm were deployed in the sea-based nursery attached to monofilament collectors, as in the present study. Stocking density, as well as the variability in environmental factors recorded in the present study, had a negligible effect on retrieval of scallops, even when they experienced sudden temperature changes at 12 m in experiment 1. Dickie and Medcof (1963) reported mass mortalities of adult scallops *Placopecten magellanicus* in Atlantic Canada, due to a sudden change in the position of the thermocline. In southern Brazil, *N. nodosus* under culture at 12 m can experience sudden temperature shocks semi-daily of the same magnitude as seasonal temperature variation, but such variation did not cause spat mortality. Temperatures in the present study did not reach lethal levels, as determined in Chapter V. In Chapter III, it was postulated that wave action affecting the surface long-line may have contributed to detachment of postlarval scallops from the collectors and consequent losses through the mesh, and it was proposed that the use of a sub-surface long-line could improve spat retrievals. In the present study, the buoys and the main line were located 3 m below surface, resulting in little, if any, influence of wave action on the spat bags. Two of the field-based nursery experiments described in Chapter

III, which resulted in percentage retrievals of 15.2 % and 42.8 %, were carried out concomitantly with the present study. The higher percentage retrievals recorded from the sub-surface long-line indicate that wave action may have indeed increased detachment of spat in the Chapter III experiments, although the effects of other factors (i.e., salinity, turbidity) may also have contributed to low retrieval in the experiments undertaken in different seasons.

IV.4.3. Environmental variables

During the first 2–3 weeks of experiment 1 (March), scallops at 12 m were influenced by an oscillating water mass with thermal characteristics different from the upper layer, with the result that temperature variability at 12 m was greater than at 4 m. A colder bottom water mass frequently influenced the 12 m layer, but only occasionally reached 4 m. These differences in water masses between depths resulted in a temperature change of 10.4°C between 4 m and 12 m in less than 24 h, resulting in a semi-daily variability in temperature equivalent to that observed on a seasonal time scale (Chapter III). Such cold water intrusions disappeared towards the end of the experiment 1 (April) and were not observed in experiment 2 (June–July). Furthermore, maximum values of chlorophyll-*a* were recorded in March, when concentrations at 12 m were higher than at 4 m. Such spatial variation in temperature and chlorophyll-*a* is in agreement with the patterns reported in other studies for the inner continental shelf when SACW is present (Brandini 1990, Borzone et al. 1999). There was strong evidence that scallops held at 12

m in experiment 1 were under the oscillating influence of a water mass that could be identified as predominantly SACW. SACW has been characterised as a cold water mass (10–20°C) affecting lower layers of the continental shelf (Matsuura 1986, Castro and Miranda 1998) with relatively higher concentrations of nutrients and chlorophyll-*a*, lying underneath warmer Coastal Waters (CW), which during summer are reported to be relatively oligotrophic (chlorophyll-*a* < 0.4 μg/L) off Santa Catarina State (Brandini 1990). Borzone et al. (1999) indicated that from December to April (summer–early autumn), the water column on the inner continental shelf displayed a marked thermocline, with near-surface temperature reaching 27°C, whereas below the thermocline, temperature was 16°C and maximum chlorophyll-*a* was recorded (ca. 3 μg L^{-1}). This gradient was attributed to SACW intrusions. Conversely, from May to September (late autumn–winter–early spring) the temperature gradient disappeared with vertical mixing of the water column (Borzone et al. 1999). During this period SACW retreats toward the shelf break (Castro and Miranda 1998) and the inner continental shelf becomes influenced by cold (< 20°C), low salinity water (29–35 psu) of sub-Antarctic origin (SAW), leading to a vertically mixed water column (Sunyé and Servain 1998). These observations are in agreement with the recorded differences in temperature between experiments 1 and 2 in the present study. The salinity reported for SACW is around 35 psu (Matsuura 1986, Castro and Miranda 1998), and salinity at 12 m remained close to 33 psu throughout the present study. According to Castro and Miranda (1998), mixing of SACW and CW occurs across the continental shelf during summer. The water resulting from this interaction, maintaining the major characteristics of SACW (relatively low temperature

and high chlorophyll-*a* but with slightly lower salinity) is probably the water mass that repeatedly affected the culture site at 12 m during summer, causing strong thermal instability. Moreover, during summer, when the upper layers of the inner continental shelf are dominated by warm CW, the only reports of waters colder than 20°C in the whole SBB are for areas affected by SACW (Castro and Miranda 1998), providing further evidence that the cold waters affecting lower layers of the coastal aquaculture site originated from SACW.

The oscillation of the cold water mass in the study site during summer may have been a result of tidal influence or wind stress. Penetration of SACW onto the continental shelf has been related to situations in which prevailing winds blow offshore, generating a cross-shelf circulation towards shore in the deeper layers (Castro and Miranda 1998). However, the short-term variability in the position of SACW has not been investigated in detail. The factors influencing hydrographic processes in shallow coastal areas are complex and vary greatly on short spatial and temporal scales (Mann and Lanzier 1991). The study region is characterised by a micro-tidal regime, which may have contributed to the oscillation of the cold water mass, but the small tidal amplitude may not have been sufficient to destabilise the thermocline and generate vertical mixing throughout the water column. The spatial and temporal dynamics of SACW along the southeastern coast of Brazil are still poorly understood, and the implications of SACW intrusions on aquaculture requires further study.

Another important environmental factor that markedly differed between 4 and 12 m in experiment 1, but not in experiment 2, was PIM, which was higher at 12 m. No previous study carried out in southern Brazil has investigated the organic and inorganic

contents of the seston associated with different water masses. The higher percent PIM near the bottom recorded in experiment 1, but not in experiment 2, is possibly associated with SACW, suggesting that during the process of SACW intrusion into the lower layer across the continental shelf, a frictional shear on the substrate resuspends bottom sediments, resulting in the higher particulate inorganic matter of SACW. According to Borzone et al. (1999), the bottom sediments of the inner continental shelf of SBB are formed by quartz sand with a silt-clay fraction that increases with depth. These fine sediments could be resuspended in the water column, increasing PIM levels of SACW. A further indication of the deeper origin of the water mass affecting the bottom layers is the dissolved oxygen, which was lower at 12 m on two occasions, once falling below 50 %. According to Longhurst and Pauly (1987), waters originated from deeper layers of the ocean often contain less oxygen than surface waters.

In conclusion, the influence of environmental factors associated with depth at an aquaculture site in southern Brazil varied seasonally, depending on the sub-surface intrusion of SACW. The characteristics of this water mass were less favourable for postlarval scallop growth than those of near-surface waters. During summer–early autumn, lower temperature and sudden temperature changes, together with a higher inorganic content of the seston associated with SACW, resulted in slower growth of postlarval *N. nodosus* at 12 m than at 4 m. Furthermore, on several occasions the vertical thermal gradient from 4 to 12 m (ca. 10°C) was similar to the seasonal temperature variation. Conversely, during late autumn–early winter, the water column was vertically mixed and scallop growth was similar at both depths. Higher temperatures explained higher growth rates near surface during summer–early autumn, compared with lower

growth in late autumn–early winter at both depths. Furthermore, growth of postlarval scallops cultured in spat bags showed an inverse relationship with stocking density, but the differences were small and it would be more advantageous to grow postlarval scallops at the highest density tested. It is suggested that during summer–early autumn, if the site is subject to SACW intrusions, suspended nursery culture should be undertaken above the thermocline in order to maximise growth.

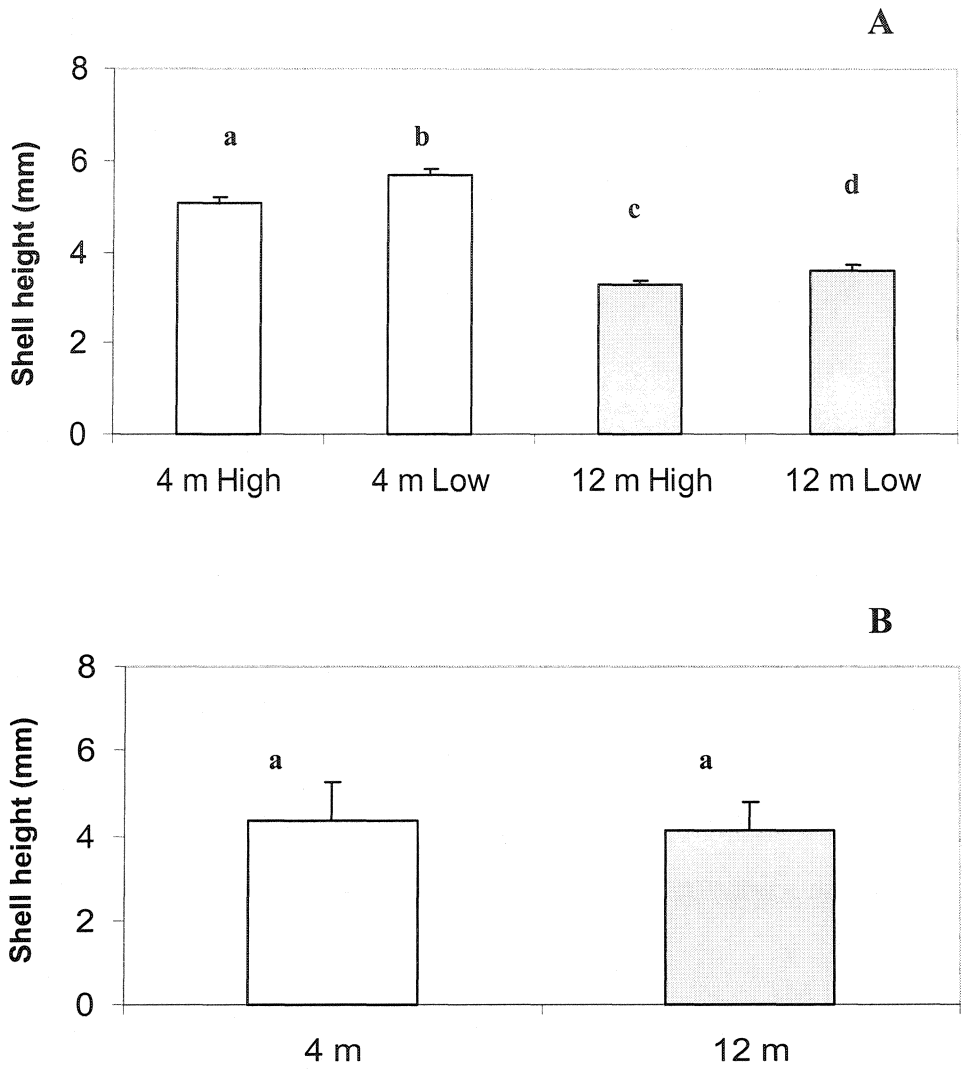


Figure IV.1. **A** - Final shell heights (mm) of scallops cultured at 4 m and 12 m depth at high (350 spat/collector) and low (140 spat/collector) stocking densities for 26 days off Porto Belo Point - experiment 1 (March – April 2001). **B** - Final shell heights (mm) of scallops cultured at 4 m and 12 m depth for 27 days off Porto Belo Point - experiment 2 (June – July 2001). (Common letters denote no significant differences). (mean +se).

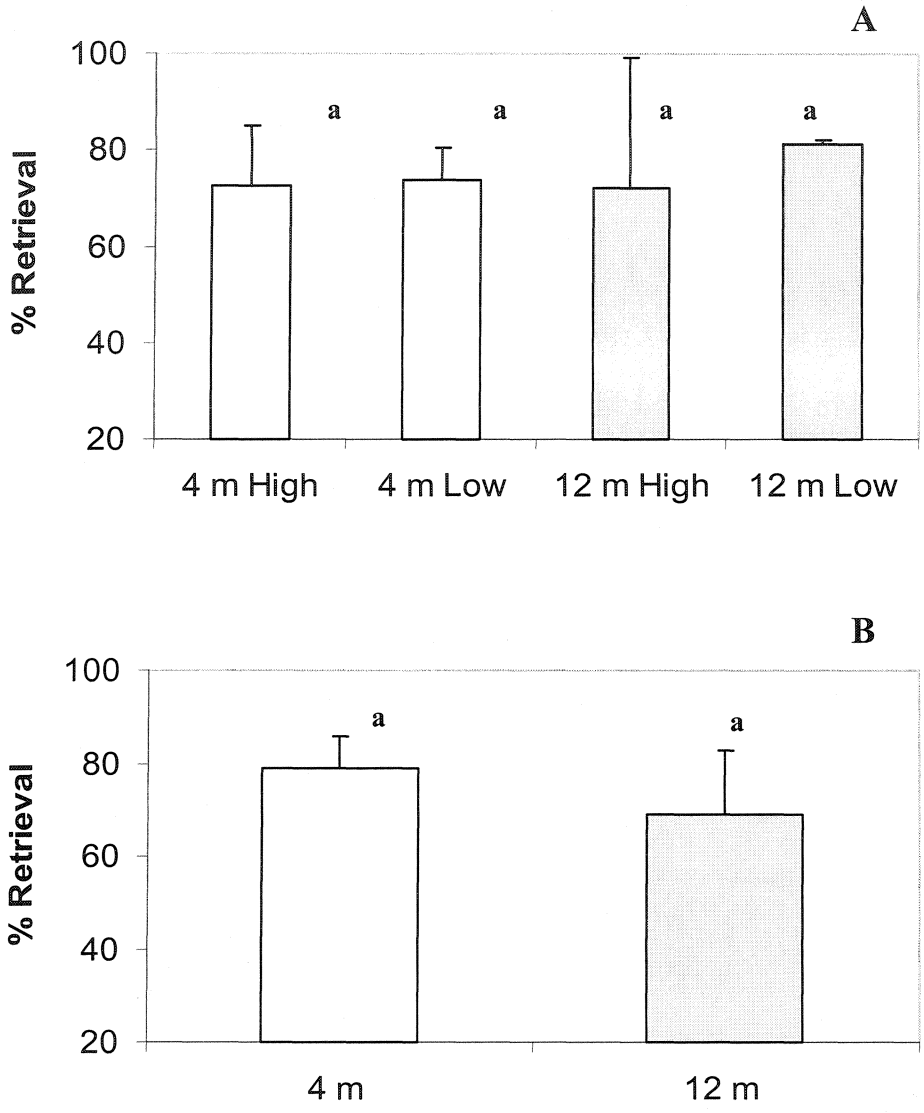


Figure IV.2. **A** - Percentage retrieval of scallops cultured at 4 m and 12 m depth at high (350 spat/collector) and low (140 spat/collector) stocking densities for 26 days off Porto Belo Point - experiment 1 (March – April 2001). **B** - Percentage retrieval of scallops cultured at 4 m and 12 m depth for 27 days off Porto Belo Point - experiment 2 (June – July 2001). (Common letters denote no significant differences) (mean + se).

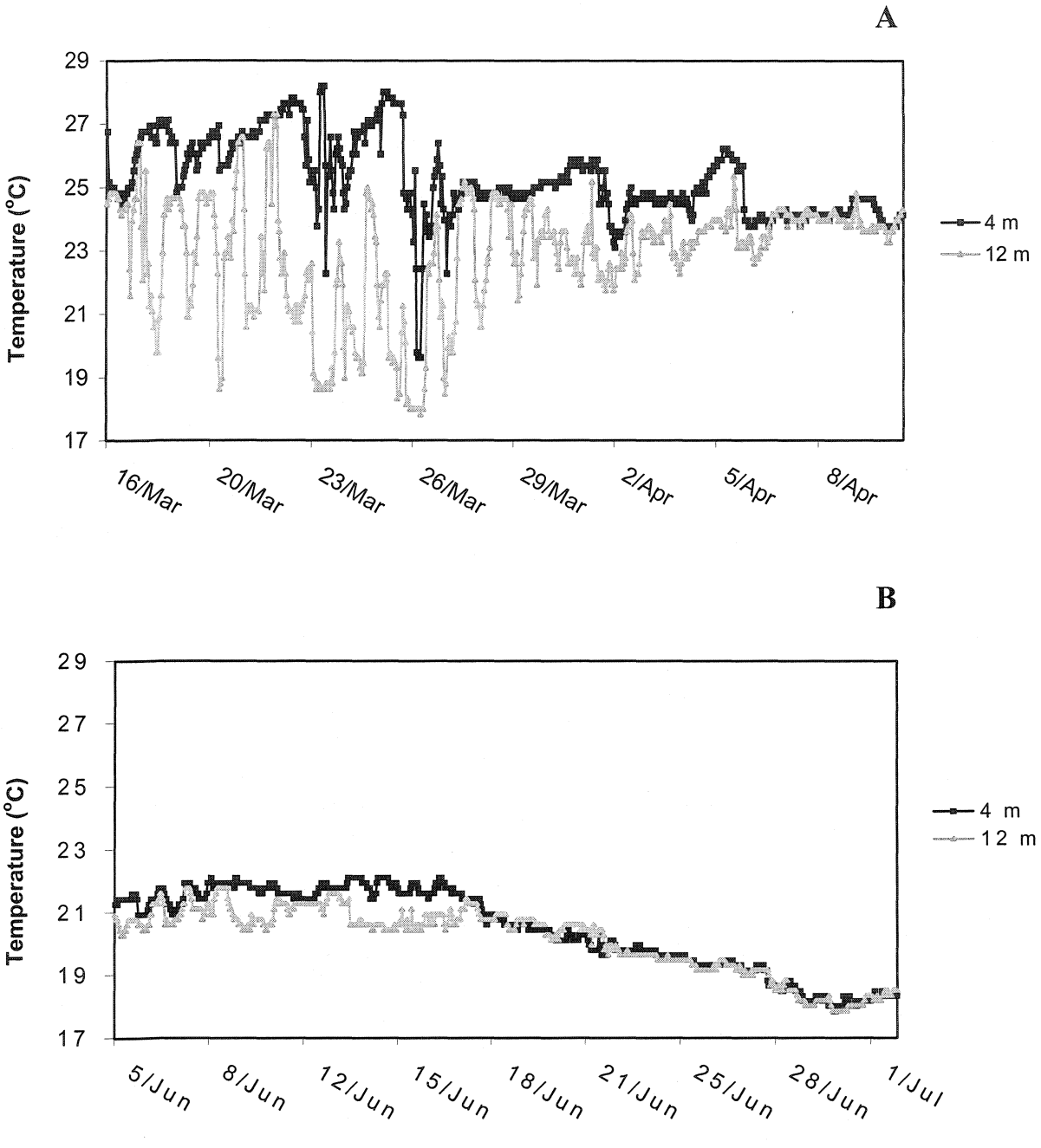


Figure IV.3. Hourly temperature at 4 m and 12 m depth off Porto Belo Point: (**A**) experiment 1 (March – April 2001); (**B**) experiment 2 (June – July 2001).

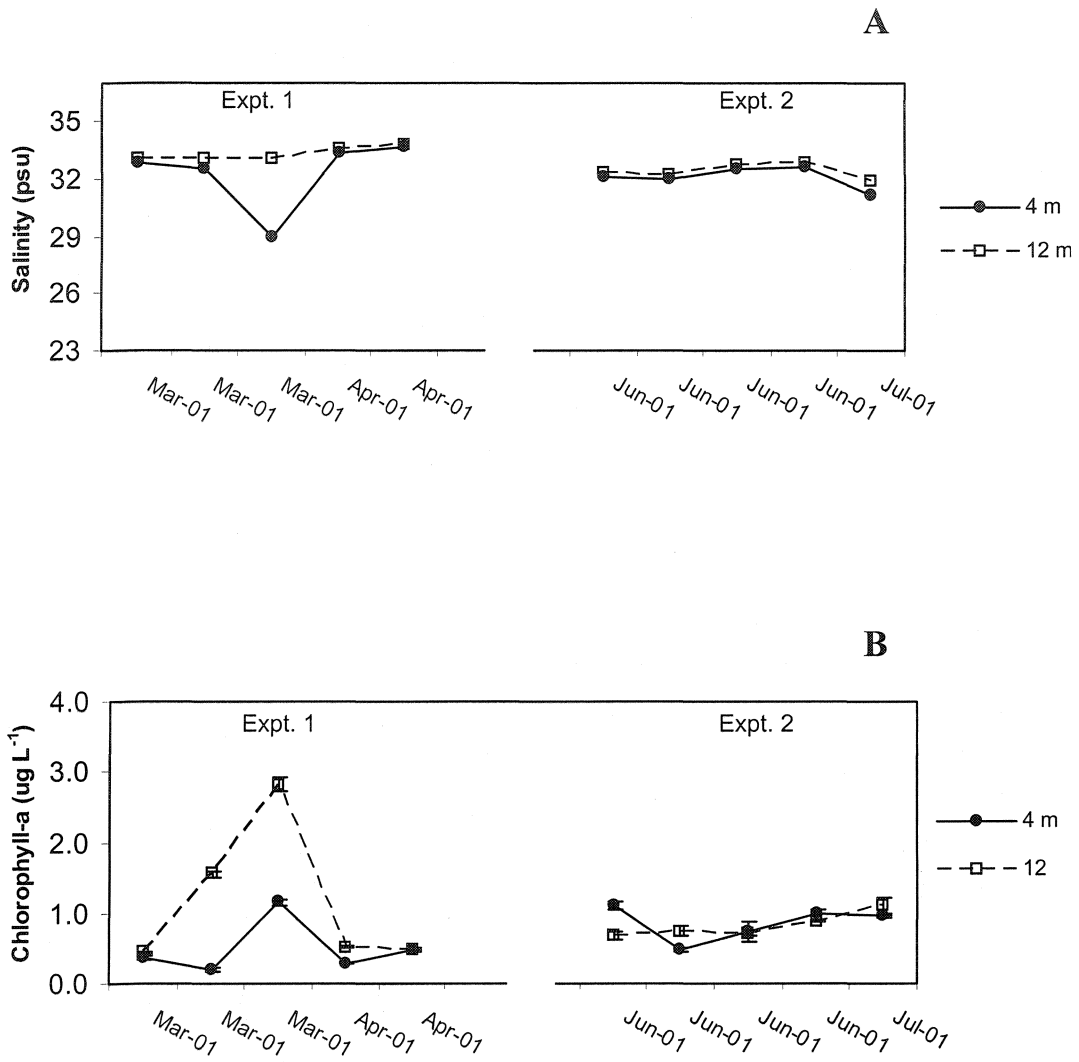


Figure IV.4. Salinity (psu) (**A**) and chlorophyll-*a* (μg L^{-1}) (**B**) at 4 m and 12 m depth off Porto Belo Point, during experiments 1 (March – April 2001) and 2 (June – July 2001). (mean ± se; where bars not seen, se is too small to plot).

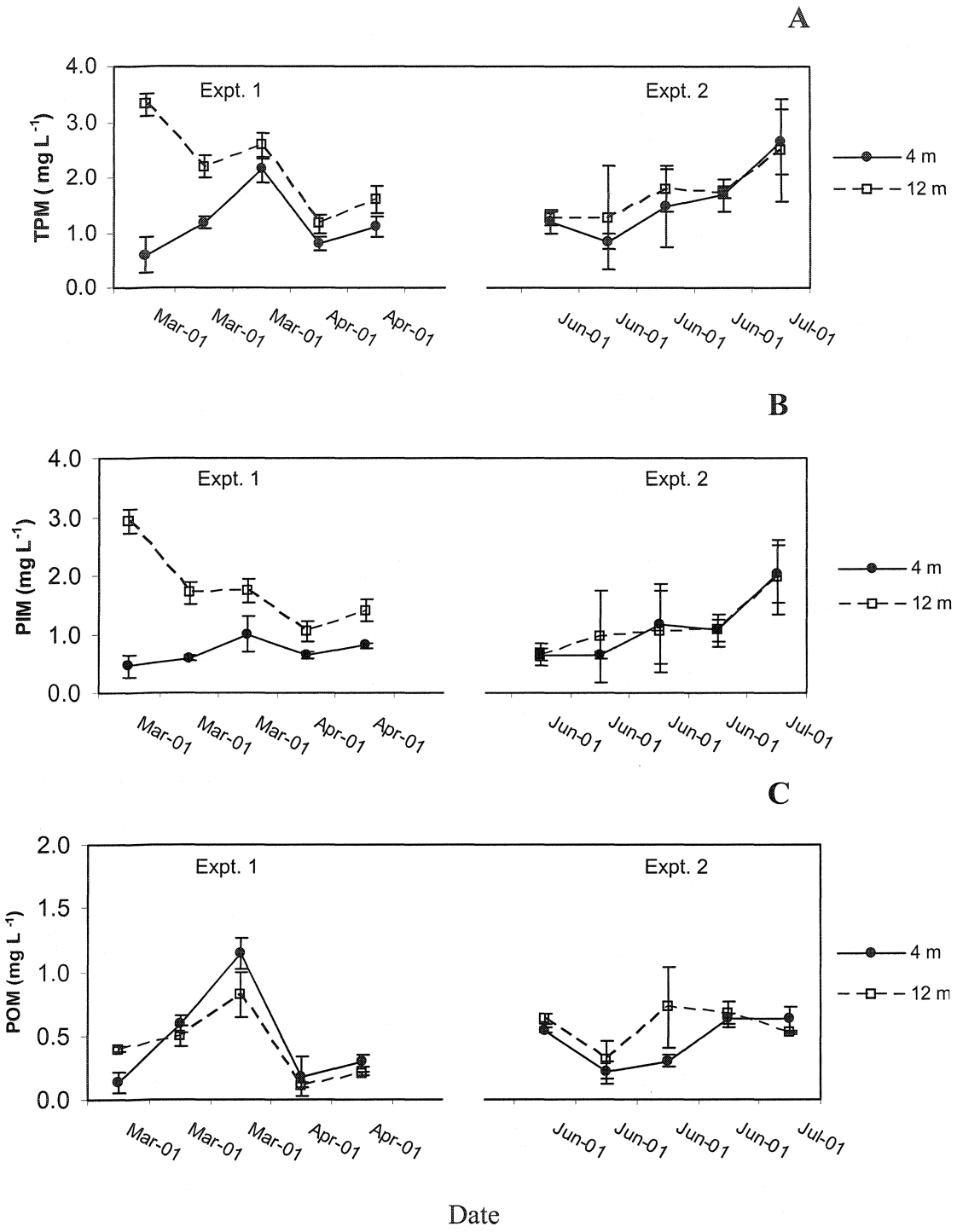


Figure IV.5. Total particulate matter (TPM) (**A**), particulate inorganic matter (PIM) (**B**) and particulate organic matter (POM) (mg L^{-1}) (**C**) at 4 m and 12 m depth off Porto Belo Point during experiments 1 (March – April 2001) and 2 (June – July 2001). (mean ± sd).

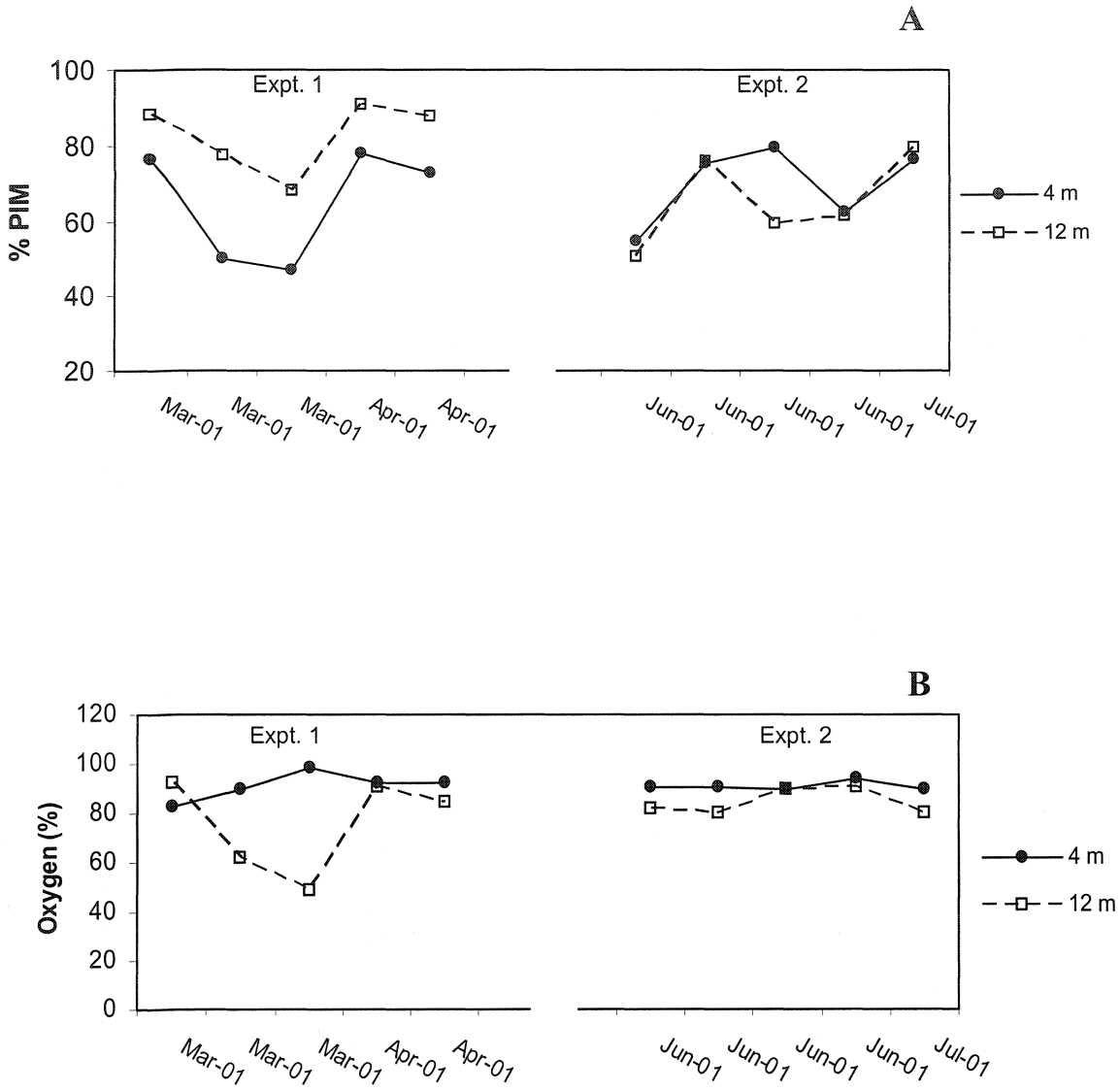


Figure IV.6. Percentage of inorganic matter in relation to total seston (% PIM) (**A**) and percent oxygen saturation (**B**) at 4 m and 12 m depth off Porto Belo Point during experiments 1 (March – April 2001) and 2 (June – July 2001).

CHAPTER V

Effects of Salinity and Temperature on the Survival and Byssal Attachment of *Nodipecten nodosus* at its Southern Distribution Limit

V.1. Introduction

The capacity of an organism to survive in its environment is restricted by its limits of tolerance to abiotic factors. Temperature and salinity are primary abiotic variables affecting survival, activity and distribution of marine organisms, and the effect of one variable can modify, or be modified, by the other (Kinne 1964, 1970a,b). The lion's paw scallop *Nodipecten nodosus*, is a subtidal, non-estuarine bivalve which inhabits shallow tropical waters in the Caribbean region (Smith 1991), extending its geographic distribution discontinuously as far south as the surroundings of Santa Catarina Island (Lat. 27.5° S), in southern Brazil (Rupp 1994). This area is within a warm-temperate marine biogeographic region which is a distribution boundary for several tropical species (Briggs 1995). In tropical areas, marine organisms are generally exposed to a narrower range of temperature, whereas at the high latitude distribution limit, tropical species are exposed to a broader temperature variation (Levinton 2001). In the Caribbean, *N. nodosus* can be exposed to a maximum thermal amplitude of about 6°C at a given depth (Lodeiros et al. 1998), and the arid conditions result in negligible salinity changes at coastal aquaculture sites (Lodeiros and Himmelman 1994). At the southern limit of distribution, on the other hand, the lion's paw scallop is subject to major oceanographic events leading to a seasonal temperature variation of at least 12.5°C (Chapter III), a magnitude comparable to that experienced by some scallop populations in temperate environments. Furthermore, sudden temperature changes (ca. 10°C) in the water column caused by sub-surface intrusions of cold waters during summer–early autumn affect coastal sites and scallop growth (Chapter IV). In addition to these remarkable thermal shifts, coastal aquaculture

areas in southern Brazil can be affected by continental runoff during severe rain, resulting in periods of decreased salinity (Chapter III). Whereas intertidal and estuarine bivalves are generally tolerant to sudden and large changes of temperature and salinity (Dame 1996), scallops are more vulnerable, and mass mortalities caused by temperatures and salinities beyond their tolerance limits have been well documented (Brand 1991, Orensanz et al. 1991). To date, no studies have focused on the effects of temperature and salinity on *N. nodosus* or other subtidal bivalves on the warm temperate coast of Brazil.

Thermal stress due to sudden change in the position of the thermocline may cause mass mortalities of *Placopecten magellanicus* (Dickie and Medcof 1963) and *Argopecten gibbus* (Miller et al. 1981). Likewise, severe cold winters have caused massive mortalities of *Chlamys opercularis* in European waters (Crisp 1964). Furthermore, the geographic distribution of many scallop species is clearly associated with the thermal characteristics of major ocean currents (Brand 1991). At the high-latitude distribution limit, low temperature may be an important factor limiting distribution (Briggs 1995). Knowledge of the lethal and sublethal temperature limits for *N. nodosus* at its distribution boundary is therefore ecologically important in assessing habitat suitability and possible thermal influences on distribution of the species.

Within pectinids, the capacity to tolerate exposure to low salinities varies among species. Whereas juvenile *Argopecten irradians* (10–30 mm) display high survival (> 80 %) at a salinity of 15 psu over a wide range of temperatures for up to 48 h (Mercaldo and Rhodes 1982), juvenile *Mimachlamys asperrima* do not survive a salinity of 25 psu for 24 h, nor an exposure to 30 psu for about 72 h (O'Connor and Heasman 1998). In temperate and boreal scallops, the limits of tolerance to reduced salinities depend on the exposure

temperature (Paul 1980a, Mercaldo and Rhodes 1982, Strand et al. 1993), but in tropical and subtropical species it is not clear if the range of temperatures experienced by the scallops in the natural habitat results in differences in salinity tolerance. According to some studies (Paul 1980a, Heasman et al. 1996, O'Connor and Heasman 1998) tolerance to temperature and salinity in scallops decreases with increasing age or body size for benthic life-history stages, but there are also reports that smaller bivalves are killed more rapidly than larger conspecifics at lethal salinities (Castagna and Chanley 1973). In aquaculture settings, scallops of different size, i.e., spat, juveniles and adults, are subject to different culture techniques (nursery culture, intermediate culture and final growout), which are often carried out in different water depths, on the bottom, or at different sites. Knowledge of the limits of tolerance for different life stages is therefore essential for aquaculture.

Increased interest in developing aquaculture of *N. nodosus* in coastal shallow areas of Brazil requires a knowledge of the lethal and sublethal effects of temperature and salinity, in order to establish ecological criteria for adequate site selection, as well as to develop culture strategies leading to optimisation of survivorship and growth. Furthermore, the approach of transferring hatchery-produced spat to the sea-based nursery has been demonstrated to be a suitable alternative for lion's paw scallop production (Chapters III and IV). At this stage individuals are attached to artificial collectors by their byssus. Such an approach requires knowledge of the influence of temperature and salinity on the synthesis of byssal threads, in order to understand the observed variability in spat retrievals and to optimise aquaculture strategies in an environment displaying marked gradients on spatial and temporal scales. Furthermore, the degree of byssal attachment has

been used as an indicator of favourable environmental conditions for scallops (Paul 1980a,b, O'Connor and Heasman 1998, Christophersen and Strand 2003), and the highest percentage of byssal attachment was recorded at temperatures supporting the highest growth and survival rates (Heasman et al. 1996).

The objective of this study was to investigate the influences of abiotic environmental factors on survivorship and byssal attachment of hatchery-produced lion's paw scallops at the high latitudinal distribution limit in the Southern Hemisphere. The lethal and sublethal effects of salinity, temperature and salinity-temperature combinations were studied in the laboratory for three size-classes of scallops, through long- and short-term exposure bioassays. The following hypotheses were tested: a) the low salinity levels recorded at a coastal aquaculture site in southern Brazil (Chapter III) approach the limits for survival and byssal attachment of *N. nodosus*; b) the temperature limits at the distribution boundary of the species approach lethal thresholds, and determine differences in byssogenesis and tolerance to low salinities of scallops acclimated at different temperatures, and c) temperature and salinity tolerances vary for individual *N. nodosus* of different sizes.

V.2. Materials and Methods

Experiments to determine lethal and sublethal effects of salinity and temperature were carried out with different size scallops *Nodipecten nodosus* at the Laboratory for the Culture of Marine Molluscs (LCMM-UFSC), Florianópolis, Santa Catarina State, Brazil. All scallops were produced in the hatchery (LCMM) using techniques described in Chapter II and maintained, prior to the experiments, in suspended culture using nylon bags (spat) or lantern nets (juveniles and adults) off João da Cunha Island, (Lat. 27° 08' S) Porto Belo (Santa Catarina State) (Fig. I.2). Broodstock was obtained from a local population off the coast of Santa Catarina State. All experiments were carried out during summer–early autumn 2000 and 2002. Experimental procedures were based on standard methods for static acute bioassays with aquatic invertebrates (Rand and Petrocelli 1985).

V.2.1. Maintenance of scallops prior to the experiments

One to two weeks before initiation of the bioassays, scallops were transferred from the ocean to the laboratory using insulated containers, which maintained ambient seawater temperature at the field site during transport. In the laboratory, scallops were cleaned of epibionts and placed in holding tanks until the start of the experiments. Juveniles (with no functional gonads, shell height 20–30 mm), adults (with functional gonads, shell height 50–60 mm) and spat (shell height 5–8 mm), were maintained at ambient temperature in 100-L tanks with static aerated filtered (1 μm) seawater. When scallop spat were to be used in salinity experiments at temperatures that were different than ambient, they were

previously acclimated in 10-L plastic chambers immersed in a 100-L water bath, with circulating chilled water or thermostatic immersion heaters, permitting a gradual thermal adjustment (Table V.1) until the desired constant-temperature (16 or 28°C) was reached. Scallops were fed daily a mixture of (1:2:1) *Isochrysis* sp. (clone T-iso), *Chaetoceros calcitrans* and *C. muelleri* at final concentrations of $3x10^4$ to $5x10^4$ cells day^{-1}. All chambers were aerated with air diffusers and seawater was changed daily. Replacement seawater was brought to the desired temperature with a titanium chiller-heater before being added to the chambers. A constant room temperature was maintained with an air conditioner. Only scallops showing healthy signs and normal behaviour (normal shell gape and protrusion of mantle borders and tentacles) were used in the bioassays. An initial sample of 15 individuals was randomly selected prior to each bioassay for measurements of shell height and dry tissue weight.

V.2.2. Salinity bioassays

V.2.2.1. Effect of low salinity on different size scallops at ambient temperature

Acute salinity bioassays were carried out for different size scallops at a constant temperature of 23.5°C, when ambient seawater temperature in the field site was 23–25°C. Scallops of three size-classes, adults (mean shell height 54.4 mm, sd = 4.5), juveniles (mean shell height 25.5 mm, sd = 1.7) and spat (mean shell height 6.11 mm, sd = 0.5) (Table V.1), were placed inside plastic chambers with a volume of 8, 5 and 1.8 L of seawater, respectively. For each size-class, scallops were exposed to salinities of 33, 29,

25, 21, 17 and 13 psu. For each salinity, there were triplicate chambers with 5–7 scallops each. The experimental salinities were obtained by diluting filtered seawater (1 μm) at ambient salinity (33 psu) with distilled water, and salinities were measured with a digital WTW Multiline P4 water quality meter. Treatments were randomly assigned to the chambers and scallops were haphazardly assigned to each treatment. The experiments were started by transferring the scallops directly to the experimental salinities. Mortality was recorded after 12, 24, 36, 48, 72 and 96 h. Individuals with a permanent wide valve gape with retracted mantle borders and which were not responsive to the touch of a glass rod or fine brush were considered dead. Mortality of juveniles and adults was confirmed by placing the scallops in seawater at a salinity of 33 psu and observing possible recovery after 24 h. Due to their small size, spat were observed under a dissecting microscope to confirm mortality. Spat with a wide shell gape and no byssal attachment were placed in a Petri dish and were considered dead when they lacked response to touch and had no heart beat, which could be seen through the translucent shells. During the experiments carried out with spat, the number of individuals attached by byssal threads was also recorded at 12, 24 and 48 h. Byssal attachment was confirmed by a gentle touch with a fine-haired brush. Water in all chambers was gently aerated with air diffusers and was changed at 48 h, or earlier for the adults if spawning was detected in a chamber. Scallops were not fed during the 96 h bioassay.

V.2.2.2. Effects of salinity-temperature interactions on spat

Salinity tolerance bioassays were also carried out with spat acclimated to temperatures of 16 and 28°C, using similar procedures as in the experiments carried out at ambient temperature (Table V.1). The experimental temperatures chosen approximated the extreme limits of seawater temperature recorded off Santa Catarina State (Chapter III). Prior to the initiation of the experiments, scallops were acclimated in the laboratory at constant experimental temperatures of 16 and 28°C for a minimum of 7 days. Acclimation temperatures were reached by a gradual decrease (2°C day^{-1}) or increase (2.3°C day^{-1}) until the desired temperature was reached. During the bioassays, temperatures were kept constant by holding the experimental chambers in a water bath with circulating chilled water (16°C), or with thermostatic immersion heaters (28°C). Spat used in the experiments at both temperatures were from the same spawning and were transferred from the sea-based nursery to laboratory at the same time. At the beginning of the experiments, the group held at 16°C had a mean shell height of 6.73 mm (sd = 0.58) and those used in the 28°C experiments had a mean shell height of 7.51 mm (sd = 0.71).

V.2.2.3. Effect of short-term exposure to low salinity at different temperatures

Further experiments on short-term exposure to low salinities were carried out, since it was found in the previous experiments that all spat exposed to salinities of 17 and 13 psu died within 12 h. Experiments of 1-h exposure to salinities of 17 and 13 psu were carried out at temperatures of 16 and 28°C. After exposure, spat were returned to normal

seawater salinity of 33 psu at the respective temperatures, and mortality was checked after 12, 24, 36, 48, 72 and 96 h. For each salinity and temperature there were triplicate chambers with seven scallops each. Spat were selected from the same groups acclimated to 16°C and 28°C used for the 96-h exposure experiments.

V.2.3. Temperature bioassays

V.2.3.1. Lethal and sublethal effects of temperature on different size scallops

Acute temperature bioassays were undertaken when seawater temperature in the field ranged from 25–28°C. All scallops were then maintained in the laboratory at a constant temperature of 27.0°C for 7 days prior to initiation of the experiments. Three size-classes, adults (mean shell height 54.2 mm, sd = 4.27), juveniles (mean shell height 27.2 mm, sd = 1.67) and spat (mean shell height 6.1 mm, sd = 0.49) (Table V.2) were placed inside plastic chambers with a volume of 8, 5 and 1.8 L of seawater, respectively. For each size-class, triplicate chambers were used for each temperature. All trials were carried out using 1 μm filtered seawater at a salinity of 33 psu. In order to determine the range of tolerance for a given exposure time, the higher experimental temperatures selected exceeded the normal maximum temperature normally experienced by the scallops in the field. Likewise, the lower experimental temperatures were below the range normally experienced by *Nodipecten nodosus* in southern Brazil. Experimental temperatures for adult scallops were: 11, 15, 20, 27, 31 and 35°C, and for spat and juveniles 11, 15, 19, 23, 27, 31 and 35°C. Target temperatures above ambient temperature

were obtained by placing the experimental chambers inside 250-L water baths containing thermostatic immersion heaters. Temperatures of 19°C and 15°C, were obtained by placing the experimental chambers inside 100-L tanks with a seawater flow at the desired temperature, controlled by a titanium chiller. To achieve a temperature of 11°C, experimental units were placed inside a 100-L water bath in an insulated chamber containing water re-circulated through a portable chiller unit.

The experiments started by transferring the scallops directly to the experimental chambers at controlled temperatures. Mortality was recorded after 6, 12, 24, 48, 72 and 96 h. The criteria for mortality were the same as those used in the salinity tolerance bioassays. However, lower temperature tolerance is usually more difficult to determine because of induction of chill coma (Kinne 1970a), when scallops may not respond to touch, but still be alive. Thus, after the 96 h exposure to temperatures of 11°C remaining scallops were gradually returned to 23°C over a 24 h period and mortality recorded. The number of scallops attached by byssal threads was also recorded at intervals of 6, 12, 24 and 48 h for the three size-classes, using the same procedures as in the salinity tolerance experiments. Water in the experimental chambers was changed at 48 h, or earlier (6-12 h) if spawning or abundant mucus caused by high mortality was detected in a chamber (treatments at 31°C and 35°C). Scallops were not fed during the 96-h bioassay.

V.2.3.2. Effect of short-term exposure to high temperatures

A further experiment to determine the effect of short-term exposure of spat and juveniles to high temperature was carried out, since it was noted in the previous

experiment (section *V.2.3.1.*) that scallops displayed high mortality at 35°C, but had high survival at 31°C, during a 6-h exposure. Scallops previously maintained at 27°C were exposed to a temperature of 33°C for periods of 2 and 4 h, after which they were returned to the initial ambient temperature. For each size-class (spat and juveniles) there were duplicate chambers with twelve scallops each. After the initial exposure period (2 h), six of the scallops were transferred to ambient temperature, and after 4 h the remaining scallops from each chamber were transferred to ambient temperature. Mortality was recorded every 12-h over a 48-h period.

V.2.4. Data analyses

For a given temperature or salinity, the percentage survival of scallops at different treatments was plotted against the exposure period. Acute lethal effects of low salinities on different size scallops held at different temperatures were analysed by determining the median lethal concentration (LC_{50}), which represents the salinity estimated to cause 50 % mortality of a test population over a specific time period (Rand and Petrocelli 1985). This approach was also applied to determine the acute lethal temperature (LT_{50}) of scallops exposed to high temperature. Probit analysis (SPSS 10.0) was used to calculate the LC_{50} and LT_{50} for each exposure time. Lethal effects of scallops exposed to low temperature were determined 24 h after returning the scallops to ambient temperature, subsequent to the 96-h exposure experiment. Sublethal effects of scallop exposure to different salinities and temperatures were examined by determining the percentage of individuals attached by byssal threads to the surfaces of the experimental chambers or to adjacent scallops.

V.3. Results

V.3.1. Low salinity tolerance

V.3.1.1. Lethal effects on different size scallops

Nodipecten nodosus of all size classes were highly susceptible to low salinity. At an intermediate temperature of 23.5°C, mortality was recorded at salinities of 25 psu and below (Figs. V.1A, B, C). At this temperature, all scallops died within 12 h in salinities of 17 or 13 psu. At salinities of 29 and 33 psu, all animals tested survived. Spat had a higher tolerance of low salinities than larger scallops. Whereas no juveniles or adults survived a 36-h exposure to a salinity of 21 psu, spat had a 76 % survival, and after 96-h exposure spat survival was 54.2 %. The median lethal salinity (LC_{50}) for a 24-h exposure was 21.9, 22.7 and 20.1 psu, respectively, for adults, juveniles and spat (Table V.3). For a 96-h exposure, LC_{50} was 23.6 psu for adults and juveniles and 21.2 psu for spat.

V.3.1.2. Effects of salinity-temperature interactions on halotolerance

Spat were more tolerant to low salinity at low temperature (16°C) than at high temperature (28° C) (Figs. V.2A and B). At 21 psu, all spat held at 16°C had survived for up to 36 h, but only 66 % survived at 28°C. Furthermore, after 96-h exposure, 65 % of the spat survived at 16° C, but only 19 % survived at 28°C. The LC_{50} for a 24-h exposure was 18.9 and 20.2 psu for spat maintained at 16° C and 28°C, respectively (Table V.3). After

an exposure of 96 h, the LC_{50} was 20.5 and 22.9 psu, respectively, for 16°C and 28°C. As at ambient temperature, no spat survived an exposure of 12 h to a salinity of 17 psu at 28°C, but at this salinity and period 20 % of spat survived at 16°C. Furthermore, at a salinity of 29 psu some mortality (5 %) was recorded at 28°C, in contrast to ambient temperature (no mortality).

V.3.1.3. Effects of short-term exposure to low salinity

The extreme susceptibility of *N. nodosus* to low salinity was further demonstrated by the short-term exposure (1 h) to salinities of 13 and 17 psu (Fig. V.3). The exposure temperature also strongly influenced mortality. At 28°C, no spat survived a 12-h exposure to either salinity. At 16°C, on the other hand, 100 % mortality of spat exposed to 13 psu was recorded 36 h later. At this temperature 28.5 % of spat were alive 96 h after the short-term exposure to 17 psu.

V.3.1.4. Byssal attachment

The capacity of spat to synthesise byssal threads was maximal at a salinity of 33 psu and decreased as salinity decreased (Fig. V.4). Byssal attachment increased with time for salinities of 29 psu and above. At 25 psu, byssal attachment was about 30–60 % lower than at 33 psu throughout the experiment. Likewise, at 29 psu percentage byssal attachment was about 20–40 % lower than at 33 psu. At a salinity of 21 psu, only 6 % of

the spat were attached at a 48-h exposure, and below this salinity no byssal attachment was recorded.

V.3.2. Upper and lower thermal tolerance bioassays

V.3.2.1. Lethal effects on different size scallops

Nodipecten nodosus adults, juveniles and spat displayed high survival at 33 psu when exposed to a wide temperature range (15 to 27°C) during the 96-h bioassays, but high mortality occurred at 31°C and above. Tolerance to high temperature, however, decreased as body size increased (Figs. V.5A, B, C,). Whereas all adult scallops died at 31°C within 72 h of exposure, juveniles and spat displayed 28.6 and 66.7 % survival, respectively. After 96-h exposure, 23.8 % of the juveniles and 42.9 % of the spat were still alive at 31°C. No scallops survived 6-h at 35°C. The median lethal temperatures (LT_{50}) for a 24-h exposure were 30.8, 30.5 and 32.8°C for adults, juveniles and spat, respectively (Table V.4). For a 96-h exposure, LT_{50} was 28.0, 29.4 and 30.2°C, for adults, juveniles and spat, respectively.

Lower temperature limits are more difficult to determine under laboratory conditions due to the induction of chill coma (Kinne 1970a). Scallops exposed to 15°C displayed no mortality for 96 h after the acute shock. Immediatelly after the acute exposure, scallops exhibited thermal shock, with a small shell gape and retraction of the mantle and tentacles. After 12 h, however, scallops at 15°C started to protrude the mantle border and tentacles, but the mantle was never as fully extended as in scallops at

temperatures of 19°C to 27°C. At the termination of the experiments, scallops previously exposed to 15°C were gradually returned to ambient temperature of 23°C over a 24-h period, and no mortality was recorded. At 11°C, on the other hand, scallops clearly displayed signs of cold-induced lethargy. Immediately after exposure, scallops displayed retracted mantle border and tentacles but a wide shell gape, which subsequently diminished. Over a 96-h period, scallops did not return to normal behaviour, displaying retracted mantle borders and a small shell gape throughout. The response to touch was extremely weak compared with scallops exposed to higher temperatures. Furthermore, 24 h after the end of the experiment, when scallops were gradually returned to the ambient temperature of 23°C, significant mortality was recorded (78, 62 and 80 % in adults, juveniles and spat, respectively). The surviving scallops, although responsive to touch and therefore considered alive, showed mantle borders detached from the shells, indicating limited viability.

V.3.2.2. Effects of short-term exposure to high temperature

Juveniles and spat were all alive immediately after exposure to 33°C for 2 and 4 h. Forty-eight hours later, however, spat displayed 44 and 75 % mortality for 2- and 4-h exposure to high temperature, respectively. Likewise, juveniles displayed 90 and 100 % mortality 48 h later for 2- and 4-h exposure, respectively.

V.3.2.3. Byssal attachment

Unlike many other scallops, *Nodipecten nodosus* retains the ability to produce byssal threads at the adult stage. However, the percentage of attachment for adults was lower than that for juveniles and spat (Figs. V.6A, B, C). After 12-h exposure, adults were byssally attached only at temperatures of 20°C (28.5 %) and 27°C (21.5 %) (Fig. V.6A). After 96 h, 50 % and 42 % of the adults were byssally attached at 20°C and 27°C, respectively. Juveniles had the highest percentage byssal attachment at temperatures between 23°C and 27°C (Fig. V.6B). The percentage byssal attachment of spat was initially lower at lower temperatures (Fig. V.6C), but after 48 h it increased, exceeding 75 % over a wide range of temperatures (15–27°C), and negligible attachment was recorded at the extreme temperatures (11 and 31°C). For all size classes, the rate of byssus production increased with temperature (Figs. V.7A, B, C), except at 31°C, when the opposite was recorded. Furthermore, spat had the lowest rate of byssal attachment at 15°C, and juveniles displayed the lowest rate of attachment at 15°C and 19°C.

V.4. Discussion and Conclusions

A substantial amount of ecological, aquaculture and biogeographic information can be obtained by determining lethal and sublethal responses of *N. nodosus* to salinity and temperature. The approach of examining sudden changes to experimental temperatures and salinities is controversial since it gives no opportunity for the animals to acclimate physiologically to the new condition, and most wild subtidal populations are unlikely to experience such sudden changes (Davenport et al. 1975). When the animal is gradually acclimated to the experimental conditions, the tolerance range tends to be broader (Kinne 1970a). In southern Brazil, however, shifts in the position of the thermocline can occur at coastal aquaculture sites in depths from 10–12 m during summer to early autumn, causing sudden temperature changes of ca. 10°C (Chapter IV). Such conditions also affect wild populations, which occur at depths from 6–30 m in the region (Rupp 1994). Furthermore culturing scallops in coastal shallow waters, can expose them to sudden salinity changes due to continental freshwater run-off (Chapter III). Therefore, the results of the present study give an ecologically meaningful, indication of the scallops' responses to sudden environmental changes in the natural environment in southern Brazil.

V.4.1. Salinity tolerance

Nodipecten nodosus is a stenohaline species, and high mortality occurs at salinities of 25 psu and below, depending on temperature and body size. Previous studies on other pectinids have recorded lower salinity tolerance limits ranging from 15 to 30 psu (Paul

1980a, Mercaldo and Rhodes 1982, Strand et al. 1993, O'Connor and Heasman 1998, Frenette and Parsons 2001). Scallops, unlike several other bivalves, are incapable of completely closing their valves due to the byssal notch opening (Brand 1991), and the osmotic concentration of the hemolymph comes into equilibrium with the concentration of the external medium after a certain lag period (Shumway 1977, Signoret-Brailovsky et al. 1996). A relationship between body size and salinity tolerance has seldom been demonstrated in bivalves. In the present study, *N. nodosus* spat had a higher percentage survival to low salinity (21 psu) and survived for a longer period than juveniles and adults. Paul (1980a) reported that *Chlamys opercularis* spat (5–10 mm) displayed greater tolerance to low salinity than larger scallops (> 30 mm). Likewise, O'Connor and Heasman (1998) found that 18 mm *Mimachlamys asperrima* survived longer at low salinities than 28 mm scallops, and postulated that the additional metabolic demand due to the onset of gonad maturation could explain such results, as also suggested by Kinne (1970a) for other invertebrates. Gonads of *N. nodosus* are not functional until juveniles attain a size of about 35 mm, and immature gonads first become discernible at a size of ca. 20 mm (pers. obs.). The present results, therefore, are consistent with the hypothesis that increased susceptibility to low salinity is related to the onset of gonadic differentiation, possibly due to the increased energy requirements for gametogenesis. In order to obtain 100 % survival for up to 3 days during culture of *N. nodosus* salinity should be above 25 psu for spat (nursery culture) and above 29 psu for growout of juveniles and adults.

The approach of determining the LC_{50} for a given exposure period and temperature using standard methods is useful for inter-comparison of environmental tolerances among different species or populations of the same species. However, only a few studies have

used such an approach, and although several authors have studied the effects of salinity on scallops (as previously noted), differences in methodology make it difficult to compare results. Bergman et al. (1996) found that at low temperature (1°C) LC_{50} for juvenile *Placopecten magellanicus* was 11.4 psu for a 96-h exposure. In addition, Frenette and Parsons (2001) recorded 96-h LC_{50} for *P. magellanicus* ranging from 13 to 14.6 psu at temperatures of 3–18°C, indicating that this temperate-boreal scallop is significantly more tolerant of low salinity than the tropical/subtropical *N. nodosus* (96-h LC_{50}, 20.5 to 23.6 psu) at the ambient temperatures of their respective habitats. Paul (1980a) recorded LC_{50} for an exposure of 24 h ranging from 16 to 28 psu for *Chlamys opercularis*, depending on size and acclimation temperature.

Tolerance of the lion's paw scallop to low salinity decreased as temperature increased. For a 96-h exposure, LC_{50} increased from to 20.5 to 23 psu as exposure temperature increased from 16 to 28°C. Likewise, the tolerance of *C. opercularis*, *Argopecten irradians* and *P. magellanicus* to low salinity decreases at increasing temperatures (Paul 1980a, Mercaldo and Rhodes 1982, Frenette and Parsons 2001). However, greater susceptibility to low salinity at lower temperatures has also been observed (Strand et al. 1993, Christophersen and Strand 2003). In addition, O'Connor and Heasman (1998) found no temperature-salinity interaction for juvenile *Mimachlamys asperrima*, which showed a negligible change in tolerance to low salinity over a 10°C temperature range. According to Kinne (1964), several marine species can tolerate subnormal salinity at the lower part of their temperature range, but others survive low salinity better at higher temperatures. Nevertheless, tolerance to low salinity tends to decrease at extreme temperatures, when temperature becomes the factor limiting survival.

The apparent divergence among studies may be explained by the range of temperatures tested and the plasticity of temperature tolerance of the species. In the present study, however, the experimental temperatures covered the range normally found in coastal waters, so the observed responses are ecologically meaningful and useful for establishing aquaculture criteria. It could be argued that, the higher tolerance to low salinity at lower temperature in the long exposure experiment did not exclude the possibility that scallops could take longer to die at low temperature. The short-term experiments however, further emphasised the effect of temperature. Whereas *N. nodosus* spat did not recover from a 1-h exposure to 17 psu at 28°C, at 16°C, 28.5 % of the scallops survived. Thus, the seasonal temperature variation normally recorded at coastal sites in southern Brazil, which may range from 15°C to 29°C in some places, influences the tolerance limits of the spat of lion's paw scallop to low salinity. The present data suggest that in shallow waters *N. nodosus* spat were more vulnerable to low salinity events during summer. During years of strong influence of the El Niño Southern Oscillation (ENSO), intense rainfall may occur during late winter to early spring, causing catastrophic floods in southern Brazil (CLIMERH 2003), but in non-Niño years higher precipitation usually occurs in summer (Sunyé and Servain 1998), when heavy rain is often concentrated in short periods of time, causing hyposaline events or a freshwater surface lens in coastal aquaculture areas.

A reduction in salinity also affected synthesis of byssal threads by *N. nodosus* spat. Byssal attachment was greatest in salinities above 29 psu. Although spat mortality was not severe at salinities between 25 and 29 psu, byssal attachment was significantly reduced, suggesting that below 29 psu growth of spat may also be inhibited. Christophersen and Strand (2003) also found reduced byssal attachment within this

salinity range in *Pecten maximus*, together with a significant reduction in growth and clearance rate. Likewise, *Argopecten purpuratus* displayed negative scope for growth at 27 psu (Navarro and Gonzalez 1998), and Laing (2002) found that a salinity of 28 psu resulted in significantly lower growth rates of *Pecten maximus*, depending on temperature. In Chapter III, a salinity episode of 27 psu was recorded in a coastal aquaculture site, coinciding with a period when spat retrieval was low and growth was less than was predicted by temperature. It is possible that the low salinity may have physiologically stressed the scallops, reducing growth, and possibly affected byssal attachment, causing loss of spat. In this manner, although spat were shown to be more tolerant than adults to low salinity exposure, in order to achieve maximum growth and retrievals during nursery culture, *N. nodosus* spat should only be grown in areas where salinity does not drop below 29 psu, as is the case for juveniles and adults.

V.4.2. Temperature tolerance

Since temperature responses of ectotherms depend on temperature acclimation and the thermal regime to which animals were previously exposed (Kinne 1970a, Newell and Branch 1980) LT_{50} values are valid only for a particular acclimation temperature. All temperature tolerance experiments in the present study were carried out from summer to early autumn, when scallops in the field were exposed to temperatures of 25–28°C prior to the start of the experiments, i.e., were "summer-acclimated". This is the period when temperatures are maximal in shallow coastal waters as well as in the upper layers of shelf waters, due to the influence of the warm Brazil Current (Sunyé and Servain 1998).

Furthermore, during this period sudden temperature changes often occur in the waters off southern Brazil due to sub-surface intrusions of cold South Atlantic Central Water (SACW) (Chapter IV, Matsuura 1986, Brandini 1990, Castro and Miranda 1998). During this period, scallops may therefore be exposed to temperatures close to the upper tolerance limits, as well as to sudden temperature shock.

The lion's paw scallop displayed high survival within the normal range of temperatures experienced by wild populations at the southern distribution limit, but the recorded temperatures in the sea approached the upper lethal limit. At some coastal aquaculture sites, seawater may occasionally reach 29–30°C, i.e., above the lethal threshold, depending on scallop size and exposure period. The highest experimental temperature at which no mortality was recorded was 27°C, and all size-classes displayed high mortality at 31°C. Spat, however, were more tolerant to high temperatures than juveniles and adults. The lethal temperature to kill 50 % of the scallops (LT_{50}) decreased as exposure time increased. This is in agreement with previous studies on other scallops (Dickie 1958, Paul 1980b). A 48-h exposure period has been proposed to determine lethal temperature adequately, since shorter exposures may result in tolerance of higher temperature (Paul 1980b). In the present study, exposures of 24 h and longer consistently resulted in LT_{50} values approximately 2°C higher for spat than for adults, juveniles displaying an intermediate LT_{50} after 48-h exposure. This size-tolerance relationship obtained for *N. nodosus* is in agreement with a previous study on *Chlamys opercularis* (Paul 1980b), in which spat displayed higher tolerance to high temperatures than juveniles and adults at sizes comparable to those in the present study. The results with *N. nodosus* also suggest that the additional metabolic demand due to gonad maturation reduces

tolerance to high temperature. This is consistent with aquaculture studies carried out in the Caribbean region, in which the scallops *N. nodosus* and *Euvola ziczac* displayed higher mortality after sexual maturation during periods of high water temperature (ca. 28°C) and lower phytoplankton abundance (Lodeiros et al. 1998, Lodeiros and Himmelman 2000). Mortality of *N. nodosus* in the sea can therefore occur at temperatures near the upper lethal limit, during periods of high metabolic demand and poor nutritional conditions associated with warm and oligotrophic tropical waters. Such conditions can influence nearshore regions off the southeastern coast of Brazil (Brandini 1990, Sunyé and Servain 1998).

An acute temperature drop from 27°C to 15°C, as is likely to occur during sudden changes in the position of the thermocline during summer, was not lethal to *N. nodosus* spat, although it caused a significant thermal shock to the organisms, as reflected by behavioural responses. The present experiments, however, were not designed to assess the effects of cyclic thermal oscillations caused by fluctuations of the thermocline, which could cause physiological stress and possible mortality, as reported by Dickie and Medcof (1963) and Miller et al. (1981) for other scallops. Further studies should focus on the effects of cyclic temperature variation on growth, survival and physiological responses of the lion's paw scallop, in order to assess the effects of the cyclic cold water intrusions, which affect aquaculture sites in this subtropical environment (Chapter IV).

Lower temperature limits have seldom been determined for scallops. Lower lethal limits are generally more variable and more difficult to determine than upper limits (Kinne 1970a). At low temperature scallops may exhibit chill coma, in which they display no contractile response to touch, yet remain alive. On the other hand, animals may respond

to stimuli, but be irreversibly impaired. Although significant mortality was not detected during the 96-h experiment in any size-class, *N. nodosus* was severely damaged when exposed to temperatures of 11°C, displaying minimal response to touch throughout the experiment, retracted mantle borders and tentacles and a sub-normal shell gape, symptoms of cold lethargy. At the end of the experiment, after ambient water temperature was gradually restored, mortality was high for all size-classes and the few survivors were irreversibly damaged due to the extensive detachment of the mantle border from the shell. Ninety-six hour exposure to 11°C were therefore lethal for the lion's paw scallop. Scallops maintained at 15°C initially displayed symptoms of thermal shock similar to those exhibited at 11°C, but after 12 to 24 h shell gape was wider and the mantle border and tentacles protruded, but never as extensive as at higher temperatures, also indicating temperature-induced lethargy. The lower temperature limit for *N. nodosus* therefore lies between 11°C and 15°C. Further studies should focus on narrower temperature intervals within this range, not only during summer, but also during winter, in order to determine the lower thermal limit of this southern population. Although temperatures below 15°C have not been recorded at an experimental aquaculture site off Santa Catarina State (Lat. 27° S) (Chapters III and IV), not far south of the study site, between Cabo Santa Marta (Lat. 28.5° S) and Arroio Chui (Lat. 33° S), surface waters are dominated by SAW during winter which reach temperatures below 15°C (Castro and Miranda 1998), and the northward flow of this current reaches latitudes as low as 23.3° S in some years (Campos et al. 1996), possibly influencing wild populations of *N. nodosus* as well as aquaculture sites. Furthermore, during summer, water temperatures below 15°C have been recorded during upwelling events off Cabo Frio, Rio de Janeiro State (Lat. 23° S) (Gonzalez-

Rodriguez et al. 1992), as well as in southern parts of Santa Catarina Island (Lat 27.5° S) (pers. obs.).

Short-term exposure to a temperature of 33°C indicated that immediately after the exposure scallops were alive but physiologically impaired, as reflected by the high mortality recorded 24 to 48 h later. Irreversible physiological damage was therefore caused by a 2-h exposure to a temperature not far above the maximum recorded at some coastal areas. Furthermore, during the process of thinning stock and changing the nets in aquaculture operations, scallops are brought to surface and transferred to a basin filled with seawater, where they may be held for one or more hours. This is a risky practice under the hot subtropical sun, when the water temperature in the basin could rapidly reach a temperature at which mortality would not be immediately seen but would subsequently occur.

The thermal range within which growth and normal physiological function can occur is usually narrower than the tolerance limits (Kinne 1970a, Newell and Branch 1980). In the present study, scallops displayed a higher percentage of byssal attachment within a narrower range of temperatures than those at which mortality was recorded. Byssogenesis in scallops is very sensitive to environmental conditions (Brand 1991), and it has been suggested that the highest rate of byssal attachment occurs at the optimum temperature for growth (Paul 1980b, Heasman et al. 1996). As the byssal threads are composed of complex proteinaceous molecules (Bubel 1984), the rate of byssal synthesis may reflect the overall rate of protein synthesis, which is temperature regulated (Somero and Hochachka 1976), as well as other physiological functions of the organism. Optimum temperatures for byssal synthesis are therefore likely to indicate the most favourable

temperature range for growth. A knowledge of the optimum temperature range for growth of bivalves is essential to an adequate selection of aquaculture sites (Lodeiros et al. 2001), and determination of the optimum temperature for byssal attachment may be a simple way to estimate the temperature range most favourable for growth.

Unlike several other scallops in which adults lose the capacity to synthesise byssus, small adult lion's paw scallops (size range of 50 to 60 mm) retained this capacity, although it decreased as scallop size increased. This is in agreement with Caddy (1972), who reports that small *Placopecten magellanicus* are more active in byssus formation than larger individuals. The range of temperatures at which *N. nodosus* was byssally attached, however, was wider for spat than for juveniles and adults. Adults displayed higher percent attachment between 20°C to 27°C and juvenile attachment was highest between 23°C and 27°C. However, differences between adults and juveniles in the temperature range for maximum attachment may be explained by logistic constraints that prevented experiments with adults to be carried out using narrower temperature intervals, and may therefore be more apparent than real. Spat, on the other hand, displayed maximum percent attachment over a wider range of temperatures (15 to 27°C) than adults and juveniles. These results agree with others in the present study, in which spat displayed a wider temperature and salinity tolerance than juveniles and adults. The proportion of attached spat reached an equilibrium over time within a wide range of temperatures, which is in agreement with the observations of Caddy (1972). The rate at which scallops become byssally attached at a given temperature is therefore more meaningful for estimating optimum temperatures than the final percent attachment. Spat exposed to a temperature of 15°C had a slower rate of byssal attachment than those exposed to temperatures from 19°C

to 27°C. Whereas spat exposed to 19°C to 27°C reached a maximum attachment at 12-h exposure, those at 15°C took 48 h to reach a similar percent attachment. At 31°C byssal attachment decreased with time, strongly suggesting that the spat were physiologically stressed. Thus the optimum temperature for synthesis of byssal threads in spat lies between 19°C and 27°C, which could be an indicator of the most favourable temperatures for physiological function and consequently growth. These results agree well with findings obtained in Chapter III, where growth rates of *N. nodosus* spat in the field-based nursery were significantly reduced when seawater temperature was below 19°C even though food availability was high. Juvenile *N. nodosus* in the present experiment displayed a significantly lower rate of byssal attachment at 19°C, and never reached the percent attachment attained at 23°C and 27°C, suggesting that the most favourable temperatures for juvenile growth lie between 23°C and 27°C. The results for adults, are less conclusive, since data were not obtained for temperature intervals between 20°C and 27°C. A decrease in percent attachment was recorded at 27°C, between 24 and 48 h after exposure, suggesting that this temperature may be stressful for the adults. In summary, the temperature range at which byssal synthesis was maximised in adults and juveniles was narrower than that for spat.

Several studies have demonstrated that growth is a complex function of several environmental factors, food availability and temperature being of major importance (Bricelj and Shumway 1991, Thompson and MacDonald 1991). Estimates of optimum temperatures for byssal attachment from the present study may be a good indicator of the range of temperatures most favourable for growth of *N. nodosus* at different life stages. However, such results should be interpreted with care, and may be predictive of growth in

the field only in situations where food availability is not limiting, and in the absence of other factors impeding the normal physiological functions of food acquisition and utilisation. Further studies using physiological integration indices such as the scope for growth (Thompson and MacDonald 1991) or scope for activity (Bayne et al. 1976, Sicard et al. 1999), as well as measuring growth rates at different temperatures, would be useful to establish a relationship with byssal attachment, and provide further insights into the applicability of the rate of byssal attachment as a predictor of favourable conditions for growth in scallops.

The present results for lethal and sublethal temperatures and salinities suggest that from a biogeographic perspective these factors may be important determinants of the distribution of *N. nodosus* along the Brazilian coast. It is likely that the cold sub-Antarctic waters, with temperature below 15°C, that influence southern Brazil during winter (Castro and Miranda 1998), along with low salinity (ca. 29 psu) water advected from Rio de la Plata, Lagoa dos Patos (Sunyé and Servain 1998) and other coastal lagoons, are important barriers preventing the distribution of the lion's paw scallop further south than the surroundings of Santa Catarina Island. Furthermore, the discontinuous distribution of *N. nodosus* along the Brazilian coast (Smith 1991) has not received much attention. From Venezuela to southeastern Brazil, there are only scattered reports of specimens collected in Recife (Pernambuco State) and Salvador (Bahia State), and no records of the existence of populations. Based on the tolerance limits of salinity and temperature, the discontinuous distribution of *N. nodosus* on the northern, northeastern and eastern coasts may be explained by two major features: the presence of large rivers such as the Amazon, São Francisco and others, from which low salinity waters extend several miles offshore;

and high temperatures, in some areas exceeding 30°C, which together with the oligotrophic Brazil Current, may be unsuitable for the establishment of this species.

In conclusion, *N. nodosus* from a population at the high-latitude limit of distribution can be characterised as eurythermal, but stenohaline tropical species. Adults have a lower tolerance to salinity and temperature than spat. Salinities and temperatures at a coastal aquaculture site (Chapter III) in southern Brazil reach sublethal levels, causing a decrease in the rate of byssal attachment, and therefore possibly inhibiting growth of spat. Furthermore, there was a significant interaction between salinity and temperature, so that scallops were more vulnerable to low salinity events when exposed to summer temperatures. Lethal temperatures and salinities for *N. nodosus* were not far from the extreme values recorded in coastal waters in southern Brazil, lethal limits possibly being reached at some of the shallow aquaculture sites. Maximum rate of byssal attachment occured at 19–27°C and 23–27°C for spat and juveniles, respectively, and these temperatures are suggested to be the most favourable for growth, assuming that there are no other inhibiting factor. The results of the present study will assist in the establishment of criteria for adequate site selection for scallop aquaculture in Brazil.

Table V.1. Experimental conditions for the salinity tolerance bioassays with different size scallops (adults, juveniles, spat).

Size class	Experimental temperature (°C)	Test salinities (psu)	No. scallops / container	Container volume (L)	Mean shell height (mm) (± sd)	Mean dry tissue weight (mg) (± sd)	Temp. in the field (°C) [a]	Acclimation period at experimental temperature (days)
Adults	23.5	33, 29, 25, 21, 17, 13	7	8	54.4 (4.5)	1,760 (500)	22.8	4
Juveniles	23.5	33, 29, 25, 21, 17, 13	5	5	25.5 (1.7)	100 (20)	22.8	9
Spat	23.5	33, 29, 25, 21, 17, 13	7	1.8	6.1 (0.5)	2.1 (0.32)	25.7	3 [b]
Spat	16	33, 29, 25, 21, 17, 13	7	1.8	6.7 (0.58)	3.12 (0.85)	25.7	8 [c]
Spat	28	33, 29, 25, 21, 17, 13	7	1.8	7.5 (0.71)	5.1 (1.3)	25.7	7 [d]

(a) when scallops were retrieved from the field site.
(b) temperature decrease of 2.2°C day^{-1} (1 day) prior to reaching experimental temperature.
(c) temperature decrease of 2°C day^{-1} (4 days) prior to reaching experimental temperature.
(d) temperature increase of 2.3°C day^{-1} (1 day) prior to reaching experimental temperature.

Table V.2. Experimental conditions for the temperature tolerance bioassays with different size scallops (adults, juveniles, spat).

Size class	Test temperature (°C)	Salinity (psu)	No. scallops / container	Container volume (L)	Mean shell height (mm) (± sd)	Mean dry tissue weight (mg) (± sd)	Temp. in the field [a] (°C)	Temp. acclimation in lab
Adults	35, 31, 27, 20, 15, 11	33	7	8	54.2 (4.27)	1,680 (320)	25.2	27.0 [b]
Juveniles	35, 31, 27, 23, 19, 15, 11	33	7	3.8	27.2 (1.67)	180 (57)	27.5	27.0
Spat	35, 31, 27, 23, 19, 15, 11	33	7	1.8	6.1 (0.49)	2.14 (0.32)	27.5	27.0

(a) when scallops were retrieved from the field site.
(b) Acclimation in laboratory for 7 days at experimental temperature prior to beginning of the experiment.

Table V.3. Median lethal salinities (LC_{50}) (psu), for *Nodipecten nodosus* spat at acclimated temperatures of 16°C, 23.5°C and 28°C, and juveniles and adults at a temperature of 23.5°C.

	Size class				
	Adults	Juveniles	Spat	Spat	Spat
Experimental temperature (°C)	23.5°C LC_{50}	23.5°C LC_{50}	23.5°C LC_{50}	16°C LC_{50}	28°C LC_{50}
Exposure time					
12-h	20.5	21.7	20.08	18.37	19.67
24-h	21.9	22.7	20.1	18.92	20.24
36-h	22.9	23.3	20.11	19.3	20.55
48-h	23.2	23.6	20.11	19.85	21.33
72-h	23.2	23.6	20.42	20.35	22.37
96-h	23.6	23.6	21.22	20.47	22.94

Table V.4. Median lethal temperature (LT_{50}) (°C) for *Nodipecten nodosus* spat, juveniles and adults for exposure periods from 6 to 96 h.

Exposure time	Adults	Juveniles	Spat
6-h	32.56	33.03	33.67
12-h	31.64	31.94	32.72
24-h	30.84	30.47	32.81
48-h	29.87	29.89	31.83
72-h	29.16	30.33	31.42
96-h	28.04	29.44	30.26

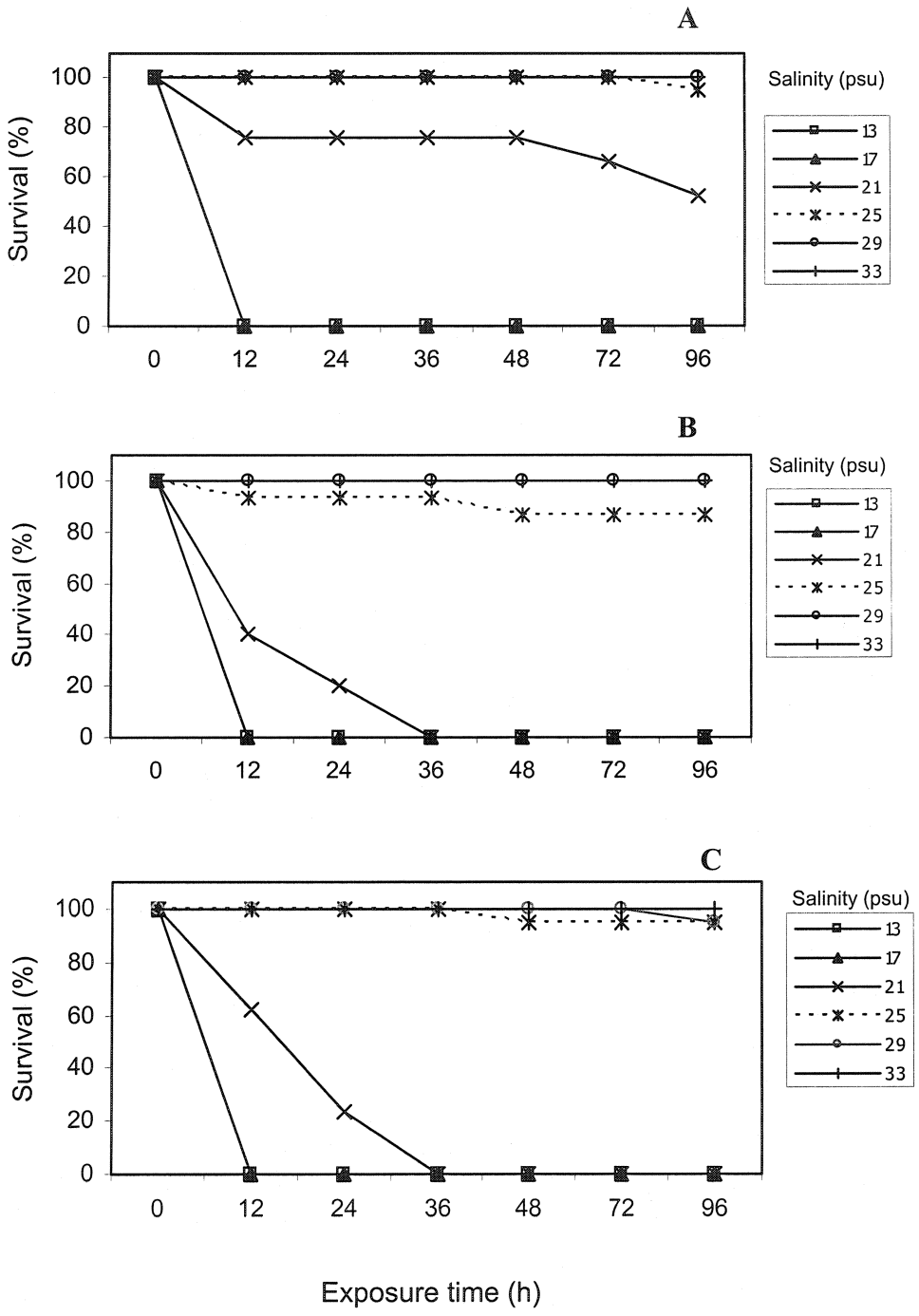


Figure V.1. Percentage survival of *Nodipecten nodosus* exposed to different salinities at an ambient temperature of 23.5°C. **A** – spat, **B** – juveniles, **C** – adults.

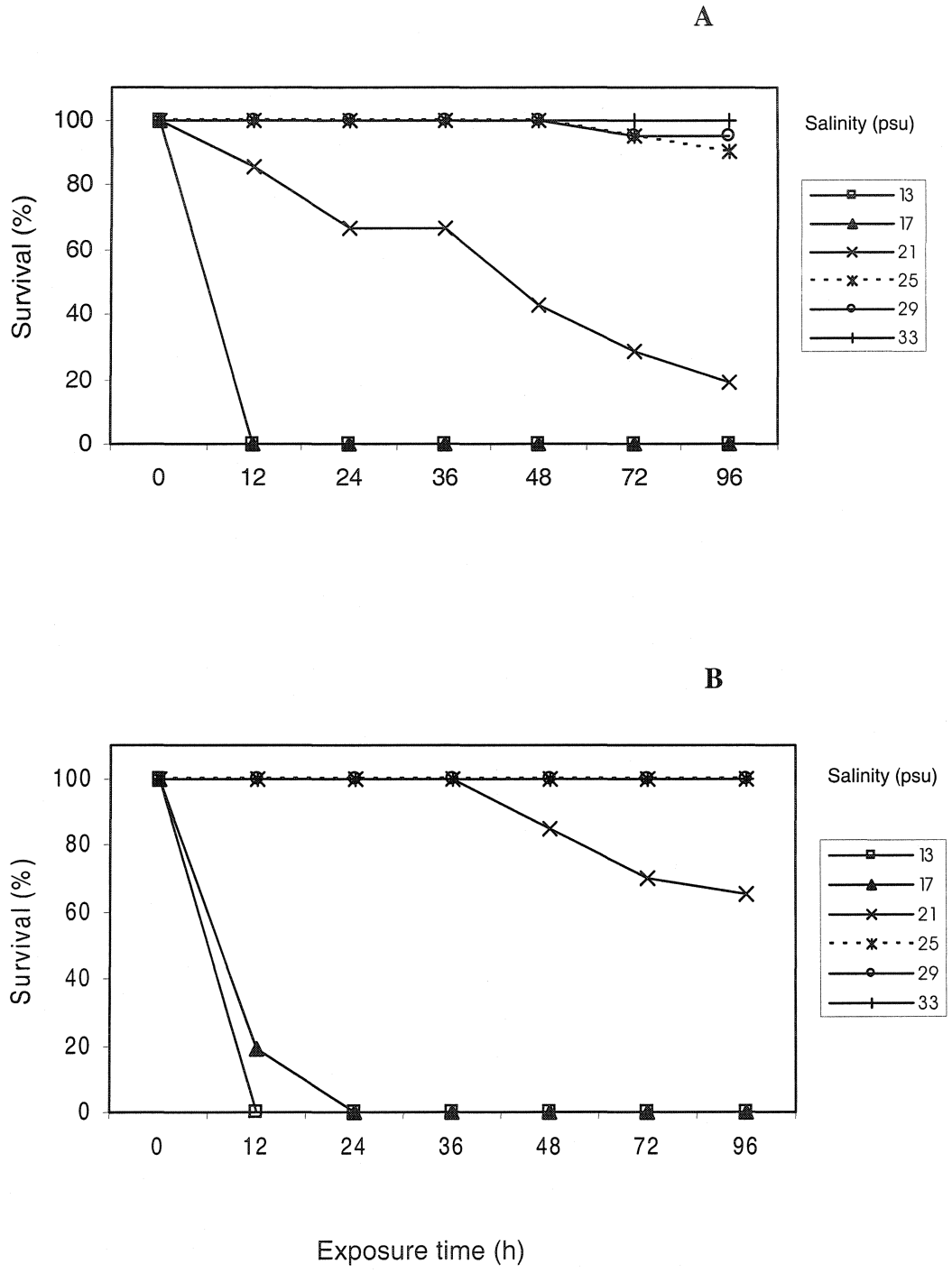


Figure V.2. Percentage survival of *Nodipecten nodosus* spat exposed to different salinities at 28°C (**A**) and 16°C (**B**).

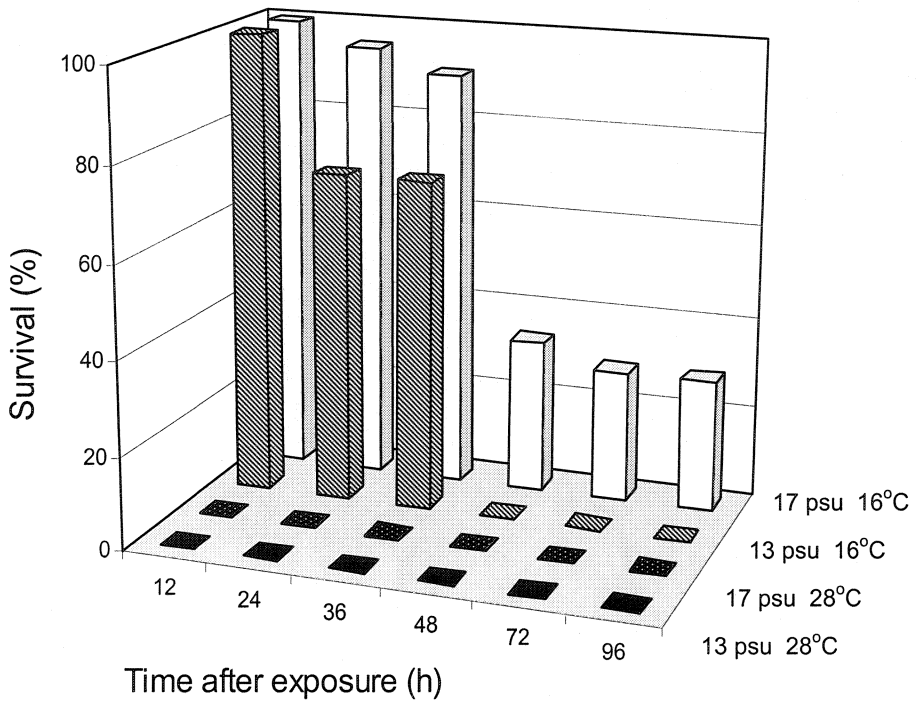


Figure V.3. Percentage survival of *Nodipecten nodosus* spat at different intervals, after a 1-h exposure to salinities of 13 and 17 psu, at temperatures of 16 and 28°C.

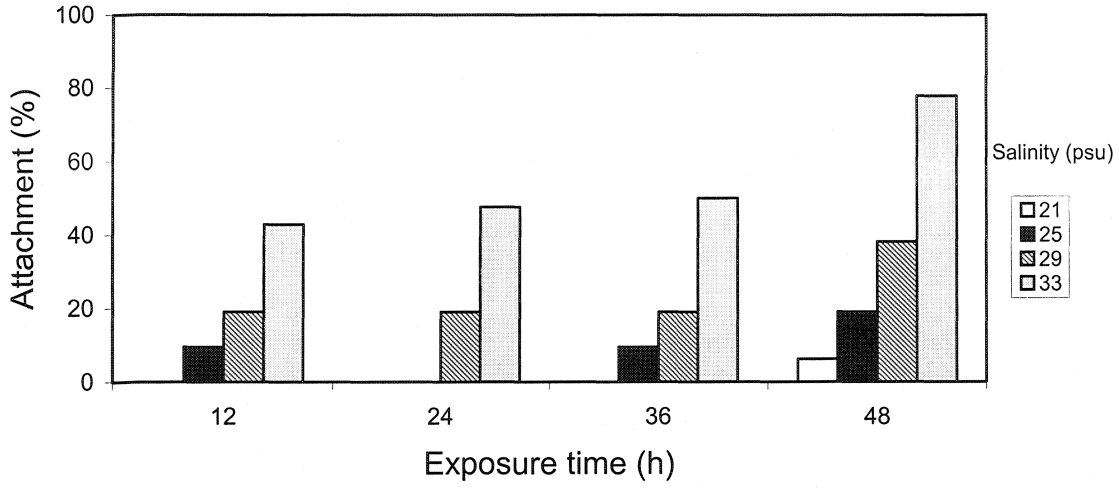


Figure V.4. Percentage of *Nodipecten nodosus* spat that were attached by byssal threads at different experimental salinities for different exposure periods at a temperature of 23.5°C.

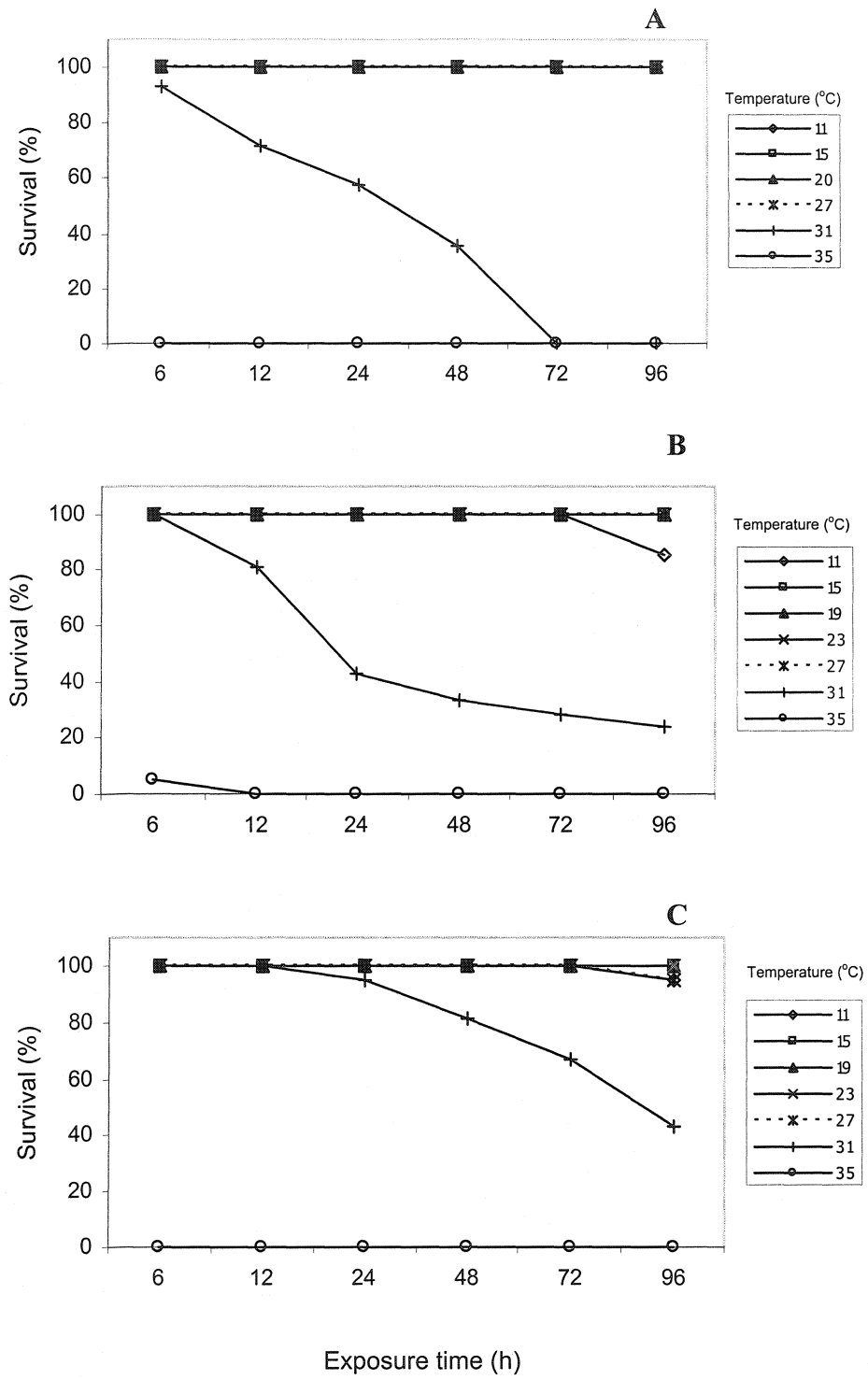


Figure V.5. Percentage survival of *Nodipecten nodosus* adults (**A**), juveniles (**B**) and spat (**C**) exposed to temperatures from 11 to 35°C for different periods at salinity of 33 psu.

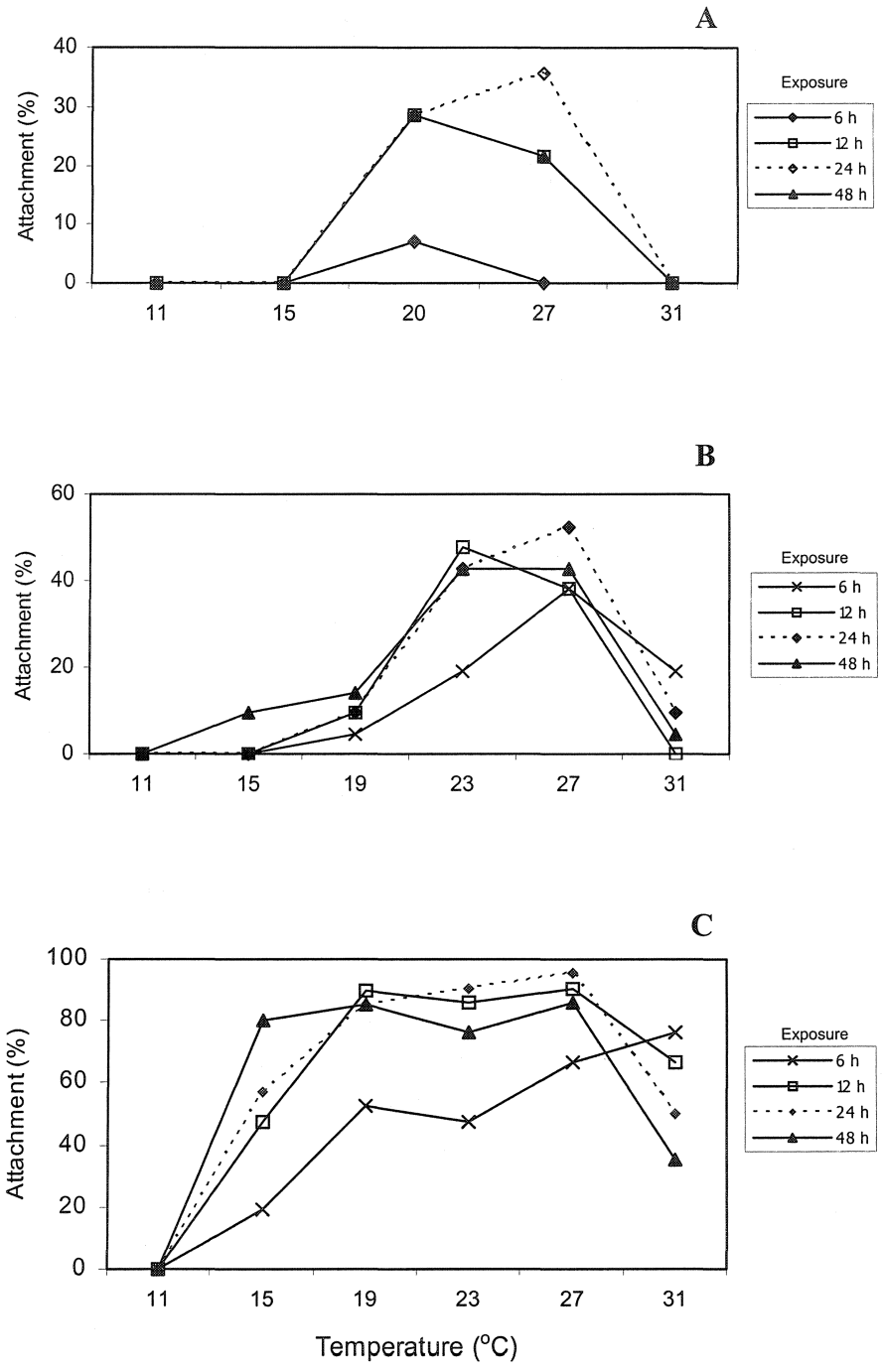


Figure V.6. Percentage of *Nodipecten nodosus* adults (**A**), juveniles (**B**) and spat (**C**) that were byssally attached at different experimental temperatures for different exposure periods at salinity of 33 psu.

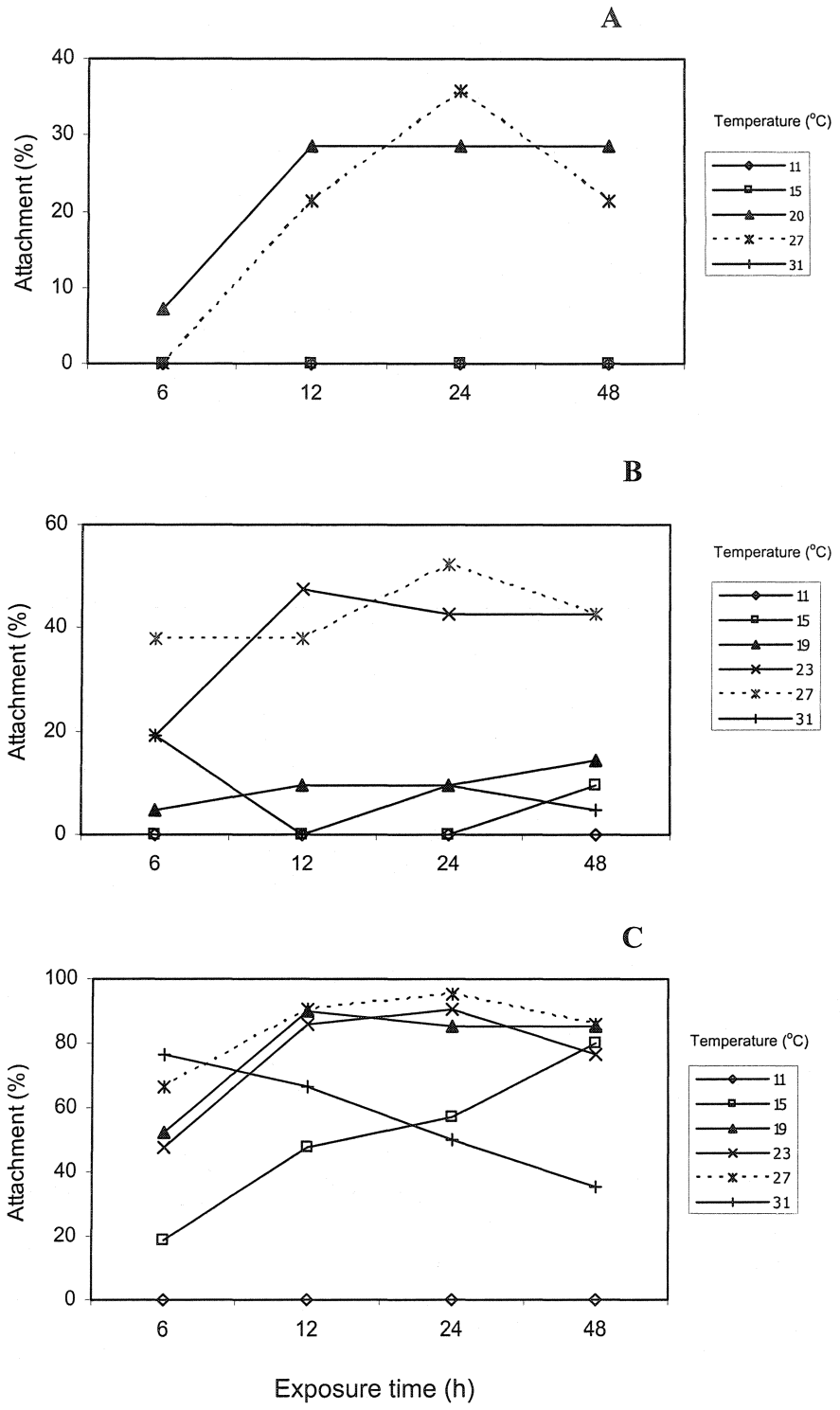


Figure V.7. Percentage of *Nodipecten nodosus* adults (**A**), juveniles (**B**) and spat (**C**) attached by byssal threads at different experimental temperatures during exposures of 6 to 48 h at salinity of 33 psu.

CHAPTER VI

Summary

The main objective of the present study was to understand how variation in food availability, temperature and salinity affect growth, survival and byssal attachment of a subtidal bivalve, the scallop *Nodipecten nodosus*, from a subtropical coastal environment during early benthic life stages. The optimization of growth and survival of *N. nodosus*, a prime candidate species for aquaculture in Brazil, will be essential for the establishment of a successful aquaculture industry, and the present study provides much of the biological information necessary to facilitate the development of scallop farming in Brazil.

Survival of marine bivalves is only possible within certain limits of abiotic and biotic environmental factors (Dame 1996), and growth, which results from the integration of the physiological processes of energy acquisition and expenditure (Bayne and Newell 1983), occurs within a narrower range than survival (Kinne 1970a). Several abiotic environmental factors affect growth and survival of marine invertebrates, including temperature, salinity, dissolved gases, turbidity and water movement (Kinne 1970a). Most lamellibranch bivalves, including pectinids, are suspension feeders, relying on phytoplankton as the main food source (Bricelj and Shumway 1991), except for a period during or early after metamorphosis, when the feeding structures and digestive system are under development and possibly not functional (Sastry 1965, Hodgson and Burke 1988).

The early benthic life stage, when feeding structures are not fully developed (Beninger et al. 1994), is critical for bivalves, especially for pectinids, in which gill development is more protracted than in other bivalves (Veniot et al. 2003). Few studies have focused directly on the influence of food availability on scallops under laboratory

conditions early after metamorphosis (O'Foighil et al. 1990, Reid et al. 1992, Whyte et al. 1992), and the contribution of endogenous energy reserves and different food sources to growth remains unclear. Furthermore, few studies have considered the importance of a wide array of environmental factors in the field during the postlarval and early juvenile stages (Bourne and Hodgson 1991, Grecian et al. 2000), and none have been carried out with a tropical species at its high-latitude distribution limit. At the limits of distribution, tropical species are exposed to the broadest temperature variation of its geographic range (Levinton 2001). In addition, in southern Brazil, an unusual pattern of variation of temperature and food availability in opposite directions may occur, and the question remains whether seasonal signals are strong enough to significantly influence growth and survival of postlarval and juvenile scallops, an important consideration in aquaculture.

The present study examined the influence of food availability on survivorship and growth of postlarval *Nodipecten nodosus* (Chapter II) and the influence of several environmental factors on the performance of postlarval and juvenile scallops (Chapters III, IV and V) as well as adults (Chapter V), under laboratory and field conditions.

In Chapter II, it was shown that the presence of a biofilm on the surface of the collectors significantly enhanced postlarval settlement, but conferred no nutritional benefit, as reflected by minimal shell growth. Furthermore, energy reserves accumulated during the larval stage were sufficient to sustain metamorphosis, limited shell growth and high survival in the absence of exogenous food for several days, giving no indications of food deprivation-induced mortality for at least nine days post-set. However, 5 days after settlement, at a shell height of about 250 μm, postlarvae fed a low algal concentration (ca. $4.7x10^3$ cells mL^{-1}) were significantly larger than those in the other treatments, providing

evidence that exogenous suspended food becomes important for sustaining growth, but a high algal concentration (ca. $4x10^4$ cells mL^{-1}) was detrimental. Approximately ten days after settlement (shell height ca. 300 μm), the feeding demand of postlarval scallops significantly increases, as demonstrated by the higher content of pigments in the gut. An alternative explanation for the high mortality of scallops often recorded early after metamorphosis in aquaculture settings, other than the depletion of endogenous reserves, is proposed in the light of recent studies on the immune system, which suggest that scallops are more vulnerable to bacterial infections than some other bivalves.

Chapter III demonstrated that in a coastal subtropical aquaculture site in southern Brazil seawater temperature, which displayed a magnitude of variation similar to that recorded in some temperate-boreal regions (c.a. 12.5°C), was the major environmental factor affecting growth of postlarval *N. nodosus*. Food availability, as expressed by concentrations of chlorophyll-*a* and seston indices, had a negligible influence on growth. Low salinity and high turbidity, concurrent with a period of heavy rainfall, reduced growth rates and possibly percentage retrievals. In southern Brazil there is a wide window of opportunity for deployment of scallops in the sea-based nursery, with favourable conditions for growth occurring throughout most of the year, except when the temperature is below 20°C for part of the winter – early spring. Furthermore, at a size of 0.5 mm (ca. 2 weeks post-set), scallops deployed in the sea-based nursery begin to grow faster than those maintained under laboratory conditions for longer periods. As a result, scallops deployed in the sea at about 0.5 mm attained a larger shell height at retrieval than those deployed at sizes ranging from 0.7–1.1 mm, thereby reducing the culture period required to achieve a size (ca. 5 mm) at which scallops could be transferred to pearl nets

in about 2–3 weeks. In addition, percentage retrievals were similar when scallops deployed at different sizes (0.5–1.1 mm) were retrieved simultaneously from the sea-based nursery, so it is more advantageous to deploy spat in the sea approximately two weeks after settlement.

In Chapter IV it is demonstrated for the first time that sub-surface intrusions of cold and phytoplankton-rich South Atlantic Central Water (SACW), which normally influences the inner continental shelf, invade a near-shore (< 0.5 km) coastal environment in late summer–early autumn. Furthermore, despite the higher phytoplankton biomass recorded at 12 m as a result of this water mass, growth of postlarval scallops was highest at 4 m, where there was no influence of SACW. The observed reduction in growth at 12 m was explained by a wide temperature oscillation (ca. 10°C in less than 24 h), together with higher inorganic content of the seston. Furthermore, growth was similar at both depths in late autumn–early winter, when SACW was absent. A stocking density of 340 spat collector^{-1} resulted in a slight reduction in growth compared with a density of ca. 150 spat collector^{-1}, but survival was similar.

In Chapter V, a laboratory-based ecophysiological approach was used to determine lethal and sublethal effects of temperature and salinity stress on different size scallops. As a result, *N. nodosus* can be characterised as a eurythermal but stenohaline tropical species. Adults had lower tolerance to temperature and salinity than spat, and this increased susceptibility coincided with the onset of sexual maturation. Salinity and temperature at a coastal aquaculture site in southern Brazil can reach levels that result in sublethal effects such as a decreased rate of byssal attachment, possibly reducing growth and retrievals of cultured spat at certain times of the year. Furthermore, there is a

significant interaction between salinity and temperature, so that scallops are more vulnerable to low salinity events when exposed to summer high temperatures. Lethal temperatures and salinities for *N. nodosus* are not far from the extreme values recorded in coastal waters in southern Brazil, and may occasionally be reached in some of the bivalve aquaculture sites. The maximum rate of byssal attachment occurs at 19–27°C and 23–27°C for spat and juveniles, respectively, and these temperatures are probably the most favourable for growth, assuming the absence of other adverse factors. In order to obtain high growth and survival during nursery, intermediate culture and final growout, farming of *N. nodosus* requires sites at which salinity does not fall below 29 psu and temperature does not exceed 28°C.

In summary, this study demonstrated that exogenous algal food becomes important to growth of *N. nodosus* approximately 3–5 days after metamorphosis (ca. 230–250 μm shell height). Approximately 2 weeks after settlement, when a shell height of 500 μm is attained, deployment of spat to the sea-based nursery becomes a feasible aquaculture approach. At this size postlarval scallops rely on phytoplankton as the major food source, displaying significantly higher growth rates when deployed in the sea. Furthermore, at this stage seasonal environmental changes significantly affect growth of postlarvae in a coastal subtropical marine environment, in which food is not a limiting factor, but growth is primarily determined by temperature. Other factors resulting from meteorological processes, such as a decrease in seawater salinity and an increase in turbidity, are also important. Temperature and salinity in aquaculture sites in southern Brazil may reach levels leading to lethal and sublethal effects on scallops, and must therefore be considered when establishing aquaculture sites and practices.

The findings of the present investigation expand current knowledge about the importance of different food sources for scallops early after metamorphosis, and increase our understanding of the spatial and temporal variability of environmental factors in a coastal subtropical marine ecosystem. Furthermore, this study enhances our comprehension of the effects of food availability, temperature and salinity on survival, growth and synthesis of byssal threads of a tropical scallop at its high latitude distribution limit. Finally, this study provides an important biological basis to assist in the development of scallop farming, proposes strategies to enhance nursery culture, and establishes criteria for adequate site selection, thereby advancing scallop aquaculture in Brazil.

In summary, the major results from the present study are:

- The presence of epiflora on the spat collectors significantly increases settlement but not growth of postlarvae. Either no pedal feeding behaviour is displayed by *Nodipecten nodosus* or the microalgae attached to the spat collectors (*Melosira* sp.) are unsuitable for this scallop.
- Suspended algae start to influence growth 3 to 5 days post-set, at a shell height between 200 and 250 μm, and a high cell concentration ($4x10^4$ cells mL^{-1}) is detrimental to growth and survival early after metamorphosis.
- Absence of exogenous food does not limit survival for at least 9 days post-set and acquisition of exogenous food starts well before that. Therefore the exhaustion of endogenous reserves does not explain postlarval mortality.

- There is a sharp increase in feeding demand between 10 and 16 days post-set, at a shell height of 300 – 400 μm, which is probably associated with critical events in gill morphogenesis.
- Observed variation in growth of postlarval scallops in the subtropical waters off Santa Catarina State is related to variation in seasonal environmental factors, temperature being the major factor affecting growth.
- During periods when the temperature is below 20°C, growth of postlarval scallops is significantly reduced, but food availability, as expressed by concentrations of chlorophyll-*a*, is not a factor limiting growth.
- Periods of low salinity (< 29 psu) and high turbidity associated with heavy rainfall are detrimental to growth and possibly retrievals of postlarvae.
- There is a wide window of opportunity (temperature > 20°C) to deploy postlarvae to the sea-based nursery, in which growth is maximized.
- Growth of postlarvae is significantly enhanced after transfer to the sea-based nursery 2 weeks post-set (0.5 mm), and there is a trade-off between higher growth in the sea-based nursery and higher percentage retrieval in the land-based nursery. However, retrievals of postlarvae deployed in the sea-based nursery at sizes ranging from 0.5 to 1.1 mm are similar, so it is more advantageous to deploy them at 0.5 mm.
- The greatest loss of spat occurs during transport and/or early after deployment in the sea-based nursery, due to detachment from the spat collectors.
- Subsurface intrusions of SACW affected the coastal site (<0.5 Km) at a depth of 12 m during late summer-early autumn, but not during the late autumn-early winter.

- In late summer – early autumn, high PIM and oscillating temperature (ca. 10°C) reduced growth of postlarvae at 12 m, but not survival.
- Postlarvae cultured at high density (340 spat/bag) displayed a slightly smaller shell height than at 150 spat/bag, but survival was similar between densities, resulting in higher yields (no. spat / bag) at higher density.
- Deploying postlarvae in the sea-based nursery using a sub-surface long-line resulted in high percent retrievals (70–80 %) at 4 and 12 m.
- *N. nodosus* at its southern distribution limit is a eurythermal but stenohaline tropical species. High survival occurs at temperatures between 15 and 27°C. Although high survival is recorded at 29 psu, the percentage byssal attachment is significantly lower than at 33 psu, indicating that scallops are physiologically stressed at 29 psu and below.
- Postlarvae (shell height 0.5–0.8 mm) had greater tolerance to high temperature and low salinity than juveniles (shell height 20–30 mm) and adults (shell height 50–60 mm), probably due to the additional energy demand of sexual maturation in the larger scallops.
- *N. nodosus* is more vulnerable to low salinity at higher temperatures, and therefore, during summer scallops may be more susceptible to periods of low salinity.
- Salinity and temperature at a coastal site in Santa Catarina can reach levels resulting in sublethal effects (reduced byssal attachment), possibly influencing growth and retrieval of cultured scallops.
- Lethal temperatures and salinities for *N. nodosus* are not far from that recorded in coastal sites off Santa Catarina State and may be occasionally reached at some aquaculture sites. Therefore, criteria for adequate site selection for culturing *N. nodosus* should be based on the results of the present study.

- The greater percentage of byssal attachment for postlarvae occurs at 19 – 27°C, and for juveniles and adults at 23 – 27°C, which can be considered the most favourable temperature ranges for growth of *N. nodosus*.

References

Abarca, A. 2001. Scallop hatcheries of *Argopecten purpuratus* (Lamark 1819) in Chile. A survey of the present situation. Abstracts. 13th International Pectinid Workshop, 18–24 April 2001, Universidad Católica del Norte, Coquimbo, Chile. pp. 74–75.

Allen, D.M. 1979. Biological aspects of the scallop, *Argopecten gibbus*, determined by spat monitoring. Nautilus, 94: 107–118.

Andersen, S., and Naas, K.E. 1993. Shell growth and survival of scallop (*Pecten maximus* L.) in a fertilised, shallow water pond. Aquaculture, 110: 71–86.

Bacon, G.S., MacDonald, B.A., and Ward, J.E. 1998. Physiological responses of infaunal (*Mya arenaria*) and epifaunal (*Placopecten magellanicus*) bivalves to variations in the concentration and quality of suspended particles I. Feeding activity and selection. J. Exp. Mar. Biol. Ecol. 219: 105–125.

Baker, S.M., and Mann, R. 1994. Feeding ability during settlement and metamorphosis in the oyster *Crassostrea virginica* (Gmelin, 1791) and the effects of hypoxia on post-settlement ingestion rates. J. Exp. Mar. Biol. Ecol. 181: 239–253.

Bamstedt, U., Gifford, D.J., Irigoien, X., Atkinson, A., and Roman, M. 2000. Feeding. *In* Zooplankton Methodology Manual. *Edited by* R.P. Harris, P.H. Wiebe, J. Lenz, H.R. Skjoldal, and M. Huntley. Academic Press, London. pp. 297–399.

Barré, C., Bricelj, M.V., and Veniot, A. 2002. Comparison of morphogenesis during early development of two pectinids: *Placopecten magellanicus* (Gmelin,1791) and *Argopecten irradians* (Lamark, 1819). Proceedings of the 2002 Annual Research Conference of AquaNet, Moncton, NB, Canada. pp. 64.

Bayne, B. L. 1965. Growth and delayed metamorphosis of the larvae of *Mytilus edulis* (L.). Ophelia, 2: 419–443.

Bayne, B.L. 1976. Marine mussels: their ecology and physiology. Cambridge University Press. 506 p.

Bayne, B.L., and Newell, R.C. 1983. Physiological energetics of marine molluscs. *In* The Mollusca. *Edited by* A.S.M. Saleuddin and K.M. Wilbur. Vol. IV. Academic Press Inc., New York. pp. 407–415.

Bayne, B.L., Thompson, R.J., and Widdows, J. 1976. Physiology I. *In* Marine Mussels: their Ecology and Physiology. *Edited by* B.L. Bayne. Cambridge Scientific Press, UK. pp. 121–206.

Bem, M.M., Rupp, G.S., Pereira, A., and Poli, C.R. 2001a. Effect of antibiotics on larval survival and microbial levels during larval rearing of the tropical scallop *Nodipecten nodosus*. Abstracts. 13th International Pectinid Workshop. Universidad Católica del Norte, Coquimbo, Chile. pp. 114–116.

Bem, M.M., Rupp, G.S., Vandenberghe, J., Sorgeloos, P., and Ferreira, J. 2001b. Effects of marine bacteria on larval survival of the tropical scallop *Nodipecten nodosus* (Linnaeus, 1758) (Bivalvia : Pectinidea). Abstracts. 13th International Pectinid Workshop. Universidad Católica del Norte, Coquimbo, Chile. pp. 111–113.

Beninger, P.G. 1991. Structures and mechanisms of feeding in scallops: paradigms and paradoxes. *In* An International Compendium of Scallop Biology and Culture. *Edited by* S.E. Shumway and P. Sandifer. World Aquaculture Workshops, No. 1. World Aquaculture Society, Baton Rouge, LA. pp. 331–340.

Beninger, P.G., Dwiono, S.A.P., and Le Pennec, M. 1994. Early development of the gill and implications for feeding in *Pecten maximus* (Bivalvia:Pectinidae). Mar. Biol. 119: 405–412.

Beninger P.G., and Le Pennec, M. 1991. Functional anatomy of scallops. *In* Scallops: Biology, Ecology and Aquaculture. *Edited by* S.E. Shumway. Elsevier, Amsterdam. pp. 133–224.

Bergman, C., Parsons, G.J., and Couturier, C. 1996. Tolerance of the giant sea scallop, *Placopecten magellanicus*, to low salinity. Bull. Aquacul. Assoc. Canada. 96: 62–64.

Borzone, C.A., Pezzuto, P.R., and Marone, E. 1999. Oceanographic characteristics of a multi-specific fishing ground of the central south Brazil bight. Mar. Ecol. 20: 132–146.

Bourne, N., and Hodgson, C.A. 1991. Development of a viable nursery system for scallop culture. *In* An International Compendium of Scallop Biology and Culture. *Edited by* S.E. Shumway and P. Sandifer. World Aquaculture Workshops, No. 1. World Aquaculture Society, Baton Rouge, LA. pp. 273–280.

Bourne, N., Hodgson, C.A., and Whyte, J.N. 1989. A manual for scallop culture in British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. No. 1694. 215 pp.

Brand, A. 1991. Scallop ecology: distribution and behaviour. *In* Scallops: Biology, Ecology and Aquaculture. *Edited by* S.E. Shumway. Elsevier, Amsterdam. pp. 517–584.Brandini, F.P. 1990. Hydrography and characteristics of the phytoplankton in shelf and oceanic waters off southeastern Brazil during winter (July/August 1982) and summer (February/March 1984). Hydrobiologia, 195: 111–148.

Brandini, F.P., Silva, A.S., and Proença, L.A. 2000. Oceanografia e maricultura. *In* Aquicultura no Brasil. *Edited by* W.C. Valenti, C.R. Poli, J.A. Pereira, and J.A. Borghetti. Minitério da Ciência e Tecnologia, Brasília, DF, Brazil. pp. 107–141.

Bricelj, M.V., and Malouf, R.E. 1984. Influence of algal and suspended sediment concentrations on the feeding physiology of the hard clam *Mercenaria mercenaria*. Mar. Biol. 85: 155–165.

Bricelj, M.V., and Shumway, S. 1991. Physiology: Energy acquisition and utilization. *In* Scallops: Biology, Ecology and Aquaculture. *Edited by* S.E. Shumway. Elsevier, Amsterdam. pp. 305–376.

Briggs, J.C. 1995. Global biogeography. Developments in Paleontology and Stratigraphy, 14. Elsevier, Amsterdam. 452 p.

Broom, M.J., and Mason, J. 1978. Growth and spawning of the pectinid *Chlamys opercularis* in relation to temperature and phytoplankton concentration. Mar. Biol. 47: 277–285.

Bubel, A. 1984. Mollusca. *In* Biology of the integument. I. Invertebrates. *Edited by* J. Bereiter-Hahn, A.G. Matoltsy and K.S Sylvia Richards. Springer-Verlag, Berlin. pp. 421–447.

Burke, R.D. 1983. The induction of metamorphosis of marine invertebrate larvae: stimulus and response. Can. J. Zool. 61: 1701–1719.

Caddy, J.F. 1972. Progressive loss of byssus attachment with size in the sea scallop, *Placopecten magellanicus* (Gmelin). J. Exp. Mar. Biol. Ecol. 9: 179–190.

Campos, E.J.D., Ikeda, Y., Castro, B.M., Gaeta, S.A., Lorenzzetti, J.A., and Stevenson, M.R. 1996. Experiment studies circulation on the Western South Atlantic. EOS trans. Am. Geophys. Union, 77: 253–259.

Campos, E.D.J., Lorenzzetti, J.A., Stevenson, M.R., Stech, J.L., and Souza, R.B. 1996. Penetration of waters from the Brazil – Malvinas confluence region along the South American continental shelf up to 23°S. Anais Acad. Bras. Ciências. 68: 49–58.

Castagna, M., and Chanley, P. 1973. Salinity tolerance of some marine bivalves from inshore and estuarine environments in Virginia waters on the western mid-Atlantic coast. Malacologia, 12: 47–96.

Castagna, M., and Duggan, W. 1971. Rearing the bay scallop, *Aequipecten irradians* (Lamark). Proc. Nat. Shellfish. Assoc. 61: 80–85.

Castro, B.M., and Miranda, L.B. 1998. Physical oceanography of the western Atlantic continental shelf located between 4° N and 34° S. *In* The Global Coastal Ocean. *Edited by* A.R. Robinson and K.H. Brink. The Sea, No. 11. pp. 209–251.

Chauvaud, L., Thouzeau, G., and Paulet, Y. 1998. Effects of environmental factors on the daily growth rate of *Pecten maximus* juveniles in the Bay of Brest (France). J. Exp. Mar. Biol. Ecol. 227: 83–111.

Cheng, T.C. 1981. Bivalves. *In* Invertebrate Blood Cells. *Edited by* N.A. Ratcliffe and A.F. Rowley. Academic Press, London. Vol. 1. pp. 223–300.

Chia, F.S. 1977. Perspectives: settlement and metamorphosis of marine invertebrate larvae. *In* Settlement and Metamorphosis of Marine Invertebrate Larvae. *Edited by* F.S. Chia and M. Rice. Elsevier, New York. pp. 283–285.

Christophersen, G., and Magnesen, T. 2001. Effects of deployment time and acclimation on survival and growth of hatchery reared scallop (*Pecten maximus*) spat transferred to the sea. J. Shellfish Res. 20: 1043–1048.

Christophersen, G., and Strand, O. 2003. Effect of reduced salinity on the great scallop (*Pecten maximus*) spat at two rearing temperatures. Aquaculture, 215: 79–92.

Claus, C. 1981. Trends in nursery rearing in bivalve molluscs. *In* Nursery culturing of bivalve molluscs. *Edited by* C. Claus, N. De Pauw, and E. Jasper. European Mariculture Society Special Publication No. 7. Belgium. pp. 1–33.

CLIMERH. 2003. Centro Integrado de Meteorologia e Recursos Hídricos de Santa Catarina. Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina. Governo do Estado de Santa Catarina. www.climerh.rct-sc.br.

Corpe, W.A. 1970. Attachment of marine bacteria to solid surfaces. *In* Adhesion in Biological Systems. *Edited by* R.S. Manley, Academic Press, New York. pp. 72–87.

Côté, J., Himmelman, J., and Claereboudt, M. 1994. Separating the effects of limited food and space on growth of the giant scallop *Placopecten magellanicu* in suspended culture. Mar. Ecol. Progr. Ser. 106: 85–91.

Côté, J., Himmelman, J., Claereboudt, M., and Bonardelli, J. 1993. Influence of density and depth on the growth of the sea scallop (*Placopecten magellanicus*) in suspended culture. Can. J. Fish. Aquat. Sci. 50: 1857–1869.

Coutteau, P., and Sorgeloos, P. 1992. The use of algal substitutes and the requirement for live algae in the hatchery and nursery rearing of bivalve molluscs: an international survey. J. Shellfish Res. 11: 467–476.

Crisp, D.J. 1964. The effect of the severe winter of 1962-63 on marine life in Britain. J. Anim. Ecol. 33: 165–210.

Dabinett, P., Caines, J., and Crocker, K. 1999. Hatchery production of sea scallop spat (*Placopecten magellanicus*) in Newfoundland, Canada. Abstracts. 12th International Pectinid Workshop, Institute of Marine Research. Bergen, Norway. pp. 63–64.

Dagg, M.J., and Wyman, K.D. 1983. Natural ingestion rates of the copepods *Neocalanus plumchrus* and *N. cristatus* calculated from gut contents. Mar. Ecol. Prog. Ser. 13: 37–46.

Dame, R.F. 1996. Ecology of marine bivalves - An ecosystem approach. CRC Press, Boca Raton. 245 p.

Davenport, J., Gruffydd, Ll.D., and Beaumont, A.R. 1975. An apparatus to supply seawater of fluctuating salinity and its use in the study of the salinity tolerances of larvae of the scallop *Pecten maximus* L. J. Mar. Biol. Ass. U.K. 55: 391–410.

Davis, R.L., and Marshall, N. 1961. The feeding of the bay scallop, *Aequipecten irradians*. Proc. Nat. Shellfish. Assoc. 52: 25–29.

De la Roche, J. P., Marín, B., Freites, L., and Vélez, A. 2002. Embryonic and larval and postlarval growth of the tropical scallop *Nodipecten* (=*Lyropecten*) *nodosus* (L. 1758) (Mollusca:Pectinidae). Aquaculture Res. 33: 819–827.

De Pauw, N. 1981. Use and production of microalgae as food for nursery bivalves. *In* Nursery culturing of bivalve molluscs. Edited by C. Claus, N. De Pauw, and E. Jasper. pp. 35–69.

Dickie, L.M. 1958. Effect of high temperature on survival of the giant scallop. J. Fish. Res. Board Canada, 15: 1189–1211.

Dickie, L.M., and Medcof, J. C. 1963. Causes of mass mortality of scallops (*Placopecten magellanicus*) in the southeastern Gulf of St. Lawrence. J. Fish. Res. Bd. Canada, 20: 451–482.

Duerr, E.O., Molnar, A., and Sato, V. 1998. Cultured microalgae as aquaculture feed. J. Mar. Biotechnol. 7: 65–70.

Duggan, W. 1973. Growth and survival of the bay scallop, *Argopecten irradians*, at various locations in the water column and at various densities. Proc. Natl. Shellfisheries Assoc. 63: 68–71.

Elston, R. 1984. Prevention and management of infectious diseases in intensive mollusc husbandry. J. World Mar. Soc. 15: 28–300.

Elston, R., and Leibovitz, L. 1980. Pathogenesis of experimental vibriosis in larval American oysters *Crassostrea virginica*. Can. J. Fish. Aquat. Sci. 37: 964–978.

Emerson, C.W., Grant, J., Mallet, A., and Carver, C. 1994. Growth and survival of sea scallops *Placopecten magellanicus*: effects of culture depth. Mar. Ecol. Prog. Ser. 108: 119–132.

Emilsson, I. 1961. The shelf and coastal waters of southern Brazil. Bolm. Inst. Oceanogr. S. Paulo, 11: 101–112.

Farias, A. 2001. Nutrición en los moluscos pectínidos. *In* Los Moluscos Pectínidos de Iberoamérica: Ciencia y Acuicultura. *Edited by* A.N. Maeda-Martinez. Editorial LIMUSA, México. pp. 89–104.

Farias, A., Uriarte, I., and Castilla, J.C. 1998. A biochemical study of the larval and postlarval stages of the Chilean scallop *Argopecten purpuratus*. Aquaculture, 166: 37–47.

Fréchette, M., and Daigle, G. 2002. Reduced growth of Iceland scallops *Chlamys islandica* (O.F. Müller) cultured near the bottom: a modeling study of alternative hypotheses. J. Shellfish Res. 21: 87–91.

Freites, L., Lodeiros, C., Vélez, A., and Bastardo, J. 1993. Vibriosis en larvas de la vieira tropical *Euvola* (*Pecten*) *ziczac*. Caribb. J. Sci. 29: 89–98.

Frenette, B., and Parsons, G.J. 2001. Salinity-temperature tolerance of juvenile giant scallops, *Placopecten magellanicus*. Aquacul. Assoc. Canada Spec. Publ. 4: 76–78.

Gerdes, D. 1983. The Pacific oyster *Crassostrea gigas*. Part I. Feeding behaviour of larvae and adults. Aquaculture, 31: 221–231.

Gonzalez-Rodriguez, E., Valentin, J.L., André, D.J., and Jacob, S.A. 1992. Upwelling and downwelling at Cabo Frio (Brazil): comparison of biomass and primary production responses. J. Plankton Res. 14: 289–306.

Grant, J. 1996. The relationship of bioenergetics and the environment to the field growth of cultured bivalves. J. Exp. Mar. Biol. Ecol. 200: 239–256.

Grecian, L.A., Parsons, G.J., Dabinett, P., and Couturier, C. 2000. Influence of season, initial size, depth, gear type and stocking density on the growth rates and recovery of the sea scallop, *Placopecten magellanicus*, on a farm-based nursery. Aquaculture International, 8: 183–206.

Griffiths, C.L., and King, J.A. 1979. Some relationships between size, food availability and energy balance in the ribbed mussel *Aulacomya ater*. Mar. Biol. 51: 141–149.

Hadfield, M.G. 1977. Metamorphosis in marine molluscan larvae: an analysis of stimulus and response. *In* Settlement and metamorphosis of marine invertebrate larvae. *Edited by* F. Chia and M.E. Rice. Elsevier, New York. pp. 165–175.

Hasle, G.R., and Syvertsen, E.E. 1997. Marine Diatoms. *In* Identifying Marine Phytoplankton. *Edited by* C. Tomas. Academic Press, San Diego. pp. 5–385.

Heasman, M.P., O'Connor, W.A., and Frazer, A.W. 1996. Ontogenic changes in optimal rearing temperatures for the commercial scallop, *Pecten fumatus* Reeve. J. Shellfish Res. 15: 627–634.

Heasman, M.P., O'Connor, W.A., Frazer, A.W., Languet, Y., and O'Connor, S.J., 2002. Alternative means of nursery culture for commercial scallop (*Pecten fumatus* Reeve) spat. Aquaculture, 213: 323–338.

Hine, P.M. 1999. The inter-relationships of bivalve hemocytes. Fish Shellf. Immunol. 9: 367–385.

Hodgson, C.A., and Bourne, N. 1988. Effect of temperature on larval development of the spiny scallop, *Chlamys hastata* Sowerby, with a note on metamorphosis. J. Shellfish Res. 7: 349–357.

Hodgson, C.A., and Burke, R.D. 1988. Development and larval morphology of the spiny scallop, *Chlamys hastata.* Biol. Bull. 174: 303–318.

Holland, D.L., and Spencer, B.E. 1973. Biochemical changes in fed and starved oysters *Ostrea edulis* L. during larval development, metamorphosis and early spat growth. J. Mar. Biol. Assoc. U.K. 53: 287–298.

Kennedy, V.S. 1996. Biology of larvae and spat. *In* The eastern oyster, *Crassostrea virginica. Edited by* V.S. Kennedy, R.I.E. Newell and A.F. Eble. Maryland Sea Grant Publication, College Park, Maryland. pp. 371–421.

King, J.J. 1986. Juvenile feeding ontogeny of the geoduck *Panope abrupta* (Bivalvia:Saxicavacea) and comparative ontogeny and evolution of feeding in bivalves. M.Sc. Thesis, University of Victoria, British Columbia. 281 p.

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

Kinne, O. 1970a. Temperature – Invertebrates. *In* Marine Ecology. *Edited by* O. Kinne. Vol I, Part 1. Wiley-Interscience, London. pp. 405–514.

Kinne, O. 1970b. Salinity – Invertebrates. *In* Marine Ecology. *Edited by* O. Kinne. Vol I, Part 2. Wiley-Interscience, London. pp. 821–995.

Kitchen, J.C., Zaneveld, J.R.V., and Pak, H. 1982. Effect of particle size distribution and chlorophyll content on beam attenuation spectra. Applied Optics, 21: 3913–3918.

Kleinman, S., Hatcher, B.G., Sheibling, R.E., Taylor, L. H., and Hennigar, A.W. 1996. Shell and tissue growth of juvenile sea scallops (*Placopecten magellanicus*) in suspended and bottom culture in Lunenburg Bay, Nova Scotia. Aquaculture, 142: 75–97.

Laing, I. 2000. Effect of temperature and ration on growth and condition of king scallop *Pecten maximus*) spat. Aquaculture, 183: 325–334.

Laing, I. 2002. Effect of salinity on growth and survival of king scallop spat (*Pecten maximus*). Aquaculture, 205: 171–181.

Leighton, D.L. 1979. A growth profile for the rock scallop *Hinnites multirugosus* held at several depths off La Jolla, California. Mar. Biol. 51: 229–232.

Levinton, J.S. 2001. Marine Biology - Function, Biodiversity and Ecology. Oxford University Press, Oxford. 513 p.

Lodeiros, C., and Himmelman, J. 1994. Relations among environmental conditions and growth in the tropical scallop *Euvola (Pecten) ziczac* (L.) in suspended culture in the Golfo de Cariaco, Venezuela. Aquaculture, 119: 345–348.

Lodeiros, C., and Himmelman, J. 1996. Influence of fouling on the growth and survival of the tropical scallop, *Euvola (Pecten) ziczac* (L. 1758) in suspended culture. Aquaculture Research, 27: 749–756.

Lodeiros, C., and Himmelman, J. 2000. Identification of factors affecting growth and survival of the tropical scallop *Euvola (Pecten) ziczac* in the Golfo de Cariaco, Venezuela. Aquaculture, 182: 91–114.

Lodeiros, C., Maeda-Martinez, A., Freites, L., Uribe, E., Lluch-Cota, D., and Sicard, M.T. 2001. Ecofisiologia de pectínidos iberoamericanos. *In* Los Moluscos Pectínidos de Iberoamérica: Ciencia y Acuicultura. *Edited by* A.N. Maeda-Martinez. Editorial LIMUSA, México. pp. 77–88.

Lodeiros, C., Rengel, J., Freites, L., Morales, F., and Himmelman, J. 1998. Growth and survival of the tropical scallop *Lyropecten* (*Nodipecten*) *nodosus* maintained in suspended culture at three depths. Aquaculture, 165: 41–50.

Longhurst, A.R., and Pauly, D. 1987. Ecology of Tropical Oceans. Academic Press Inc., San Diego, CA. 407 p.

Loosanoff, V.L., and Davis, H.C. 1963. Rearing of bivalve mollusks. Adv. Mar. Biol. 1: 1–136.

Lu, Y., and Blake, N.J. 1996. Optimum concentrations of *Isochrysis galbana* for growth of larval and juvenile bay scallops, *Argopecten irradians concentricus* (Say). J. Shellfish Res. 15: 635–643.

Lu, Y., and Blake, N.J. 1997a. Clearance and ingestion rates of *Isochrysis galbana* by larval and juvenile bay scallops, *Argopecten irradians concentricus* (Say). J. Shellfish Res. 16: 47–54.

Lu, Y., and Blake, N.J. 1997b.The culture of the southern bay scallop in Tampa Bay, an urban Florida estuary. Aquaculture International, 5: 439–450.

Lu, Y., Blake, N., and Torres, J.J. 1999. Biochemical utilization during embryogenesis and metamorphosis in the bay scallop, *Argopecten irradians concentricus* (Say). J. Shellfish Res. 18: 425–429.

Luna-Gonzales, A., Maeda-Martinez, A.N., Sainz, J.C., and Ascencio-Valle, F. 2002. Comparative susceptibility of veliger larvae to four bivalve mollusks to a *Vibrio alginolyticus* strain. Dis. Aquat. Org. 49: 221–226.

MacDonald, B.A., and Bourne, N.F. 1989. Growth of the purple-hinge rock scallop, *Crassodoma gigantea* Gray, 1825 under natural conditions and those associated with suspended culture. J. Shellfish Res. 8: 179–186.

MacDonald, B.A., and Thompson, R.J. 1985. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. I. Growth rates of shell and somatic tissue. Mar. Ecol. Progr. Ser. 25: 279–294.

MacDonald, B.A., and Thompson, R.J. 1986. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. III. Physiological ecology, the gametogenic cycle, and scope for growth. Mar. Biol. 93: 37–48.

MacDonald, B.A., and Ward, J.E. 1994. Variation in food quality and particle selectivity in the sea scallop *Placopecten magellanicus* (Mollusca:Bivalvia). Mar. Ecol. Progr. Ser. 108: 251–264.

Maeda-Martinez, A.N., Reynoso-Granados, T., Monsalvo-Spencer, P. Sicard, M.T., Mazón-Suástegui, J.M., Hernández, O., Segovia, E., and Morales, R. 1997. Suspension culture of the catarina scallop *Argopecten ventricosus* (=*circularis*) (Sowerby II, 1942), in Bahia Magdalena, Mexico, at different densities. Aquaculture, 158: 235–246.

Manahan, D.T. 1983. Nutritional implications of dissolved organic material for laboratory culture of pelagic larvae. *In* Culture of Marine Invertebrates: Selected Readings. *Edited by* C.J. Berg. Hutchinson Ross Pub. Co., New York, NY. pp. 179–191.

Mann, K.H., and Lanzier, J.R.N. 1991. Dynamics of Marine Ecosystems. Blackwell Scientific Publications, Cambridge, MA. 466 p.

Manzoni, G.C., Poli, C.R., and Rupp, G.S. 1996. Período reproductivo del pectinido *Nodipecten nodosus* (Mollusca: Bivalvia) en los alrededores de la Isla do Arvoredo (27° 17'S – 48° 22'W) – Santa Catarina – Brasil. *In* Acuicultura en Latinoamérica. IX Congresso Latinoamericano de Acuicultura / 2do Simposio Avances y Perspectivas de la Acuicultura en Chile. *Edited by* A. Silva and G. Merino. Universidad Católica del Norte. Asociación Latinoamericana de Acuicultura, Coquimbo, Chile. pp. 197–201.

Martinez, G., Torres, M., Uribe, E., Diaz, M.A., and Perez, H. 1992. Biochemical composition of broodstock and early juvenile Chilean scallops, *Argopecten purpuratus*, Lamark, held in two different environments. J. Shellfish Res. 11: 307–313.

Matsuura, Y. 1986. Contribuição ao estudo da estrutura oceanográfica da região sudeste entre Cabo Frio (RJ) e Cabo de Santa Marta Grande (SC). Ciencia e Cultura, 38: 1439–1450.

Mercaldo, R.S., and Rhodes, E.W. 1982. Influence of reduced salinity on the Atlantic bay scallop *Argopecten irradians* (Lamark) at various temperatures. J. Shellfish Res. 2: 177–181.

Miller, G.C., Allen, D.A., and Costello, T.J. 1981. Spawning in the calico scallop *Argopecten gibbus* in relation to season and temperature. J. Shellfish Res. 1: 17–21.

Mitta, G., Vandenbulke, F., Hubert, F., Salzet, M., and Roch, P. 2000. Involvement of mytilins in mussel antimicrobial defense. J. Biol. Chem. 275: 12954–12962.

Møhlenberg, F., and Riisgard, H.U. 1978. Efficiency of particle retention in 13 species of suspension feeding bivalves. Ophelia, 17: 239–246.

Navarro, J.M., and Gonzalez, C.M. 1998. Physiological responses of the Chilean scallop *Argopecten purpuratus* to decreasing salinities. Aquaculture, 167: 315–327.

Navarro, J.M., and Winter, J.E. 1982. Ingestion rate, assimilation efficiency, and energy balance in *Mytilus chilensis* in relation to body size and different algal concentrations. Mar. Biol. 67: 255–266.

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

Newell, R.I.E., and Langdon, C.J. 1996. Mechanisms and physiology of larval and adult feeding. *In* The Eastern Oyster *Crassostrea virginica. Edited by* V.S. Kennedy, R.I.E. Newell, and A.F. Eble. Maryland Sea Grant Program. College Park, Maryland. Chapter 5. pp. 185–229.

Nicolas, J.L., Corre, D., Gauthier, G., Robert, R., and Ansquer, D. 1996. Bacterial problems associated with scallop *Pecten maximus* larval culture. Dis. Aquat. Org. 27: 67–76.

Nicolas, L. and Robert, R. 2001. The effect of food supply on metamorphosis and post-larval development in hatchery-reared *Pecten maximus*. Aquaculture, 192: 347–359.

O'Connor, W.A., and Heasman, M.P. 1998. Ontogenic changes in salinity and temperature tolerance in the doughboy scallop, *Mimachlamys asperrima*. J. Shellfish Res. 17: 89-95.

O'Foighil, D., Kingzett, B., O'Foighil, G., and Bourne, N. 1990. Growth and survival of juvenile japanese scallops, *Patinopecten yessoensis,* in nursery culture. J. Shellfish Res. 9: 135–144.

Orensanz, J.M., Parma, A.M., and Iribarne, O.O. 1991. Population dynamics and management of natural scallops. *In* Scallops: Biology, Ecology and Aquaculture. *Edited by* S.E. Shumway. Elsevier, Amsterdam. pp. 625–713.

Palmer, R.E., and Williams, L.G. 1980. Effect of particle concentration on filtration efficiency of the bay scallop *Argopecten irradians* and the oyster *Crassostrea virginica*. Ophelia, 19: 163–174.

Parsons, G.J., and Dadswell, M.J. 1992. Effect of stocking density on growth, production, and survival of the giant scallop, *Placopecten magellanicus*, held in intermediate suspension culture in Passamaquoddy Bay, New Brunswick. Aquaculture, 103: 291–309.

Parsons, G.J., Dadswell, M.J., and Roff, J.C. 1993. Influence of biofilm on settlement of the sea scallop, *Placopecten magellanicus* (Gmelin, 1791), in Passamaquoddy Bay, New Brunswick, Canada. J. Shellfish Res. 12: 279–283.

Parsons, G.J., Robinson, S.M.C., Roff, J.C., and Dadswell, M.J. 1993b. Daily growth rates as indicated by valve ridges in postlarval giant scallop (*Placopecten megellanicus*) (Bivalvia:Pectinidae). Can. J. Fish. Aquat. Sci. 50: 456–464.

Paul, J.D. 1980a. Salinity-temperature relationships in the queen scallop *Chlamys opercularis*. Mar. Biol. 56: 295–300.

Paul, J.D. 1980b. Upper temperature tolerance and the effects of temperature on byssus attachment in the queen scallop, *Chlamys opercularis*. J. Exp. Mar. Biol. Ecol. 46: 41–50.

Peña, J.B. 2001. Taxonomía, morfología, distribuición y hábitat de los pectínidos Iberoamericanos. *In* Los Moluscos Pectínidos de Iberoamérica: Ciencia y Acuicultura. *Edited by* A.N. Maeda-Martinez. Editorial LIMUSA, México. pp. 1–25.

Pilditch, C.A., and Grant, J. 1999. Effects of temperature fluctuations and food supply on the growth and metabolism of juvenile sea scallops (*Placopecten magellanicus*). Mar. Biol. 134: 235–248.

Prieur, D., Mével, G., Nicolas, J.L., Plusquellec, A., and Vignuelle, M. 1990. Interactions between bivalve molluscs and bacteria in the marine environment. Oceanogr. Mar. Biol. Annu. Rev. 28: 277–352.

Rand, G.M., and Petrocelli, S.R. 1985. Fundamentals of aquatic toxicology: methods and applications. Hemisphere Pub. Corp., Washington. 666 p.

Reid, R.G.B., McMahon, R.F., Ó Foighil, D., and Finnigan, R. 1992. Anterior inhalent currents and pedal feeding in bivalves. Veliger, 35: 93–104.

Rhodes, E.W., Goldberg, R., and Widman, J.C. 1981. The role of raceways in mariculture systems for the bay scallop *Argopecten irradians irradians*, and the surf clam *Spisula solidisima*. *In* Nursery culturing of bivalve molluscs. *Edited by* C. Claus, N. De Pauw, and E. Jasper. European Mariculture Society Special Publication No. 7. Belgium. pp. 227–251.

Riisgard, H.U. 1988. Feeding rates in hard clam (*Mercenaria mercenaria*) veliger larvae as a function of algal (*Isochrysis galbana*) concentration. J. Shellfish Res. 7: 377–380.

Riisgard, H.U. 1991. Filtration rate and growth in the blue mussel, *Mytilus edulis* Linnaeus, 1758: dependence on algal concentration. J. Shellfish Res. 10: 29–35.

Rios, E.C. 1994. Seashells from Brazil. Fundação Cidade do Rio Grande; Museu Oceanográfico, Rio Grande, Brasil. 328 p.

Riquelme, C., Hayashida, G., Toranzo, A.E., and Chavez, P. 1995. Pathogenicity studies on a *Vibrio anguillarum*-related (VAR) strain, causing an epizootic in *Argopecten purpuratus* larvae cultured in Chile. Dis. Aquat. Org. 22: 135–141.

Rodriguez, J.L., Sedano, F.J., Garcia-Martin, L.O., Perez-Camacho, A., and Sanchez, J.L. 1990. Energy metabolism of newly settled *Ostrea edulis* spat during metamorphosis. Mar. Biol. 106: 109–111.

Román, G., Campos, M.J., Acosta, C.P., and Cano, J. 1999. Growth of the queen scallop (*Aequipecten opercularis*) in suspended culture: influence of density and depth. Aquaculture, 178: 43–62.

Rose, R.A., and Dix, T.G. 1984. Larval and juvenile development of the doughboy scallop, *Chlamys (Chlamys) asperrimus* (Lamark) (Mollusca:Pectinidae). Aust. J. Mar. Freshwater Res. 35: 315–323.

Rupp, G.S. 1994. Obtenção de reprodutores, indução a desova, cultivo larval e pós larval de *Nodipecten nodosus* (Linnaeus, 1758) (Mollusca: Bivalvia). M.Sc. Thesis. Universidade Federal Santa Catarina Florianópolis (UFSC), Departamento de Aquicultura. Florianópolis, SC, Brazil. 132 p.

Rupp, G.S. 1997. Desenvolvimento de tecnologia de produção de sementes de *Nodipecten nodosus* (Linnaeus,1758) (BIVALVIA:PECTINIDAE). Program RHAE/PIBIO/UFSC. 71 p. (Final Report CNPq).

Rupp, G.S., de Bem, M.M., and Poli, C.R. 2000a. Larviculturas experimentais de *Nodipecten nodosus* realizadas no LCMM entre 1995 e 1996. Abstracts. Aquaculture Brazil – 2000 / Brazilian Symposium of Aquaculture. Florianópolis, SC. November 2000 - CD ROM.

Rupp, G.S., de Bem, M.M., and Poli, C.R. 2000b. Resultados de assentamentos e produções experimentais de sementes de *Nodipecten nodosus* (Linnaeus 1758) (Bivalvia : Pectinidae) realizadas no LCMM em 1996. Abstracts. Aquaculture Brazil – 2000 / Brazilian Symposium of Aquaculture. Florianópolis, SC. November 2000 – CD ROM.

Rupp, G.S., and Poli, C.R. 1994. Spat production of the sea scallop *Nodipecten nodosus* (Linnaeus, 1758), in the hatchery: initial studies in Brazil. Can. Tech. Rep. Fish. Aquat. Sci. No. 1994 (2): 91–96.

Rupp, G.S., Vélez, A., Bem, M.M., and Poli, C.R. 1997. Effect of temperature on conditioning and spawning of the tropical scallop *Nodipecten nodosus* (Linnaeus, 1758). Abstracts. 11th International Pectinid Workshop, 10–15 April 1997, Centro Interdisciplinario de Ciencias Marinas, La Paz, BCS, Mexico. pp. 132–133.

Sainz, J.C., Maeda-Martinez, A.N., and Ascencio, F. 1999. Experimental vibriosis induction with *Vibrio alginolyticus* of larvae of the catarina scallop (*Argopecten ventricosus* = *circularis*) (Sowerby II, 1842). Microb. Ecol. 35: 188–192.

Sastry, A.N. 1965. The development and external morphology of pelagic larval and post-larval stages of the bay scallop, *Aequipecten irradians concentricus* Say, reared in the laboratory. Bull. Mar. Sci. 15: 417–435.

Sastry, A.N. 1979. Pelecypoda (excluding Ostreidae). *In* Reproduction of Marine Invertebrates. *Edited by* A.C. Giese and J.S. Pearse. Academic Press, New York. pp. 113–292.

Shumway, S.E. 1977. Effect of salinity fluctuation on the osmotic pressure and Na^{+}, Ca^{2+} and Mg^{2+} ion concentrations in the hemolymph of bivalve molluscs. Marine Biology, 41: 153–177.

Shumway, S.E. 1996. Natural environmental factors. *In* The Eastern Oyster *Crassostrea virginica. Edited by* V.S. Kennedy, R.I.E. Newell, and A.F. Eble. Maryland Sea Grant Program. College Park, Maryland. Chapter 13. pp. 467–513.

Shumway, S.E., Barter, J., and Stahlnecker, J. 1988. Seasonal changes in oxygen consumption of the giant scallop, *Placopecten magellanicus* (Gmelin). J. Shellfish Res. 7: 77–82.

Shumway, S.E., Cucci, T.L., Lesser, M.P., Bourne, N., and Bunting, B. 1997. Particle clearance and selection in three species of juvenile scallops. Aquaculture International, 5: 89–99.

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

Sicard, M.T., Maeda-Martinez, A.N., Ormart, P., Granados, T., and Carvalho, L. 1999. Optimum temperature for growth in the catarina scallop (*Argopecten ventricosus-circularis,* Sowerby II, 1842). J. Shellfish Res. 18: 385–392.

Signoret-Brailovsky, G., Maeda-Martinez, A., 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. Shellfish Res. 15: 623–626.

Smith, J.T. 1991. Cenozoic giant pectinids from California and the Tertiary Caribbean Province: *Lyropecten*, "*Macrochlamis*," *Vertipecten*, and *Nodipecten* species. U.S. Geological Survey Professional Paper No. 1391: 136 p.

Soares, F.S. 1983. Estudo do fitoplancton de águas costeiras e oceânicas da região de Cabo Frio - RJ (23° 31'S; 41° 52'W) até Cabo de Santa Marta Grande – SC (28° 43'S; 47° 57'W). M.Sc. Thesis. Universidade de São Paulo. Instituto Oceanográfico. São Paulo, SP, Brazil. 118 p.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry: The Principles and Practices of Statistics in Biological Research. W. H. Freeman and Co., New York, USA. 887 p.

Somero, G.N., and Hochachka, P.W. 1976. Biochemical adaptations to temperature. *In* Adaptation to Environment. *Edited by* R.C. Newell. Butterworths, London. pp. 125–190.

Sprung, M. 1984. Physiological energetics of mussel larvae (*Mytilus edulis*). II. Food uptake. Mar. Ecol. Progr. Ser. 17: 295–305.

Strand, O., Solberg, P., Andersen, K., and Magnesen, T. 1993. Salinity tolerances of juvenile scallops (*Pecten maximus* L.) at low temperature. Aquaculture, 115: 169–179.

Sunyé, P.S., and Servain, J. 1998. Effects of seasonal variations in meteorology and oceanography on the Brazilian sardine fishery. Fish. Oceanogr. 7: 89–100.

Suplicy, F.M., Schmitt, J.F., Moltschaniwskyj, N.A., and Ferreira, J.F. 2003. Modelling of filter-feeding behaviour in the brown mussel *Perna perna* (L.), exposed to natural variations of seston availability in Santa Catarina, Brazil. J. Shellfish Res. (Submitted)

Thompson, F.L., Li, Y., Gomez-Gil, B., Thompson, C.C., Hoste, B., Vandemeulebroecke, K., Rupp, G.S., Pereira, A., De Bem, M.M., Sorgeloos P., and Swings, J. 2003. *Vibrio neptunius* sp. nov., *Vibrio brasiliensis* sp. nov. and *Vibrio xuii* sp. nov., isolated from the marine aquaculture environment (bivalves, fish, rotifers and shrimps). Int. J. Syst. Evol. Microbiol. 53: 245–252.

Thompson, R.J. 1984. The reproductive cycle and physiological ecology of the mussel *Mytilus edulis* in a subarctic, non-estuarine environment. Mar. Biol. 79: 277–288.

Thompson, R.J., and Bayne, B.L. 1974. Some relationships between growth, metabolism and food in the mussel *Mytilus edulis*. Mar. Biol. 27: 317–326.

Thompson R.J., and MacDonald, B.A. 1991. Physiological integrations and energy partitioning. *In* Scallops: Biology, Ecology and Aquaculture. *Edited by* S.E. Shumway. Elsevier, Amsterdam. pp. 347–376.

Thorarinsdóttir, G.G. 1994. The Iceland scallop, *Chlamys islandica* (O.F. Müller), in Breidafjördur, west Iceland. III. Growth in suspended culture. Aquaculture, 120: 295–303.

Trittar, S., Prieur, D., and Weiner, R. 1992. Effects of bacterial films on the settlement of the oysters *Crassostrea gigas* (Thunberg, 1793) and *Ostrea edulis*, Linneaus, 1750 and the scallop *Pecten maximus* (Linnaeus 1758). J. Shellfish Res. 11: 325–330.

United States Environmental Protection Agency (USEPA). 1997. Method 445.0: *In vitro* determination of chlorophyll-*a* and pheophytin-*a* in marine and freshwater phytoplankton by fluorescence. National Exposure Research Laboratory, Cincinnati. 14 p.

Uriarte, I., Rupp, G., and Abarca, A. 2001. Producción de juveniles de pectínidos Iberoamericanos bajo condiciones controladas. *In* Los Moluscos Pectínidos de Iberoamérica: Ciencia y Acuicultura. *Edited by* A. N. Maeda-Martinez. Editorial LIMUSA, México. pp. 147–171.

Vahl, O. 1978. Seasonal changes in oxygen consumption of the Iceland scallop (*Chlamys islandica*) (O. F. Müller) from 70° N. Ophelia, 17: 143–154.

Vahl, O. 1980. Seasonal variations in seston and in the growth rate of the Iceland scallop, *Chlamys islandica* (O.F. Müller) from Balsfjord, 70 °N. J. Exp. Mar. Biol. Ecol. 48: 195–204.Vargas-Albores, F., and Barraco, M.A. 2001. Mecanismos de defensa de los moluscos bivalvos, con énfasis en pectínidos. *In* Los Moluscos Pectínidos de Iberoamérica: Ciencia y Acuicultura. *Edited by* A.N. Maeda-Martinez. Editorial LIMUSA, México. pp. 127–146.

Veniot, A., Bricelj, M.V., and Beninger, P.G. 2003. Ontogenetic changes in gill morphology and potential significance for food acquisition in the scallop *Placopecten megellanicus*. Mar. Biol. 142: 123–131.

Ventilla, R.F. 1982. Scallop industry in Japan. Adv. Mar. Biol. 20: 309–382.

Videla, J.A., Chaparro, O.R., Thompson, R.J., and Concha, I.I. 1998. Role of biochemical energy reserves in the metamorphosis and early juvenile development of the oyster *Ostrea chilensis*. Mar. Biol. 132: 635–640.

Wallace, J.C., and Reinsnes, T.G. 1985. The significance of various environmental parameters for growth of the iceland scallop *Chlamys islandica* (Pectinidae), in hanging culture. Aquaculture, 44: 229–242.

Walne, P.R. 1979. Culture of Bivalve Molluscs. Fishing News Books. England. 189 p.

Webb, K.L., and Chu, F.L. 1983. Phytoplankton as food source for bivalve larvae. *In* Biochemical and physiological approaches to shellfish nutrition. *Edited by* G.D. Pruder, C.J. Langdon and D.E. Conklin. World Mariculture Soc. Spec. Publ. No. 2, Louisiana State University, Louisiana. pp. 272–291.

Whyte, J.N., Bourne, N., Ginther, N.G., and Hodgson, C.A. 1992. Compositional changes in the larva to juvenile development of the scallop *Crassodoma gigantea* (Gray). J. Exp. Mar. Biol. Ecol. 163: 13–29.

Widdows, J. 1976. Physiological adaptation of *Mytilus edulisi* to cyclic temperatures. J. Comp. Physiol. 105: 115–128.

Widdows, J., Fieth, P., and Worrall, C.M. 1979. Relationship between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Mar. Biol. 50: 195–207.

Widman, J.C., and Rhodes, E.W. 1991. Nursery culture of the bay scallop, *Argopecten irradians irradians*, in suspended mesh net. Aquaculture, 99: 257–267.

Yentsch, C.S., and Menzel, D.W. 1963. A method for determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Res. 10: 221–231.

Yukihira, H., Klump, D.W., and Lucas, J.S. 1998. Comparative effects of microalgal species and food concentration on suspension feeding and energy budgets of the pearl oysters *Pinctada margaritifera* and *P. maxima*. Mar. Ecol. Progr. Ser. 171: 71–84.