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**Canada**

**GROWTH RATES AND RECOVERY OF HATCHERY-REARED SEA SCALLOP,  
*Placopecten magellanicus* (Gmelin 1791), SPAT UNDER A VARIETY OF NURSERY  
CONDITIONS.**

by

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## Abstract

Nursery culture of the sea scallop, *Placopecten magellanicus*, is an important transitional phase in hatchery-rearing practices. The Belleoram Sea Scallop Hatchery utilizes the farm-based mesh equipment as the nursery. The purpose of this study was to examine growth rates and recovery of scallops in the farm-based nursery. These factors were monitored with respect to time of year, depth, gear mesh size and type, stocking density, and time of deployment. Remote setting, hatchery flow-through options and ammonia toxicity were also studied for nursery-sized scallops.

Growth rates of nursery-sized scallops dropped over the winter followed by an increase in the spring. Recovery of scallops (number of live scallops still in equipment after mortality and loss through mesh), however, decreased in the autumn, and leveled off over the winter, which was attributed to handling practices, including the need for acclimation. Growth rates and recovery were highest in the scallops deployed in the largest mesh size which may have been due to better food availability as well as better acclimation by larger scallops. Growth rates were higher in 3.0 mm pearl nets than 3.0 mm collector bags, however, they exhibited the same recovery. The difference in growth may be explained by gear design. No differences in food quantity or temperature existed between 5 and 10 m, however, growth rates were greater at 5 m where fouling was always higher than 10 m. Recovery was similar at both depths. Fouling-induced flow reduction (thus better exploitation of food) or food quality may have influenced growth rates at 5 m. No density dependent effects were noted between 2600 and 5200 spat/bag. Deployment of remote set-or nursery-sized scallops in early to late summer allowed them to have

superior growth rates and recovery than deploying during the autumn when temperature and food quantity and quality have dropped. Practicing temperature acclimation and feeding scallops a diet high in essential fatty acids may improve growth and recovery during deployment to sub-optimal farm-based nursery conditions. Scallops held on mesh in flow-through tanks exhibited higher growth than scallops on solid trays. Low growth rates overall in flow-through tanks, however, suggests that flow-through may not be useful for enhancing growth of scallops in autumn sea water temperatures. Summer flow-through trials should be investigated. Ammonia toxicity bioassays suggest that scallops have an increasing tolerance to ammonia with size and that feeding is influenced by the presence of low concentrations of ammonia.

With this knowledge of important influences of the farm-based nursery, the operators of Belleoram Sea Scallop Hatchery should be able to develop new protocol for scallop nursery practices and thus improve the growth and recovery of their product.

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I thank Dr. Jay Parsons for allowing me to study under his supervision. I am honored to have worked with someone as educated and well-versed in scallop aquaculture as Jay. Many thanks for guidance in experimental set-ups, sample analyses, statistical analyses and writing this huge report on the final frontier of sea scallop aquaculture- the nursery stage. Thanks, Jay, for encouraging me to prevail when I became overwhelmed. Also great thanks to Dr. Pat Dabinett and Cyr Couturier, my committee members, for their encouraging support.

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## **Chapter One:**

### **Introduction to Nursery Culture of the Sea Scallop, *Placopecten magellanicus***

## 1.1 Introduction

The sea, or giant, scallop, *Placopecten magellanicus*, has been a candidate for aquaculture in Atlantic Canada for twenty years (Naidu 1991). As with any aquaculture endeavor, the industry has been dependent on a supply of seed. In some areas of Atlantic Canada the supply through natural collection is sufficient (Couturier et al. 1995). In Newfoundland, however, because there is low natural seed supply, there is a need for a hatchery. A commercial hatchery in Belleoram, NF was established to supply the scallop spat demand of the industry. Unfortunately, growth and survival have been limited in hatchery-reared spat due to inadequate nursery culture techniques.

Nursery culture is the rearing of post-larval scallops to a size that is easily handled by growers. Nursery-culture strategies must take into account the size of the scallops, the cost and maintenance of equipment, labour involved in operating the system, the cost of operating the system, and the quality of the environment within the culture system as well as the environment to which the system is exposed. Scallops have been reared in land-based nursery ponds, upwellers, downwellers, and raceways, and ocean- or farm-based mesh equipment including pearl nets and collector bags (Claus 1981; Bourne and Hogdson 1991; Anderson and Naus 1993).

Reports on the utilization and success of nursery strategies for *P. magellanicus* are scant (Young-Lai 1989; Neima and Kenchington 1997). The nursery strategy at the Belleoram Sea Scallop Hatchery (BSSH) was a combined hatchery-rearing on trays in tanks to 3 mm in shell height followed by transfer to collector bags and pearl nets to overwinter spat at a farm-based nursery for scallops >3.0 mm. This protocol was

inadequate because scallops were transferred late in the autumn or early winter which resulted in slow growth and high mortality.

To get reliable numbers of spat to a size that can be handled by growers, growth and survival of nursery stage scallops had to be improved. This involved an investigation into the influence of the spatial and temporal parameters involved in the development of commercially acceptable nursery culture strategies.

## **1.2 Sea Scallop Biology and Fishery**

The sea scallop is a benthic bivalve that is found from Cape Hatteras, North Carolina, to the Strait of Belle Isle, at depths of >50 m and 2-100 m, respectively (Appendix 1.1; Couturier et al. 1995). Phytoplankton, which is its main source of food, is selected from all potential food particles in the water column (Shumway et al. 1987; Beninger and LePennec 1991). The quantity and quality of food of an environment determines growth rates of sea scallops (MacDonald and Thompson 1985a; MacDonald and Ward 1994). Depending on environmental conditions, sea scallops fully mature in 3 to 5 years or >80 mm in shell height, after which they are marketable (Parsons et al. 1992; Davidson and Poussart 1998). Sea scallop biology has been explored in great detail and is discussed by Naidu (1991), Black et al. (1993), and Couturier et al. (1995).

The natural life history of the sea scallop is well-known. The sea scallop is a highly fecund, dioecious species (Langton et al. 1987; Barber et al. 1988; Couturier and Newkirk 1991). Synchronized spawning of males and females is cued by temperature, salinity, dissolved oxygen, pH, presence of gametes, tidal events, phytoplankton, water



flow, and mechanical shocks (Parsons et al. 1992; Davidson et al. 1993; Couturier et al. 1995). Spawning events occur from August to October although spawning also occurs in June in Nova Scotian and western Newfoundland stocks (Dupaul et al. 1989; Dadswell and Parsons 1992a,b; Davidson et al. 1993). Fertilization success, which depends on proximity of gametes in the water column, results in the development of veliger larvae (Orensanz et al. 1991; Couturier et al. 1995). These larvae remain in the water column for 35 days when settlement, metamorphosis and byssal attachment to substrates can occur (Figure 1.1; Culliney 1974; Couturier et al. 1995). Post-settled scallops, or spat, prefer to attach to filamentous substrates although attachment generally lasts until they reach 5 mm shell height (Naidu et al. 1981; Black et al. 1993; Parsons et al. 1996). Sea scallops are able to swim by water propulsion through the valves, which allows them to move freely to avoid predation or unfavourable environmental condition (Dadswell and Weihs 1990). Scallops less than 15 mm shell height are, however, inefficient swimmers (Manuel and Dadswell 1991). As they get older (>80 mm shell height) they swim less (G. J. Parsons, pers. comm.). Natural mortality, as high as 80%, may be due to rapid temperature changes, low oxygen levels, disease and predation (Couturier et al. 1995).

Scallops are the most important commercial molluscan species in Canada with a 1996 Atlantic Canada production of over 59 000 metric tonnes of live weight (Department of Fisheries and Oceans Statistics Board 1999). High scallop production (>90000 mt in Atlantic Canada) made the sea scallop the number one scallop in world production from 1976 to 1987, contributing 30% of the annual world production of scallop species (Naidu 1991). In some years, *P. magellanicus* contribution reached 50%, however, percentages have declined recently due to increased world-wide production of

other scallop species through both fisheries and aquaculture (Naidu 1991). Sea scallop catches also exhibit cyclical variations every 9, 18 and 21 years as a result of hydrographic, tidal or climatological conditions (Black et al. 1993). The main market for Canadian scallop landings is the United States where the value is between \$10-20/kg for adductor muscle meats depending on supply and demand (Couturier et al. 1995).

The sea scallop is an ideal candidate for artificial rearing. The variability in catch from natural stocks, the high value of the product, the well-known life history, the feeding, temperature and salinity requirements, and fast growth to market size are all features that have lead to the development of culture practices of the sea scallop.

### **1.3 Sea Scallop Aquaculture**

Culturing scallops in suspended cages or nets of various mesh sizes substantially reduces loss of scallops to bottom predation, byssal detachment and swimming. Wild collected seed, or spat, are transferred to intermediate culture in pearl nets or trays and then final growout in pearl nets, lanterns nets, ear hanging, trays, etc., depending on the desired final size. A reliable spat supply plays an important role in making sea scallop culture a viable industry.

Attempts at wild collection indicated the unreliability of a wild-collected seed supply in Newfoundland (Dabinett and Couturier 1994). Spat were first collected for scallop culture in 1968 by Memorial University (Couturier et al. 1995). Despite poor collection from wild sources, interest in culture of the sea scallop was perpetuated by fluctuations and depletion of natural fisheries catch. Scallop culture has persisted despite

limited seed supply (Figure 1.2). Other areas in Atlantic Canada were found to have a reliable seed supply (Dadswell 1989), however, not enough to supply the demand.

The well known early life history of sea scallops gave the species potential for investigating hatchery culture at Memorial University commencing in the 1970s. Université du Québec à Rimouski and Dalhousie University in Nova Scotia also showed interest. By 1995, Nova Scotia and Newfoundland had commercial hatcheries. In the autumn of 1996, the Nova Scotian hatchery ended production while at St. Augustine, Quebec, PecNord established a private scallop hatchery. The hatchery in Belleoram, Newfoundland, has initiated research of nursery culture techniques with this study.

#### **1.4 Scallop Nursery Culture Strategies**

Various definitions exist for nursery culture of bivalves, the most general being the handling of post-larvae until they reach juvenile or intermediate growout size (Mercer 1981). Ventilla (1982) defines it as the stage between 1 and 5 cm shell height for naturally collected spat of the Pacific scallop *Patinopecten yessoensis* while Mercer (1981) divides nursery culture into early stage (post-larvae to 2-3 mm shell height), intermediate stage (2-3 mm to 10-15 mm shell height), and late stage (10-15 mm to 40 mm shell height or 1/3-1/2 growout size). Bourne and Hodgson (1991) divide nursery culture of hatchery-reared *Patinopecten yessoensis* and *Crassadoma gigantea* into Primary stage (Phase I-metamorphosis to 1 mm shell height, and Phase II- 1 mm to 10 mm shell height) and Secondary stage (10 mm to 40-50 mm shell height or growout size).

For the purposes of this study, the nursery stage of sea scallops which is

undefined, but precedes intermediate culture (initial size of 5-10 mm), will be divided into Phase I (metamorphosis to 1.4 mm shell height) and Phase II (1.4 to 7 mm shell height). This is on the basis of spat >1.4 mm shell height being the minimum size that can be held in 1.2 mm (on the diagonal) mesh equipment available at the Belleoram hatchery for farm-based deployment. Spat >7 mm shell height are large enough for growers to hold in 4.5 mm mesh equipment thus will be considered to be the intermediate culture size.

Scallops have been reared using a variety of nursery strategies. *Patinopecten yessoensis* and *C. gigantea* have been reared in tanks of filtered and unfiltered seawater enhanced with cultured food, farm-based mesh cages held on long lines, upwellers or downwellers which consist of scallops set on mesh-lined containers in which water either flows up or down through mesh, and raceways or long troughs in which scallops are set on bottom as water flows over them in one direction (Bourne and Hodgson 1991). On-shore ponds with enriched natural production have been used for *Chlamys varia* and *Pecten maximus* (Mercer 1981; Rodhouse et al. 1981; Andersen and Naas 1993). *Argopecten irradians* has been reared in raceways, ocean pens or mesh nursery cages, upwellers, and tanks with cultured phytoplankton (Rhodes et al. 1981; Karney 1991).

The purpose of the nursery is to minimize impact of transferring scallops from the hatchery to the grow-out environment (Bourne and Hodgson 1991). Direct placement of hatchery-reared scallops into growout results in reduced growth and survival rates. However, a transitional phase, or nursery, is required where large numbers of scallops are stocked in a protected environment so they can acclimate to growout environment conditions with minimal mortality and maximal growth (Claus 1981). Like any stage of

culture, the nursery growth should take minimal time to reach intermediate size (Dadswell and Parsons 1991). The nursery strategy thus must address the effects of internal and external factors of the nursery culture environment on scallop performance, usually growth and survival. Some factors to consider are nursery location, and the possibility of environmental disturbances, as well as specific temporal and spatial environmental concerns including seasonal variations in food, temperature, fouling and predation, depth, gear type and stocking density.

When choosing a nursery strategy the most important factor to consider is food availability (Claus et al. 1983). Large scale production of microalgae for bivalve seed production is regarded as the main constraint in hatchery production due to light and water heating limitations as well as being one of the most expensive areas of production at 30% of total operational costs (Coutteau and Sorgeloos 1991). In addition, growth and survival of spat are limited in nursery strategies that are extensions of hatchery conditions because food quality (ie. algal species, size, essential fatty acid component) is inadequate for spat growth and development and thus cannot be overcome by feeding excessive amounts of larval food (Claus 1981; Young-Lai and Aiken 1986; Whyte et al. 1992). Ó'Foighil et al. (1990) found that a diet of cultured microalgae supplemented with natural phytoplankton resulted in better scallop growth and survival than feeding solely on cultured algae. It is not unexpected then that the trend in nursery culture is to feed spat partially or exclusively on natural phytoplankton due to lower costs and better growth and survival (Coutteau and Sorgeloos 1991).

Few reports cite strategies used for sea scallop nursery culture. An experimental sea scallop hatchery at Île-de-la-Madeleine used mesh-lined baskets in flow-through tanks

with cultured phytoplankton for nursery culture (Young-Lai 1989). Fisheries Resource Development Limited (FRDL) set sea scallop larvae on Chinese hat collectors which were transferred to the farm-based nursery after 10-14 days (Neima and Kenchington 1997). The BSSH sets spat on flat trays in static water, aerated tanks with 100% water change every three days and also has focussed some efforts on downwelling and raceway systems. Scallops are held in these systems up to 3.0 mm shell height and are then transferred to the ocean in collector bags or pearl nets until the following summer.

### **1.5 Sea Scallop Nursery Culture: Belleoram Sea Scallop Hatchery Experience**

The earliest scallop nursery practices used the natural environment for growth of naturally caught spat held in suspension. This dates back to 1935 when the Japanese used cedar twigs for natural seed settlement (Ventilla 1982). As technology improved and equipment was modified, fine mesh collector bags (rectangular 0.4 m x 0.8 m nylon mesh bag with draw string) filled with 500 g of gillnet, commonly called onion bag collectors, were utilized. Many scallop hatcheries have adapted similar nursery strategies for hatchery-reared spat. Some hatcheries choose to set spat directly on filamentous substrates (Netron®, gillnet, Kinran®, Chinese Hats, Vexar®) which are then transferred to growout in fine mesh equipment while other hatcheries grow spat in tanks to a size where they can be placed directly in the fine mesh and transferred to growout without falling through (Bourne and Hogdson 1991; Karney 1991; P. Dabinett, pers. comm.; R. Garrison, pers. comm.).

Of the few reports available on the success of nursery strategies for scallops, the

farm-based nursery offers the best growth and survival rates (Bourne and Hogdson 1991). Initially, the operators of the Belleoram Sea Scallop Hatchery (BSSH) chose to combine an extended hatchery-rearing phase up to 3 mm shell height in tanks with transfer to collector bags in bread trays held on long-lines at a farm-based nursery for culture of *P. magellanicus*. This strategy was chosen due to the success of pilot scale hatchery trials (Dabinett 1989), proximity and access to a farm-based nursery, low transfer costs, low operation costs, and limited handling.

High mortality and poor growth during the first production season (1995) at BSSH emphasized the need for studying nursery culture strategies for *Placopecten magellanicus*. With several options available for nursery culture, and costs to consider, the operators decided to refine the farm-based strategy as well as investigate other low cost options.

This study has three objectives. The primary objective of this study was to improve growth and survival of nursery-sized scallops in the existing farm-based nursery. This was achieved by determining the optimal initial size of scallops, stocking density, equipment type and mesh size, and depth for deployment to the farm-based nursery. The second objective was to determine the window of opportunity for deployment on the farm-based nursery through evaluating the effects of seasonal changes in environmental conditions on growth and survival and to consider the possibilities of expanding the window by remote set options with scallop larvae. The final objective was to determine the potential growth rates and recovery of scallops in a hatchery-based flow-through nursery system in which scallop diet was a combination of natural and cultured phytoplankton. A preliminary objective for the flow-through was to determine the

ammonia toxicity of spat with respect to holding in static or non-flow-through systems. The studies that encompassed these objectives intend to address many of the questions regarding farm-based nursery culture and possibilities for hatchery-based nursery options of sea scallops.



## **Chapter Two:**

### **Influence of Initial Size, Depth, Gear Type and Stocking Density on the Growth Rates and Recovery of Hatchery-reared Sea Scallop, *Placopecten magellanicus*, on a Farm-based Nursery**

## **2.1 Introduction**

Growth rates and survival determine the feasibility of species for aquaculture including sea scallops. Physiological activity, which subsequently determines growth and survival, is affected by biotic and abiotic factors in the culture environment and by husbandry decisions. The uncontrollable natural factors that exhibit varying conditions of environmental quality that affect the growth rates and survival of scallops in culture include water temperature, food availability, salinity, and fouling. Other factors that the grower controls include initial size, depth of culture, culture method, equipment mesh size, gear type and stocking density. Variations of these factors can be evaluated such that the more enhanced growth and survival are, compared to natural conditions, the more suitable the environment and husbandry protocols for culture. Environmental quality and husbandry protocols are thus defined by their suitability for enhancing the growth and survival of the cultured organism.

Environmental quality is known to change seasonally and cannot be controlled by the grower (Cropp and Hortle 1992; Parsons and Dadswell 1992; Emerson et al. 1994; Thorarinsdóttir 1994; Kleinman et al. 1996). Food quality and quantity and temperatures decline in the winter in Newfoundland (Dabinett and Clemens 1994; Navarro and Thompson 1995; Parrish et al. 1995; Penney and McKenzie 1996; Dabinett and Clemens 1997). Fouling, which is seasonal, affects growth and survival of scallops by decreasing food and oxygen concentrations due to decreased water flow through equipment mesh, as well as increasing competition for food (Duggan 1973; Leighton 1979; Monical 1980; Mook 1981; Wallace and Reinsnes 1985; Wildish et al. 1988; MacDonald and Bourne

1989; Bourne and Hodgson 1991; Karney, 1991; Thorarinsdóttir 1991; Cropp and Hortle 1992; Hunter 1992; Côté et al. 1993; Penney 1993; Claereboudt et al. 1994a; Lodeiros and Himmelman 1994). Other environmental parameters, including salinity, dissolved oxygen, turbidity, and light intensity vary seasonally and thus may influence the quality of the aquaculture site for growing shellfish (Dabinett and Clemens 1994; Dabinett and Clemens 1997). The effects of seasonality can be studied by monitoring changes in growth and survival of scallops exposed to the seasonal conditions.

Sea scallops grown in culture situations are not depth limited, however, increasing depth generally has lower environmental quality, although this may be site specific. Temperature and food availability diminish with depth and are limiting factors for growth and survival of scallops (Kirby-Smith and Barber 1974; Leighton 1979; Monical 1980; Vahl 1980; Richardson et al. 1982; Rodhouse and Gaffney 1984; Wallace and Reinsnes 1984; MacDonald and Thompson 1985a; Wallace and Reinsnes 1985; Hortle and Cropp 1987; Dadswell and Parsons 1991; Côté et al. 1993; Lodeiros and Himmelmann 1995). Scallop growth rates are higher in suspension than on the bottom (Duggan 1973; Leighton 1979; Monical 1980; Vahl 1980; Wallace and Reinsnes 1985; MacDonald 1986; MacDonald and Bourne 1989; Lodeiros and Himmelman 1994; Thorarinsdóttir 1994). Fouling accumulation declines with depth which may be more conducive to culture practices (Duggan 1973; Leighton 1979; Monical 1980; Wallace and Reinsnes 1985; MacDonald and Bourne 1989; Côté et al. 1993; Claereboudt et al. 1994a).

Other factors that affect growth and survival can be controlled by the grower. The grower chooses initial size and stocking density as well as gear type and mesh size. These factors can also be studied for effects on growth rates and survival of sea scallop

spat.

Rates of shell growth decline with increasing size in most bivalves (Seed 1976; Parsons et al. 1993; C. R. Newell, pers. comm.). Penney and Mills (1996) found that while large juvenile scallops maintain higher shell height after one year, smaller juveniles had higher growth rates and were able to catch up after two years. Within a smaller size range, growth rates increased with size such that scallops at 300  $\mu\text{m}$  shell height had a growth rate of 30  $\mu\text{m}/\text{d}$  (10%/d) while scallops at 1500  $\mu\text{m}$  shell height had growth rates of 60  $\mu\text{m}/\text{d}$  (4%/d; Parsons et al. 1993). For nursery culture, it would be useful to know what the effect of initial size is on growth rates and survival of sea scallop spat.

Stocking density determines the cost and time of production as a result of its effect on growth rates and survival. In intermediate culture, growth rates and survival decline with increasing density (Duggan 1973; Ventilla 1982; Dadswell and Parsons 1991; Parsons and Dadswell 1991; Dadswell and Parsons 1992a,b; Côté et al. 1993). Survival was not impacted in some studies even when densities were previously considered too high (Parsons and Dadswell 1991; Dadswell and Parsons 1992a,b; Penney 1995). Determining optimal stocking densities is necessary to develop nursery culture protocols.

Culture technique modification and use of a variety of equipment types have been studied to find ways of maximizing growth rates thus reducing cost of production (Wildish et al. 1988; Parsons and Dadswell 1994; Penney and Mills 1996; Couturier et al. 1997). The method of culture determines the growth rates of scallops in suspension. Studies in intermediate growout show that gear type influences growth rates due to different exposure to fouling, flow and handling (Parsons and Dadswell 1994; Penney

1995). In nursery culture, the time to reach the desired size should be minimized and thus gear type providing maximum growth rates are the preferred choice of culture method.

Mesh size affects growth rates by altering food delivery to the scallops. Mesh reduces water flow due to the interference with the mesh material, hence the smaller the mesh size, which has the highest amount of material per unit area, causes the greatest reduction of water flow (Walker et al. 1991; Cole et al. 1996; Devaraj and Parsons 1997; Brake and Parsons 1998). Fouling reduces flow even more by blocking the mesh openings when the equipment is suspended in the ocean (Devaraj and Parsons 1997). In a farm-based nursery strategy, mesh size is important as water flow and fouling can not be controlled very well by the grower until the culture environment is better understood.

The present study was initiated to evaluate farm-based nursery strategies for *P. magellanicus*. The objective of this study was to determine optimal growth and survival rates of scallops in a farm-based nursery on the basis of initial size, stocking density, deployment depths, and gear type. Environmental water characteristics were monitored through the studies. The specific hypotheses tested were:

(1) If growth rates of nursery-sized sea scallops cultured in suspension are influenced by environmental parameters (especially food density and temperature) then it is expected that growth rates will be low in the winter when quality and quantity is lowest.

(2) If survival of nursery-sized sea scallops held in suspension culture is influenced by a sudden decrease in environmental quality from hatchery to nursery environments then it is expected that survival will decline initially then stabilize thereafter.

(3) If growth rates of nursery-sized sea scallops held in suspension culture increase with increasing shell height and growth rates and survival depend on flow of water through equipment for food replenishment then it is expected that growth rates and survival will be highest in the largest initial size class which is held in the largest mesh equipment.

(4) If growth rates and survival of nursery-sized sea scallops held in suspension culture are influenced by temperature and food density, then the highest growth rates and survival will be at 10 m depth where slightly lower temperatures cause less fouling which will allow higher water flow thus higher exploitable food density due to the replenishment of food.

(5) If growth rates and survival of nursery-sized sea scallops are influenced by water flow, then the growth and survival will differ in pearl nets and collector bags where structural designs (mesh size and shape) of each, thus water flow through them, is different.

(6) If scallops are stocked at a density where food and space are limiting then growth and survival of scallops at the higher density are expected to be lower than at the low density.

## **2.2 Materials and Methods**

Three studies were initiated to examine factors that may influence growth rates and survival of sea scallops in a farm-based nursery. The first study, which began in October 1996, examined initial shell height and mesh size with depth and seasonality. The other two studies, a gear type study and density study, began in October 1997. All three studies were conducted on the same study site.

### **2.2.1 Study Site**

The experiments were carried out at Shell Fresh Farms Ltd., Pool's Cove, Newfoundland, at the head of Fortune Bay in North Bay ( $47^{\circ}42' \text{ N}$ ,  $55^{\circ} 26' \text{ W}$ ; Figure 2.1). Bottom substrate consisted of sand or gravel. Southwest winds prevailed over the site (D. Caines, pers. comm.). There was no winter ice other than skim ice on the site. The north east section of the site, however, had freshwater influence from the Bay du Nord River. Usable depth ranges were from 3.5 m to 24 m in the water column. The projects were located in Ladder Garden and were deployed on a shore to bottom long-line at 3 m from the surface in a depth of 14 m (Figure 2.1). Ladder Garden was sheltered from the southwesterly winds. The long-line was 400 m long with 360 m of work space.

## 2.2.2 Initial Shell Height and Depth Study

### *Experimental Design and Set-up*

This study examined four factors that affect farm-based nursery growth rates and survival. They were: initial size of scallops, mesh size of equipment, effects of seasonality, and depth of deployment. Four initial size ranges were chosen based on the ability to grow spat in the hatchery and availability of equipment at the hatchery. The size class treatments were 1.4-1.7 mm shell height, 1.7-2.0 mm shell height, 2.0-3.0 mm shell height, and >3.0 mm shell height (Appendix 2.1). The mesh size corresponded to the size classes. The four mesh sizes of equipment that were compared were 1.2, 1.5, 2.0 and 3.0 mm on the diagonal (Appendix 2.1). Four replicates of the 1.2 and 2.0 mm mesh equipment and three replicates of the 1.5 and 3.0 mm mesh equipment were held at each of two depths, 5 and 10 m. These depths were within the usable depth range on the aquaculture site as well within food and temperature limits. There were four sample dates, about every ten weeks.

Spat were raised at the Belleoram Sea Scallop Hatchery (BSSH), Belleoram, NF, from spawning batches during June 1996 at 15°C and fed 40 cells/ $\mu$ L of a mixture of cells. Scallops that had settled were removed from tanks by brushing or by strong water currents. Spat were screened on 3.0 mm and 2.0 mm Vexar® mesh and 1.2 mm (1.7 mm diagonal), and 1.0 mm (1.4 mm diagonal) Nitex® mesh (Appendix 2.1 and 2.2). Thirty spat from each size class were measured with Vernier calipers for initial shell height (distance between ventral margin and dorsal hinge; 0.1 mm accuracy). Due to preparation



time, spat were held in mesh containers at dense conditions in aerated static water tanks until transfer to the nursery the following day.

Scallops were transferred to the nursery site in water. Scallop densities were determined volumetrically (Appendix 2.3 a, b). They were below the generally accepted floor coverage limits of 30% for scallops (Appendix 2.4). Collector bags of 1.2 and 2.0 mm mesh were stuffed with 1 m of Netron® tube (Appendix 2.5) and held on bread trays (69 cm x 57 cm x 15 cm) to prevent collapsing. Trays were deployed in stacks of four. Pearl nets with 1.5 mm and 3.0 mm mesh were tied in strings of three. An extension of rope joined two sets of pearl nets or collector bags so that each string had a treatment at 5 and 10 m. Rocks in sacks were tied to trays at 10 m from below for negative buoyancy.

### *Sampling Protocol*

Sampling of the treatments was every ten weeks. Fouling of gear was measured as were scallop shell height and recovery. Fouling was removed from each unit by washing with water and filtering onto a 106  $\mu\text{m}$  screen. Fouling organisms were sub-sampled (<1% of total quantity) and preserved in vials of 40% methanol for identification (South 1975). The remaining bulk was frozen for later weighing of dry mass. Dry mass was determined by drying to constant mass at 80°C for 24 hours and weighing to 0.0001 g (Appendix 2.6). Survival was sampled by emptying and counting all spat from each unit. For collector bags, total number of spat was measured volumetrically and sub-sampled by volume (~1.0 mL) to count for live spat present (Appendix 2.6). All live spat and empty valves were counted for pearl nets. Shell height of live (n=30) and dead

(n=10) were measured in each unit. Scallops in 3.0 mm mesh were not sampled in July 1997.

Water quality at the farm-based site was monitored with a conductivity, temperature, depth meter (Sea Logger CTD with additional sensors; Model No. SBE 25-03) monthly. Using this device, temperature ( $^{\circ}\text{C}$ ), salinity, oxygen concentration (mg/L and % saturation), chlorophyll-*a* concentration ( $\mu\text{g/L}$ ), optical back-scatter (OBS) or turbidity (formazin turbidity units; FTU) and irradiance (light intensity; microeinsteins) were measured in the water column down to a depth of 14 m. When animals were sampled, temperature and salinity profiles of the water column down to 14 m were obtained with a YSI Model No. 30 S-C-T Meter. Characteristics of the water column were used for comparing the two depths and the seasonality of growth rates and survival.

### *Data Analysis*

Growth, survival, fouling and siltation data were standardized (Appendix 2.6). For fouling and siltation measurements, it is assumed that because all units were treated the same during handling that they would have lost an equal proportion of fouling and siltation present hence the portion that remains would still be comparable amongst the units. Interval growth rates refer to those growth rates between sample dates. The total number of scallops retrieved in the initial shell height/depth study depended on mortality due to poor acclimation or predation, or loss of scallops through the mesh. Because a substantial portion of the scallops were lost through mesh their survivorship was unknown and thus can't be used to ascertain an overall survival rate per unit. Survival

implies the number of live scallops as a percentage of the total number of scallops retrieved after the treatment period while recovery implies the total number of live scallops after the treatment period as a percentage of the initial number of scallops stocked per unit. Recovery is the best estimate of scallops available to the grower based on the initial size and density, and the treatment used in the nursery stage. Recovery was cumulative from initial deployment to date sampled. All percent data were arcsine-square-root transformed prior to statistical analysis (Sokal and Rohlf 1995). Fouling and siltation accumulations were based on unit area due to the different sizes of the pearl nets and collector bags. Data were analyzed using the SPSS® statistical package (Version 8.0).

Three-way ANOVAs were performed to determine the overall potential effects of depth, mesh/initial size and date on variability of growth and survival. Post-hoc Tukey-B tests were performed to determine significant differences among treatments. Environmental data were analyzed using descriptive statistics to determine if differences existed between the two depths. The relationship of growth and survival to environmental factors was explored using regression and correlation analysis.

### 2.2.3 Gear Type Study

#### *Experimental Design and Set-up*

This study compared growth of the same size class of scallops in two gear types; 3.0 mm mesh pearl nets and 3.0 mm mesh collector bags. Pearl nets (35 cm x 35 cm

base) are the traditional gear used for growing scallops, however, the collector bags (onion collector; 40 cm x 80 cm) are effective gear for collecting spat in the wild. The collector bags were filled with 1 m of Netron® tube instead of gillnet and placed in ordinary bread trays. This allowed the collector bags to be supported which prevented crowding in corners. The bread trays also protected the collector bags against direct fouling. The pearl nets, with their pyramidal shape, had direct exposure to water flow while the collector bags, which were rectangularly-shaped, were shielded from water flow by the bread trays. These two gear types were studied for their effectiveness at maximizing nursery growth of scallops. Six replicates of each treatment were used.

Scallops were obtained from the BSSH. They had been transferred to the farm-based nursery in early September 1997 and were sorted on October 24. They were size graded to obtain scallops between 3.3 and 6.4 mm shell height. An initial sample (n=90) was arbitrarily taken from the size-graded scallops for shell height measurements. The scallops were then stocked into gear by volume to attain equal coverage (Appendix 2.4). The pearl nets were stocked at approximately 500 spat/net and the collector bags at approximately 1200 spat/bag. Six pearl nets (three per string) and six collector bags (two per tray) were deployed on a long line at 5 m on the farm-based nursery (Ladder Garden) on Shell Fresh Farms Ltd., Pool's Cove, NF, on October 26, 1997.

### *Sampling Protocol*

The scallops were sampled on May 17, 1998. Scallops were emptied from each replicate and counted for survival. Thirty scallops from each replicate were also

measured for shell height (distance between ventral margin and dorsal hinge; 0.1 mm accuracy). Fouling was cleaned from equipment, frozen and later dried and weighed. Fouling was oven dried at 80°C until a constant weight was reached.

### *Data Analysis*

Analyses of variances were performed on shell height, recovery and fouling data due to gear type.

#### 2.2.4 Density Study

### *Experimental Design and Set-up*

Density in collector bags was studied using two treatments; 5200 spat/collector bag, which was the density used by BSSH, and 2600 spat/collector bag. It was unknown whether 5200 spat/collector limited growth thus the lower density was also investigated. Percent floor coverage was below reported limits for stocking densities of scallops.

Scallops deployed in early September 1997 by BSSH were obtained and re-sorted for this study on October 24. Scallops were size-graded between 2.0 and 3.3 mm shell height (Appendix 2.2). Initial shell height was measured for 90 arbitrarily sampled scallops. Scallops were stocked at 2600 and 5200 spat/bag by volume in three replicates each of 2.0 mm collector bags at floor coverages of 5.02 and 10.04%, respectively, and deployed on October 26, 1997.

### *Sampling Protocol*

Scallops were sampled on June 25, 1998. Scallops were emptied from each unit and counted for survival. Thirty scallops from each unit were measured for shell height (distance from ventral margin to dorsal hinge; 0.1mm accuracy).

### *Data Analysis*

Analyses of variances were performed on shell height and recovery data to determine variation due to density.

## 2.3 Results

### 2.3.1 Initial Shell Height and Depth Study

#### *Growth Rates*

Initial scallop shell heights were significantly different among size classes (One-way ANOVA:  $F = 327.523$ ,  $d.f.=3, 116$ ,  $P < 0.001$ ). Shell height increased over the experiment testing the effect of mesh size, depth and seasonality (Figure 2.2). Overall mean growth rate for scallops in this experiment was  $20.15 \pm 2.06$  ( $\pm S.E.$ )  $\mu\text{m}/\text{d}$ . Multiple ANOVA, with an unequal sample size, indicated that there were significant differences in growth rates due to date, depth and mesh size, which takes into account initial scallop size (Table 2.1). No significant interactions existed among these parameters. Growth rates were highest at 5 m. The highest overall interval growth rate was for the May to July interval which had a mean of  $43.77 \mu\text{m}/\text{d}$  (Figure 2.3). All other growth intervals had statistically similar interval growth rates with the lowest being from November to March at  $16.07 \mu\text{m}/\text{d}$ . Tukey's-B test indicated that the 3.0 mm pearl nets had the highest mean interval growth rate overall at  $40.79 \mu\text{m}/\text{d}$  (Figure 2.3). The lowest interval growth rate was for 1.2 mm collector bags ( $22.79 \mu\text{m}/\text{d}$ ) although no significant differences existed among interval growth rates for the three smallest mesh equipment.

## *Recovery*

Final mean recovery was  $57.45 \pm 0.037\%$  for all treatments. Multiple ANOVA indicated that there were significant interactions in recovery data due to date and equipment (Table 2.2). Date by equipment interactions were also significant. One-way ANOVAs confirmed the relationships found in the MANOVA. Again, date (One-way ANOVA;  $F=11.476$ ,  $d.f.=3,100$ ,  $P<0.001$ ), and equipment (One-way ANOVA;  $F=14.322$ ,  $d.f.=3,100$ ,  $P<0.001$ ) were significant, but depth (One-way ANOVA;  $F=0.232$ ,  $d.f.=1,102$ ,  $P=0.631$ ) was not significant. The highest recovery was after the October to November interval at  $75.58 \pm 0.035\%$  (Figure 2.4). The lowest recovery was in July at  $49.86 \pm 0.018\%$ , however, this value did not include the scallops in the 3.0 mm pearl nets. The 3.0 mm pearl nets had the highest recovery at 81.57% (Figure 2.4). The 1.5 mm pearl nets had the lowest recovery at 45.81%.

May recovery was highest in the 3.0 mm pearl nets (83.29%) and lowest in the 1.2 mm collector bags (43.67%) although this was not statistically different from the 1.5 mm pearl nets or the 2.0 mm collector bags (Figure 2.4). Equipment mesh size had a significant influence on the May recovery of the scallops, however, depth did not account for any significant variation in May recovery (Table 2.3).

Measurements of the dead scallops were made throughout the study. Figure 2.5 indicates that the shell heights of the dead scallops in the three smallest size classes was not very different from their initial shell heights. There were no significant differences between the means of the initial scallop shell height and dead scallop shell height in the May 1997 for the 1.2 mm (Independent t-test;  $t=0.794$ ,  $d.f.=138$ ,  $P=0.429$ ), 1.5 mm



(Independent t-test;  $t=-1.414$ ,  $d.f.=80.134$ ,  $P=0.161$ ), or the 2.0 mm (Independent t-test;  $t=0.830$ ,  $d.f.=128.360$ ,  $P=0.408$ ) mesh equipment. There was a significant difference between the initial live and final dead shell heights of the scallop in the 3.0 mm equipment (Independent t-test;  $t=-6.980$ ,  $d.f.=51.423$ ,  $P<0.001$ ).

### *Water Quality*

Water temperature decreased from October until February, level off until June and then began to rise (Table 2.4). The highest temperature,  $11.1^{\circ}\text{C}$ , was recorded at 5 and 10 m in October when the study was initiated while the lowest temperature recorded,  $1.3^{\circ}\text{C}$ , was recorded in April at 5 m (Table 2.4). Temperature was equal at both depths except from December to May when it was just barely higher at 10 m.

Chlorophyll-*a* concentrations remained low from October until March. A high of  $4.1\ \mu\text{g/L}$  at 10 m was measured in April which resulted in the March to May interval having the highest chlorophyll-*a* concentration (Table 2.4). Chlorophyll-*a* was higher at 5 m than 10 m except during March and April.

Other environmental factors were measured to determine if the farm-based nursery was of high water quality over the study period. Dissolved oxygen concentrations were steady with a slight increase in the spring (Table 2.4). The lowest saturation was measured in June at 77 % saturation at 5 m while the highest of 97 % was also in June at 10 m. Mean dissolved oxygen concentration over the study was  $6.7\ \text{mg/L}$  or 90.75 % saturation. Dissolved oxygen was higher at 10 m in the autumn and spring, but the same as 5 m during the winter. Turbidity declined until spring with an overall average of 7.72

FTU (Table 2.4) and was similar at 5 and 10 m throughout the study. Salinity was consistently within sea scallop tolerance range (Bergman et al. 1996). Salinity ranged from a high of 33.2 at 10 m in February to a low of 26.4 at 5 m in May. There was a slight increase over the study period (Table 2.4). Salinity was slightly greater at 10 m than 5 m throughout the study. Light intensity peaked in March and declined to lowest values in April (Table 2.4). Light intensity was consistently higher at 5 m throughout the study.

Interval growth rates were negatively correlated with dissolved O<sub>2</sub> and turbidity (Table 2.5). Recovery correlated with all parameters except dissolved O<sub>2</sub> (Table 2.5).

### *Macrofouling*

Macrofouling species present on each piece of equipment were identified (Table 2.6). The early colonizers in the late autumn were bivalve spat at low densities. The sea star, *Asterias vulgaris*, which is a predator of sea scallops, was found on all sample dates. The checklists of species present indicated that biofouling occurred on all equipment types and was greatest on the pearl nets (Table 2.6).

The nets having greater than 2.5 mg dry weight (dry wt.)/cm<sup>2</sup> fouling were heavily fouled (75% coverage) with a thick algal layer, which may have seriously impaired water flow. Fouling between 1 and 2.5 mg/cm<sup>2</sup> corresponded to between 33% and 75% coverage (Table 2.7). Lesser amounts of fouling were due to light silt and juveniles of various species which would not impede water flow as much.

Macrofouling for all gear types from October to July averaged 0.8 mg dry wt./cm<sup>2</sup>. Macrofouling was significantly influenced by date, depth and equipment mesh size (Table

2.8). Significant interactions between factors existed, however, one-way ANOVAs confirmed the significance of date (One-way ANOVA:  $F=7.088$ ,  $d.f.=3,100$ ,  $P<0.001$ ), equipment (One-way ANOVA:  $F=8.468$ ,  $d.f.=3,100$ ,  $P<0.001$ ) and depth (One-way ANOVA:  $F=4.845$ ,  $d.f.=1,102$ ,  $P=0.030$ ). The macrofouling was highest in July 1997 at  $1.94$  mg dry wt./cm<sup>2</sup> as it had been accumulating since October 1996 (Figure 2.6). Lowest fouling occurred in November at  $0.07$  mg/cm<sup>2</sup>. Highest macrofouling was measured in the 1.5 mm pearl nets at  $2.22$  mg/cm<sup>2</sup> (Figure 2.6). The 2.0 mm collector bags had the least fouling overall which was statistically similar to fouling on the 3.0 mm pearl nets and 1.2 mm collector bags (Figure 2.6). Macrofouling at 5 m was more than double that at 10 m and highest on the 1.5 mm and 3.0 mm equipment after deployment from October 1996 to May 1997 (Figure 2.6).

### *Siltation*

Silt was defined as all particles that passed through a  $106\text{-}\mu\text{m}$ -mesh screen. Mean silt accumulation over the study was  $0.92$  mg dry wt./cm<sup>2</sup>. Silt accumulation was significantly influenced by date, depth and equipment mesh size (Table 2.9). Interactions between these factors were also significant, however, one-way ANOVAs confirmed the significance of date (One-way ANOVA:  $F=5.835$ ,  $d.f.=3,100$ ,  $P=0.001$ ), equipment (One-way ANOVA:  $F=25.670$ ,  $d.f.=3, 100$ ,  $P<0.001$ ) and depth (One-way ANOVA:  $F=6.146$ ,  $d.f.=1,102$ ,  $P=0.015$ ). The highest overall accumulation of silt occurred by May 1997 at a mean of  $1.33$  mg dry wt./cm<sup>2</sup>. The least mean accumulation was measured in November 1996 at  $0.49$  mg dry wt./cm<sup>2</sup>. Highest siltation was measured in the 1.5 mm

pearl nets at 1.80 mg dry wt./cm<sup>2</sup>. The 1.2 mm and 2.0 mm collector bags had the least siltation. Silt accumulation at 5 m was 1.11 mg dry wt./cm<sup>2</sup> which was more than the 0.72 mg dry wt./cm<sup>2</sup> measured at 10 m (Figure 2.7). Silt accumulation from October to May was highest on the 1.5 mm mesh pearl nets (Figure 2.7). Silt accumulation was higher at 5 m.

### 2.3.2 Gear Type Study

Fouling had accumulated on both the 3.0 mm pearl net and collector bag gear types (Figure 2.8). It was significantly higher in pearl nets than collector bags (One-way ANOVA;  $F=38.675$ ,  $d.f.=1,10$ ,  $P<0.001$ ).

Shell height of scallops in both gear types increased over the winter (Figure 2.9). Mean growth rate of scallops in the gear type study was 46.53  $\mu\text{m}/\text{d}$  (Figure 2.10). Significant differences in final shell heights were due to gear type (Two-way ANOVA,  $F=69.870$ ,  $d.f.=1, 360$ ,  $P<0.001$ ) and replicates (Two-way ANOVA,  $F=5.364$ ,  $d.f.=3,360$ ,  $P<0.001$ ). The significant difference in replicates was due to differences found in pearl net replicates (Table 2.10), however, pearl net replicate means were greater than the means of the collector bags. Variation in pooled data was due to gear type (One-way ANOVA,  $F= 66.212$ ,  $d.f.=1, 358$ ,  $P<0.001$ ). The 3.0 mm pearl nets had the highest growth rates .

Mean percent recovery for the gear type study was 92% (Figure 2.10). Gear type significantly influenced recovery (One-way ANOVA;  $F= 0.732$ ,  $d.f.=1,10$ ,  $P =0.412$ ).

### 2.3.3 Stocking Density Study

Growth occurred in scallops in the collector bags at both densities over the period of October 1997 to June 1998 (Figure 2.11). There were no significant differences in replicates so they were pooled (Two-way ANOVA,  $F=0.252$ ,  $d.f.=2,180$ ,  $P=0.778$ ). There was no significant difference between final shell heights at the two densities (One-way ANOVA;  $F=1.196$ ,  $d.f.=1, 178$ ,  $P=0.276$ ). Growth rates of the 2600 spat/ bag and 5200 spat/bag were 21.3 and 23.8  $\mu\text{m/d}$ , respectively (Figure 2.12).

Recovery declined to 57% over the study. Recovery for 2600 and 5200 spat/bag was 56.5 and 58.0%, respectively (Figure 2.12). The recovery was not significantly different between the two densities (One-way ANOVA;  $F= 0.303$ ,  $d.f.=1, 4$ ,  $P=0.611$ ).

## **2.4 Discussion**

### **2.4.1 Growth Rates**

Growth rates were found to vary due to season, depth, mesh or initial size, and gear type. No differences were observed in growth rates due to density. The variation in growth rates due to season, mesh size and gear type were expected, however, the variation in growth rates due to depth and density were unexpected.

Throughout this study three observations, aside from the ones reported in this chapter, were made. First, similar size spat appeared to have predictable growth rates from year to year which was obvious from observing similar size classes over two consecutive years. Second, growth rates of the scallops were within the range of cultured nursery-sized scallops and wild scallops from other studies (Table 2.11). The third observation was that growth rates of nursery-sized scallops in Newfoundland were lower than those reported for similar sized sea scallops in Passamaquoddy Bay, N.B. (Parsons et al. 1993). These differences may be explained by the study period and site specific parameters (ie. temperature, current velocity, food quality, etc.) which may be different.

### **2.4.2 Recovery**

Recovery was found to vary due to two factors examined in this study. Differences in mean recovery were caused by season, and mesh or initial size, but not depth, gear type or density. The influence of season, and mesh or initial size was

expected. However, the lack of variation due to depth, gear type and density was not expected.

In addition to the examined factors, important observations were made regarding recovery. Recovery of nursery-sized scallops was much lower than in juvenile or final growout strategies (Table 2.11), but higher than in the first production season at the Belleoram Sea Scallop Hatchery (10%, pers. obs.). Small scallops are handled in larger quantities hence screening may be less efficient than that of lower quantities of larger scallops. The higher recovery than the previous production season at BSSH may be due to better health overall as in the first year of production scallops were in poor health due to poor water circulation in tanks and deployed much later in the year (P. Dabinett, pers. comm). Low recovery in nursery culture is common because not enough is understood about nursery rearing of scallops in general. An example of another scallop species with low recovery during nursery culture is *Patinopecten yessoensis* with less than 5 % recovery in the nursery stage (O'Foighil et al. 1990; Bourne and Hodgson 1991).

#### 2.4.3 Seasonal Effects on Growth Rates and Recovery

Noticeable changes in environmental parameters occurred throughout the study, although they were not below limiting values. Temperature and chlorophyll-*a* concentrations declined from October to February, remained low until June and began to rise again. This winter cycle is characteristic of Atlantic coastal areas, e.g., Mahone Bay, N.S., where Dadswell and Parsons (1991) studied intermediate sea scallop culture, although the winter temperatures in Newfoundland are a bit lower. Growth rates of the

scallops show a similar seasonal pattern of decrease until March followed by an increase over the rest of the study. This suggests that food density and temperature may have a positive effect on growth rates as when they are too low, little or no growth will occur.

Loss of scallops was inevitable in the farm-based nursery. One hundred percent recovery was not expected because of the lack of knowledge about sea scallop nursery culture protocols in addition to limited records of environmental data at the nursery site.

Percent recovery leveled off through the winter (the drop in July may be explained by the loss of the 3.0 mm mesh equipment which had high recovery) and the majority of dead scallops in May were similar to deployment size which suggests the impact of a deleterious factor early in the study. Poor handling, specifically size grading, may have resulted in the loss of scallops early in the study (Appendices 2.4 and 2.6). Time of transfer may explain the mortality event early in the study in two aspects; the ability of the scallops to acclimate to sudden and declining conditions; and the presence of sea stars, potential predators, during their natural settlement in the nursery environment. These time of transfer factors will be discussed in Chapter 3. To assess loss and mortality due to handling, sampling must be carried out shortly after deployment, however, it is necessary to ensure 100% of the scallops are alive before deployment.

#### *Effects of Food and Temperature Changes on Growth Rates*

Nursery-sized sea scallops exhibited seasonal growth patterns. This is expected as growth rate of sea scallops depends on the suitability of the environment and the integrated response of physiological activities of the organism (MacDonald and



Thompson 1985a,b; Hilbish 1986; Dadswell and Parsons 1992a,b; Parsons and Dadswell 1992). Enhanced productivity reflects more favorable food supply and/or temperature regimes of the natural growing environment (Dadswell and Parsons 1991; Côté et al. 1993). High growth rates in the present study corresponded to high temperature and food density.

Temperature and food density have been shown to influence growth in other pectinids including *Pecten fumatus* (Cropp and Hurtle 1992), *Patinopecten yessoensis* (Bourne and Hodgson 1991), Queen scallop *Chlamys opercularis* (Richardson et al. 1982), *C. islandica* (Vahl 1980; Wallace and Reinsnes 1984; Thorarinsdóttir 1994), *Pecten maximus* (Wilson 1987) and *Adamussium colbecki* (Stockton 1984). Vahl (1980) specifically attributed food related growth differences to particulate inorganic matter (PIM) content which dilutes the particulate organic matter (POM) making clearance less efficient.

Other studies have found no seasonal change of growth rates of scallops which may be attributed to a constant array of phytoplankton supplying metabolic needs and growth potential (Anderson and Naas 1993; Emerson et al. 1994). Tropical species may exhibit this growth pattern due to the constancy in availability of food. According to Kirby-Smith and Barber (1974) and Palmer and Williams (1980) *Argopecten irradians* can retain more small particles like microalgae when they are abundant suggesting that growth is possible throughout the year, even when food quality may be low. However, growth rings do occur in bay scallops which suggests seasonal variations hence extrapolations from laboratory situation are not always applicable to natural occurrences.

The specific influence that temperature and food have on growth of sea scallops is

not fully understood. For instance, Kleinman et al. (1996) indicated the influence of temperature was greater than total particulate matter (TPM) on growth, however, Parrish et al. (1995) speculate the importance of a specific essential fatty acid 22:6 $\omega$ 3. MacDonald and Thompson (1985a) previously concluded that food was more important than temperature for sea scallop growth. Further research is needed to understand the full relationship between temperature and food quality and how it affects growth.

#### *Effect of Handling on Recovery*

Handling can be an important source of mortality (Ventilla 1982; Wildish et al. 1988). Survival tends to decrease little after the first sampling interval when handling is the principle cause of mortality (Dadswell and Parsons 1991; Parsons and Dadswell 1991; Dadswell and Parsons 1992a,b; Toro et al. 1995). Juvenile sea scallop mortality, caused by handling, normally ranges from 7-9% (Parsons and Dadswell 1992; Penney 1993).

Size grading was an avoidable area of loss of marginally sized scallops (Appendix 2.7). Preliminary calculations from size grading alone indicated the possible loss of 66,000 spat (13.7%). Slight fluctuations in the recovery indicate the variability in the counting methods of the smallest size class. Variable loss also occurred through the 1.5 mm mesh of the pearl nets which was distorted and larger than it should have been in several places (pers. obs.; see also Section 2.4.5). Loss due to size grading can be avoided by ensuring that screens are not blocked by excessive numbers of scallops or by having a larger size differential between screening mesh and equipment mesh.

Sampling technique may explain the high recovery of scallops in the 1.2 mm

collector bags in November. Two of the four replicates from the November sample were analyzed by a different person which resulted in higher recovery measurements than what was volumetrically placed in bags (given 100% recovery in statistical analysis) and the recovery in the other two replicates (72.6 and 76.4%). The differences may be explained by the “approximate numbers” of scallops initially stocked in each bag and the differences in sampling technique by different individuals. Samples were taken prior to stocking to determine how much volume of spat was necessary to get the desired density as well because of the limited supply of scallops only one sample of actual spat volume was taken. Theoretically, recovery could have been >100%. Spat sampling by volume can be highly variable thus protocol should be consistent to be precise, but it is possible that other faster precise methods of electronically or volumetrically counting large numbers of bivalves are needed. At the commercial level this would be useful also from an economic perspective such that stocking densities thus annual financial projections can be more accurate.

#### 2.4.4 Depth Effects on Growth Rates and Recovery

Reduced growth rates are associated with deep water due to decreasing food density and temperature (Leighton 1979; MacDonald and Thompson 1985a; Young-Lai and Aiken 1986; Wildish et al. 1988; Claereboudt et al. 1994a; Dabinett and Clemens 1994; Dabinett and Clemens 1997). However, similarity in environmental conditions at different depths can also occur (Richardson et al. 1982; Wallace and Reinsnes 1985; Côté et al. 1993). Wallace and Reinsnes (1985) found the same temperature occurred at all

depths, but growth in Icelandic scallops was highest at 5 m. Leighton (1979) found that food densities were consistent to 60 m. Few differences in environmental parameters measured existed between the depths in the present study due to a minimal spatial separation nor did parameters go below acceptable levels for scallop culture.

The water column at Shell Fresh Farms Ltd., Pool's Cove, NF, was not stratified over the study between 5 and 10 m. Due to the minimal spatial separation, food density, temperature and salinity were similar between the depths. Light intensity, however, was always lower at 10 m than at 5 m which may explain the why less algal fouling was present at 10 m than 5 m. Only during the spring bloom were there noticeable differences in oxygen (lower at 5 m) and chlorophyll-*a* (higher at 10 m) concentrations between depths which may have been due to the depletion of nutrients near the surface i.e., 5 m.

Growth rates of scallops varied between 5 m and 10 m despite the quantitative similarities in environmental conditions. Limitations in growth rates at 10 m were most obvious in the largest size class (Figure 2.3). Variation in growth due to depth occurs occasionally (Leighton 1979; Ventilla 1982; Wallace and Reinsnes 1985; MacDonald 1986), but not always (Duggan 1973; Monical 1980; Richardson et al. 1982; Wallace and Reinsnes 1984; MacDonald and Thompson 1985; Walker et al. 1991; Cropp and Hortle 1992; Côté et al. 1993). It depends on spatial separation of animals and site hydrodynamics. The potential factors in this study may be higher food quality, food flux and/or higher exploitation of food at 5 m due to reduced flow by fouling organisms.

Variation in recovery was not influenced by depth. Emerson and Grant (1992) found similar results. Mortality, however, has been inversely related to depth due to lower wave action (Duggan 1973; Lodeiros and Himmelman 1995) or a direct

relationship between depth and deterioration in food and temperature (Dadswell and Parsons 1991; Côté et al. 1993; Gaudet 1994). This suggests that any wave action that may have occurred at 5 m or potential deterioration in food quality at 10 m was not enough to cause mortality. The loss of scallops through the mesh may not be influenced by depth either.

Others have also found fouling to cause lower survival in scallops (Duggan 1973; Heffernan et al. 1988; Thorarinsdóttir 1991; Lodeiros and Himmelman 1994) unlike this study and one by Cropp and Hurtle (1992). Fouling may have affected food quantity or quality (see Section 3.4.3).

#### *Effects of Food Quality on Growth Rates and Recovery*

Although chlorophyll-*a* was similar at the two depths, food quality and flux may have been lower at 10 m. Quality of food, defined as the potential nutritional value, is depth specific and is dependent on the relative phytoplankton composition present. Food flux, defined as total available food based on food concentration and water flow, may be higher near at the surface where wind, wave and tidal exposure is greatest. Chlorophyll-*a* has been found to be maintained with depth while POM increases while in other cases PIM increases and carbon decreases with depth (Rodhouse and Gaffney 1984; Wallace and Reinsnes 1984; Toro et al. 1995). Potentially higher PIM, which is heavier and settles out faster, at 10 m may have diluted food and reduced total energy available to the scallops causing reduced growth. This was the cause of low growth in *Ostrea chilensis* at lower depths (Toro et al. 1995). Competition and selective feeding by fouling organisms

on specific particle size ranges may alter food quality and flux (Mook 1981). Although seston was not analyzed in detail, the increased variety of fouling organisms at 5 m may suggest higher food quality and flux at 5 m. Food quality (ie. species present, nutritive value of food, POM, PIM, etc.) at Shell Fresh Farms Ltd. needs analysis to determine any differences between 5 and 10 m.

### *Effects of Fouling and Water Flow on Feeding and Growth Rates*

Fouling and light intensity were the only two environmental factors measured that were consistently different between depths throughout the study. Individual wavelengths of light penetrate to specific depths which limits the growth of light-dependent algal-fouling with depth. Decreases in fouling due to depth are common (Leighton 1979; Monical 1980; Wallace and Reinsnes 1985; MacDonald and Bourne 1989; Côté et al. 1993; Claereboudt et al. 1994a). In the present study, decreased fouling with depth was attributed to decreased light penetration because the majority of fouling was macroalgal species which require light for growth and survival (Table 2.4).

Fouling, which occurs on any unprotected solid surface in the sea, is an important limiting factor in suspension culture of many bivalves (Wildish et al. 1988; Wahl 1989; Mallet and Carver 1991; Claereboudt et al. 1994a). Côté et al. (1993) suggest that the effects of temperature and food may be negated by fouling. However, that would imply that in this study higher growth should have occurred at 10 m where fouling was less. In addition to competing for the same source of food (Mook 1981; Lesser et al. 1992; Côté et al. 1993), fouling can reduce water flow in both artificial and natural situations (Côté et

al. 1993; Claereboudt et al. 1994a; Devaraj and Parsons 1997). A reduction in water flow consequently decreases food flux.

Current velocity affects feeding and thus growth of scallops (Cahalan et al. 1989; Wildish and Saulnier 1992). In the laboratory, sea scallop growth is limited by water flow greater than 20 cm/s and less than 6 cm/s because they do not or can not feed (Wildish and Kristmanson 1988; Kean-Howie et al. 1991). Similar effects have been found in the southern bay scallop, *Argopecten irradians concentricus* (Kirby-Smith 1972; Eckman et al. 1989). In flume tanks, scallop feeding rates depend on food density and flow steadiness and velocities, however, little research has been conducted in natural settings where currents are changing all the time (Wildish and Kristmanson 1988; Wildish et al. 1992). Clearance rates adjust to ambient flow, however, filtration may be hindered by velocities above a relatively low threshold value (Wildish et al. 1992). Low water flow can become limiting due to lack of replenishment of food and filtration of the same water mass within pearl nets (Mook 1981; Wildish and Kristmanson 1985). Kean-Howie et al. (1991) found that scallops grow best in 10 cm/s velocity and 20 mg microparticulate diet/L. Wildish and Kristmanson (1985) found that a decrease in current speeds from 10 to 7 cm/s results in increased growth. Such a reduction in water flow can occur in scallop culture gear by a reduction in the mesh size of the enclosure or by an increase in the extent that the enclosure is fouled (Cole et al. 1996; Devaraj and Parsons 1997). Higher fouling at 5 m may have dampened the water flow to rates that allowed better exploitation of the food present. More investigation into the effect of dynamic flow in natural environments is necessary to confirm these speculations.

The negative buoyancy of the equipment studied kept it well-below the surface

which suggested that the effects from surface waves were minimal if at all, as the nursery site was fairly sheltered at the head of a bay. Wave action did not have any effects on *C. islandica* either (Wallace and Reinsnes 1985). Surge effects on the purple-hinge rock scallop, *Hinnites multirugosus*, were experienced at the surface, but became reduced at depth (Monical 1980). Duggan (1973) found that disturbances from wave action were reduced with depth which resulted in good growth. This suggests that wave exposure is important in assessing the usefulness of an area for a farm-based nursery. More data needs to be collected on the wave action on the farm-based nursery.

#### 2.4.5 Effects of Initial Size on Growth Rates and Recovery

This study found that the largest nursery-sized sea scallops grew almost twice as fast and had 33% higher recovery than smaller scallops. Growth rates were consistently higher in increasing size classes although they showed no significant differences in the three smallest size classes. Recovery was statistically similar in the three smallest size classes and lowest in the 1.5 mm pearl nets.

Growth patterns found in the present study are similar to post-larval scallop growth patterns. In post-larval scallops, the smaller size class has lower growth rates whereas in juvenile scallops, the smaller size classes have higher growth rates (Parsons et al. 1993; Penney and Mills 1996). Dadswell and Parsons (1991) indicate that cultured sea scallops have increasing growth rates until they are about 16 to 18 months which may explain the difference between post-settled and juvenile sea scallop growth patterns. Similar patterns are exhibited in other species. Juvenile eastern oyster and bay scallop



growth rates decrease over time while the growth rates of the giant clam, *Tridacna gigas*, are low until five months after metamorphosis (Crawford et al. 1986; Rheault and Rice 1996).

Having been deployed in farm-based nursery conditions different than the hatchery, the sea scallops did not have a chance to increase growth rates until the following spring and they may have suffered mortality. Low growth rates and recovery in the smallest size classes may have been due to size grading, mesh flaws, poor condition and lack of acclimation when coming from the hatchery, and size-selective predation (Appendix 2.7). Size grading may explain part of the loss of the scallops in the two smallest mesh sized equipment. An estimate was calculated of the loss of scallops through mesh due to undersize individuals in the initial sample (Appendix 2.7). This suggested that the 1.2 and 1.5 mm mesh gear had relatively high percentages of undersized spat thus was expected to incur the greatest loss. Effects of acclimation and predation will be discussed in Chapter 3.

#### *Effect of Mesh Size on Growth Rates and Recovery*

Mesh size has been found to give varying results on survival for scallops. Two size classes of sea scallops grown in 4.5, 6 and 9 mm pearl nets resulted in high survival with neither mesh size nor initial size having any effect (Penney and Mills 1996). Walker et al. (1991) found that scallops survive poorly in 3 mm pearl nets in comparison to 6 and 9 mm pearl nets, but there was greater loss through mesh of the 3 and 6 mm pearl nets. This was the case for the three smallest mesh sizes in this study. The low recovery may

be explained by these losses. The loss may be due to inadequate sorting of smaller animals which would allow for a relatively larger loss through mesh, predation and natural mortality.

The 1.5 mm pearl nets were a special case as they experienced the highest loss through mesh as a result of equipment construction. The greatest loss occurred in the first sample interval. The manufacturing of these pearl nets resulted in a distorted weave, which caused the mesh to be larger than was expected, and a large hole around the central cord of the pearl nets, both of which were potential places for loss.

Mesh size affects growth rates by limiting the amount of water and thus food that can pass through any given piece of equipment. As mentioned in Section 2.4.2, scallop growth is influenced by water flow due to the replenishment of food. Cole et al. (1996) found that the reduction in water flow through pearl nets was inversely related to mesh size. This was due to the smaller mesh having more material to block the flow. Cashmore et al. (1998), however, found no differences in growth rates of wild scallops grown in two mesh sizes. In my study, scallops held in the largest mesh had the highest growth rates.

Mesh becomes less efficient in allowing water flow when it becomes fouled (Devaraj and Parsons 1997), however, there was no pattern of fouling with regard to mesh size in this study possibly due to the use of different types of equipment for the four mesh sizes (Figure 2.6). Reduction in flow of water by fouling becomes more limiting for growth within small mesh holdings due to lower food replenishment and alteration of the particle size spectrum due to competition for food by the fouling community (Wildish et al. 1988; Mallet and Carver 1991; Claereboudt et al. 1994a,b). The growth rates of the

three smallest size classes were equal hence it may be that there was no difference in the flow rates between the three mesh sizes. The 3.0 mm mesh had larger openings that even when partially blocked by fouling may have allowed sufficient replenishment of food. Smaller mesh also has more material to block water flow (Cole et al. 1996). The limitations of small mesh emphasizes the need to transfer scallop to larger mesh gear as soon as possible.

#### *Effect of Siltation on Growth Rates and Recovery*

Build up of silt may be a problem with fine meshes. Silt accumulation can build up on the smaller openings obstructing flow which is further reduced by the relatively larger amount of equipment material. Macroalgal fouling may also cause silt and fecal matter to accumulate (Leighton 1979). Small bay scallops and rock scallops experience high mortality due to silt (Duggan 1973; Monical 1980; Rhodes et al. 1981). On the north-east coast of Newfoundland, the ratio of PIM to POM is highest during spring and December which coincides with rainfall and influence of silt from freshwater runoff (Penney and McKenzie 1996). Silt quantity is higher near bottom where survival of *Argopecten irradians* is low (Duggan 1973). Any effect of siltation may have been on the 1.5 mm mesh bag where fouling and siltation on the bag were high. This suggests that not only could silt be collected on the mesh, but food also, thus allowing less to pass through to the scallops. This would have impacts on survival as well as growth. The 3.0 mm mesh pearl nets had extensive silt collection on the outside of the bag, however, its larger opening may have prevented the screening of food which would allow higher

growth.

#### 2.4.6 Effects of Gear Type on Growth Rates and Recovery

Lower growth rates of scallops were observed in the collector bags than in the pearl nets, however, recovery rates were not different in the two gear types. Gear design may have initially created different patterns of flow for delivering food, but perhaps fouling accumulation aided in the reduction of flow rate to be more suitable for exploiting food. Because of the set-up, collector bags in bread tray stacks did not accumulate fouling or have evenly distributed flow in the same way as pearl nets. This may give pearl nets the growth advantage.

Scallop culture in different gear types leads to different growth rates. Penney (1993) found higher growth rates in pearl nets than lantern nets. Parsons and Dadswell (1994) found that among round pearl nets, square pearl nets, lantern nets, super lantern nets and Shibetsu nets, the super lantern net offered the best shell growth. Flow rates may explain the differences of growth in different gear types (Brake and Parsons 1998).

Flow velocities can be stifled by gear material. In 1 x 3 mm mesh pearl nets flow was reduced by 25-45%, depending on ambient external flow (Cole et al. 1996; Devaraj and Parsons 1997). This is due to the actual percent opening being small due to the amount of material necessary to make the small openings; the actual reduction in the 3 x 3 mm mesh pearl nets may not have been as high as there is only half the material used to make the small openings. Bread trays also reduce water velocity by 75% such that water flow is low on the side of the tray facing the current and high on the opposite or back side

of the tray (Brake and Parsons 1998). This is due to the solid plastic wall on the sides of the trays with no mesh openings. No studies have been conducted on reduction of flow in collector bags on stacks of bread trays, however, it is expected to reduce flow even more.

It is known then that flow reduction does occur in the gear types studied, however, the extent is not known as current velocity data has never been consistently collected for Shell Fresh Farms Ltd.. The angle of equipment on the long line suggests that currents are high as strings of pearl nets are usually drawn on an angle when tide is ebbing or flowing. The percent flow reduction is high in the collector bags in bread trays which may cause low replenishment of food to the collector bags resulting in lower growth. The low flow is expected to alternate back and forth from one side of the bread tray to another in conjunction with the ebb and flow of the tide. This may reduce growth due to the unsteady flow or lack thereof as suggested by Wildish and Kristmanson (1988) and Claereboudt et al. (1994b). The pearl net were exposed to the same natural flow reduction and tidal periodicity, however, superior growth was exhibited. Another factor such as the changes in flux within equipment may have caused the differences in growth.

The higher rate of fouling accumulation in the pearl nets may be attributed to gear exposure. The pearl nets are exposed to settlement of organisms through the water column on the slanted tops as well as on the sides. The collector bags are only exposed to fouling on the surface which itself is exposed to only a thin layer of water passing through the bread trays. Lower exposure combined with low water flow decreases the chance of fouling directly on equipment. The bread trays themselves are subjected to extensive fouling which may in turn reduce flow and food deliverance to scallops inside the stack of trays. This may impact both growth and recovery rates of scallops. Andersen and Naas

(1993) found fouling higher on cages than pearl nets hence credited it for the differences observed in growth rates.

Fouling can alter flow patterns in and around pearl nets as well as reduce flow velocity through them. Devaraj and Parsons (1997) found that fouling combined with mesh-induced flow reduction could be as high as 75% in 1 x 3 mm pearl nets (not quite this high in 3 x 3 mm mesh pearl nets). The effect on flow depends on the extent of fouling with slight fouling having very little effect. This difference is clarified by two studies of natural fouling. Andersen and Naas (1993) observed significantly different growth under pearl nets culture in light (1000 g wet weight/ unit) to heavily (4000 g wet weight/unit) fouled conditions. Claereboudt et al. (1994a) observed that where pearl net fouling ranged from none to little the growth of scallops differed by only 4.8%. Devaraj and Parsons (1997) found that simulated high fouling covered most of the pearl nets and caused the highest reduction in the water flow which would support the reduced growth found by Andersen and Naas (1993). In the present study, the growth rates may have been high because the fouling was more comparable to Claereboudt et al. (1994a) with a maximum 15 g dry weight and thus had little effect on the growth rates of sea scallops in pearl nets. Also, the majority of fouling was algal species so there was no competition for food. Accumulated fouling may have also dampened the periodicity of the tidal flow.

No measurements of flow through fouled collector bags in bread trays have been done. Less fouling is observed on the collector bags, but the bread trays themselves were fouled. The combined effects of reduced flow by the trays and collector mesh as well as the fouling on the tray may reduce flow such that there is inadequate replenishment of food within the collector bags. Brake and Parsons (1998) also suggested that because

bread trays show high variation in flow rate reduction due to position in the tray, growth rates of scallops will depend on their position in the tray, however, this did not happen in this study as indicated by low standard error of shell heights (Figure 2.2). This potential variability may have been avoided by the shifting of the flow with the periodicity of the tide or spinning of bread trays hanging on a long-line.

Other studies have made observations on the survival of scallops held in different gear types. Penney (1993) did not observe significant differences in survival in different gear types either. Handling was attributed as the cause of mortality of scallops (Penney 1993). Parsons and Dadswell (1994) found survival lower in round and square pearl nets and the super lantern net compared to the lantern net and Shibetsu net. However, they attributed these differences to the marginally sized scallops falling through the mesh as there were no empty shells to account for any mortality. The lack of difference in percent recovery in this study, despite differing growth rates due to limited food in the collector bags, suggests that low food density had little effect on the survival/recovery.

#### 2.4.7 Effect of Stocking Density on Growth Rates and Recovery

Stocking density did not influence growth or recovery rates. This suggests that the densities may have been below actual limiting densities of floor coverage. Floor coverage being determined by the biomass of scallops that can be grown in a unit without limiting space or food before the next sorting.

Gaudet (1994), Parsons and Dadswell (1994) and Penney (1993) found similar results in growth rates in density studies of juvenile scallops. This contrasts with several

studies where increasing density decreases growth rates (Duggan 1973; Monical 1980; Dadswell and Parsons 1991; Kingzett and Bourne 1991; Walker et al. 1991; Widman and Rhodes 1991; Parsons and Dadswell 1992; Côté et al. 1993; Penney 1993). Decreased growth rates may be explained by a lack of food resources due to high density and reduction in space leading to increased contact which may cause shell breakage or less feeding due to irritation and retraction of the mantle (Parsons and Dadswell 1992; Côté et al. 1994). Both may explain negative relationship between shell height and density.

The similar growth rates may have been due to the stocking densities being below critical densities for exploitation of available food. Scallops in the high density were below the Japanese limits for stocking density (33% floor coverage). In addition, with the loss of scallops due to mortality and falling through the mesh, there was also more food available per scallop that remained. The initial floor coverage dropped to 2.6 and 5.5% for each density. The final floor coverage was 23.4 and 45.5% for each density. The actual initial stocking density limits could be tested by comparing growth rates of lower and higher densities to densities studied.

Studies have observed no effects on survival for different stocking densities (Heffernan et al. 1988; Walker et al. 1991; Parsons and Dadswell 1992; Côté et al. 1993; Gaudet 1994). Higher density can be a problem when other factors come into play such as wave action and fouling (Duggan 1973; Ventilla 1982; Dadswell and Parsons 1991; Widman and Rhodes 1991). Fouling influences water flow and food supply increasingly as it accumulates (Duggan 1973). At high densities, wave disturbance can wash scallops into confined spaces (ie. corners of pearl nets) which causes mortalities when two scallops clamp other scallop shells causing soft tissues to be cut with shell margins



(called “knifing” and may also occur during handling) as well as by suffocation (Duggan 1973). High survival of *Patinopecten yessoensis* occurs in areas of wave action, however, deformed scallops are observed when densities are high (Kingzett and Bourne 1991). The cause of increased mortality in *Pecten fumatus* in high densities, however, could not be distinguished between density or fouling (Cropp and Hortle 1992).

## **2.5 Conclusions**

Different growth rates, with respect to season, mesh size, depth and gear type were exhibited by nursery-sized sea scallops. These findings were expected although growth was predicted to be higher at 10 m than 5 m. Growth rates did not differ under differing stocking densities which was unexpected although original stocking densities were not maintained. Recovery was influenced by season and mesh size which were expected. It was expected that depth, gear type and density would influence recovery, however, they did not influence recovery.

As mentioned in the introduction, environmental quality and husbandry decisions are defined by the amount they enhance scallop growth and survival, in this case recovery. This study suggests that in a farm-based nursery situation the protocol for deployment of scallops needs to take into account size class and stocking density of scallops, and deployment depth, type and mesh size of equipment to optimize scallop growth and recovery. It can be concluded that the environmental quality in the autumn/winter may not be the highest due to the decrease in temperature and food in the environment as reflected in the low growth rates of scallops. This suggests that timing of deployment of scallops and the associated factors, particularly temperature and food, that fluctuate over time, and hence may influence growth and recovery of nursery-sized scallops. Research on this subject was conducted and is discussed in Chapter 3.

### **Chapter Three:**

## **Effect of Deployment Date and Environmental Conditions on Growth Rates and Recovery and Potential for Remote Set of Hatchery-reared Sea Scallops, *Placopecten magellanicus*, at a Farm-based Nursery**

### 3.1 Introduction

To accommodate the basic requirements of a nursery, its purpose must be considered. A nursery fosters development of young animals. For scallops, it is the transitional period between a well-maintained hatchery setting and an uncontrolled growout environment. Any nursery is expected to be moderately controlled because of the transition from controlled to virtually uncontrolled environments. In land-based nurseries, environmental factors can be controlled, however, in an ocean or farm-based nursery, environmental factors cannot be maintained by a grower. This lack of control can be overcome by determining the predictability of environmental factors in the nursery.

Determining the timing of deployment at the farm-based nursery is necessary to optimize growth rates of hatchery-reared *Patinopecten yessoensis* (Bourne and Hodgson 1991). Spat deployed during optimal food density and temperature have higher growth rates and recovery. For a temperate farm-based nursery, knowing when environmental conditions are optimal allows control of exposure fluctuating and declining conditions.

The window of opportunity of deployment on the farm-based nursery must be determined by recognizing growth and recovery rates as functions of measurable natural factors such as water quality, food availability and presence of potential predators over time. When adequate nursery accommodations are provided, growth rates are maximal and the time scallops spend in the nursery decreases. Risks of mortality should be minimal in the nursery also. When growth and survival become limited, field deployment is not viable.

Limitations on the timing of hatchery production of molluscs large enough to go to the farm-based nursery can be overcome by remote set (Nosho and Chew 1991). When larvae are ready to settle or are "competent" they can be shipped to distant or remote areas where they are placed in tanks containing settlement substrate. After several days, the settlement substrate can be removed from the tanks and placed in mesh bags and transferred to the farm-based nursery. This procedure, or modified procedures, has been used for oysters, clams, scallops and mussels (Nosho and Chew 1991; Neima and Kenchington 1997; BCSGA 1998). Remote setting decreases the time that scallops are in the hatchery thus increasing the number of scallops that are deployed in optimal nursery conditions. It also makes culture possible where infrastructure, facilities and resources for a full scale hatchery do not exist. This is common practice for a bay scallop hatchery in Nantucket where Vexar® is preferred by larvae as settlement substrate (R. Garrison, pers. comm.).

The optimal farm-based nursery requirements of hatchery-reared *Placopecten magellanicus* have not been studied. Determining the optimal timing of deployment of sea scallops to the farm-based nursery can be narrowed based on conditions derived from other growth and survival studies of scallops.

Growth rates of scallops vary seasonally due to natural fluctuations in food density and temperature (Kirby-Smith and Barber 1974; Vahl 1980; Chapter 2). Growth rates of *P. magellanicus* are highest in the summer and lowest in the winter (Dadswell and Parsons 1992a,b; Côté et al. 1993; Kleinman et al. 1996) and show no increase during the autumn bloom (Emerson and Grant 1992). Sea scallops in some areas of Atlantic Canada are able to naturally produce two cohorts of which the summer (June to July)

cohort grows faster than the autumn (September to October) cohort over the entire culture period (Dupaul et al. 1989; Dadswell and Parsons 1992a,b). The higher growth rates suggest the initial exposure to the earlier food and longer period of warmer water is more favorable.

Salinity and presence of predators impact recovery of scallops. Salinity concentrations below 18 cause mortality in scallops in long term exposures (200 hours; Bergman et al. 1996). Sea stars are an important predator of scallops in suspended equipment (Dickie and Medcof 1963; Scheibling et al. 1991; Dadswell and Parsons 1992a,b; Minchin 1992; Barbeau and Scheibling 1994a).

Timing of deployment of nursery-sized spat on the farm-based nursery is critical for optimizing growth rates and recovery. The goal of this study was to find a window of opportunity for deployment of hatchery-reared sea scallops at a farm-based nursery that enhances growth rates and recovery and predicts availability of spat for intermediate growout. Based on previous research of scallops, the hypotheses for this study are:

(1) growth will be highest in scallops deployed earliest in the summer (August) when temperature and food densities are high.

(2) recovery of scallops will decline with the onset of sea star settlement.

(3) deployment of scallops set directly on substrate and placed in pearl nets will allow acceptable growth and recovery.

## **3.2 Materials and Methods**

### **3.2.1 Study Site**

Shell Fresh Farms Ltd. in Pool's Cove, NF, was the study site (see Section 2.2.1). Deployment of scallops was at Ladder Garden, North Bay. However, water quality was measured at Ladder Garden, The Run and Fox Point (Figure 2.1).

### **3.2.2 Deployment Date Study**

#### *Experimental Design and Set-up*

This study was designed to determine when the window of opportunity of deployment was for nursery-sized scallops at a farm-based nursery. To do this, scallops were deployed over consecutive treatment intervals from the time they were large enough to go out of the hatchery until the conditions became poor late in the autumn. During the intervals, water samples at the nursery were analyzed weekly for temperature and food quality and quantity.

This study commenced as soon as scallop spat greater than 1.4 mm in shell height were available from the Belleoram Sea Scallop Hatchery (BSSH). Scallops were screened between 1.4 and 2.0 mm in shell height. Initial scallop shell height was sampled (see Section 2.2.2).

Scallops were deployed at 500 spat/collector in 1.2 mm collector bags held in

bread trays at 5 m depth. The number of replicates varied from two to four, depending on scallop availability. The intervals began on August 4, August 22, September 7, September 26 and October 26. Intervals ranged from 16 to 23 days and depended on site accessibility. Each interval ended when the next began and the final interval ended on November 8, 1997.

### *Sampling Protocol and Environmental Monitoring*

At the end of each interval, scallops were counted for recovery and measured for shell height ( $n=30$ ). Scallops were re-deployed and measured again for shell height on November 8, 1997 and June 24, 1998.

Over the study, phytoplankton, total particulate matter (TPM), particulate inorganic matter (PIM), particulate organic matter (POM), and chlorophyll-*a* were sampled at 5 m depth. Temperature and salinity were measured through the water column to a depth of 10 m. Sea star settlement was also monitored throughout the study. Each parameter was sampled weekly during the short-term intervals at Ladder Garden, The Run and Fox Point.

Immediately after collection, phytoplankton samples were fixed (Appendix 3.1). Samples then sat undisturbed for at least two weeks for settling of algal particles. The top 90% of water was siphoned off and volume was measured. The remaining volume, which contained all settled algal particles, was also measured. This concentrated volume was mixed thoroughly and 10 mL was transferred to a 10 mL Utermohl settling chamber for overnight settlement. The sample was analyzed for total number of cells and species



present on a Zeiss Axiovert 35® microscope under phase contrast at 400X magnification. Cells were counted across transects until at least 300 cells were counted. The number of grids counted was noted in such case that the entire transect was not counted.

Calculations were based on the number of grids counted (Appendix 3.2).

Plankton samples were fixed and analyzed weekly (Appendices 3.1-3.2). Total plankton was divided into 8 major groups. Seven of these were on the basis of size while the final group was unidentified species. The size categories included microzooplankton including tintinnids and ciliates ( $>20\ \mu\text{m}$  in diameter), autotrophic and heterotrophic dinoflagellates (12 to  $60\ \mu\text{m}$ ), prymnesiophytes comprising small (2 to  $12\ \mu\text{m}$  in diameter) spherical nanoflagellates, auto-nanoflagellates comprising spherical flagellates from 2 to  $20\ \mu\text{m}$  in diameter, cryptophytes comprising small (8 to  $18\ \mu\text{m}$  in length) tear-drop shaped biflagellates, centric diatoms (12 to  $30\ \mu\text{m}$  in diameter, connected in long chains), and pelagic pennate diatoms ( $30\ \mu\text{m}$  in length, single cells). Phytoplankton were categorized according to Rott (1981).

For total particulate matter (TPM) and chlorophyll-*a* samples, 15 L of water from the three areas of the farm were pumped from 5 m and pre-screened at  $300\ \mu\text{m}$  into separate 20-L buckets and taken to the hatchery. Whatman GF/C 45 mm diameter glass microfibre filters had been previously combusted in a muffle furnace at  $500^\circ\text{C}$  for 4 hours to remove any carbon and pre-weighed. For the TPM, 4 L of water were vacuum filtered on 45 mm glass fibre filters in Nalgene filtration stands. The filters were frozen at  $-20^\circ\text{C}$  until further analysis could occur. The filters were oven dried at  $80^\circ\text{C}$  for 24 hours. They were then weighed for dry weight. The filters were transferred to a muffle furnace for 4 hours at  $500^\circ\text{C}$ . They were weighed again for ash-free dry weight. From

these weights TPM, PIM and POM were calculated (Appendix 3.3).

Water was filtered for chlorophyll-*a* and phaeopigment and measured accordingly using methods of Strickland and Parsons (1968) and Parrish et al. (1995). Calculations for the concentrations of chlorophyll-*a* and phaeopigment can be found in Appendix 3.4.

Sea star settlement was monitored weekly from July 15 to November 22, 1997, by deploying a string of eight pearl nets at the three areas. Each string of pearl nets was retrieved after about two weeks depending on site access. Individual pearl nets were washed and all debris greater than 250  $\mu\text{m}$  was collected and preserved in 40% methanol. Samples were analyzed on a dissecting microscope for numbers of sea stars present.

Temperature and salinity profiles of the three areas of the farm were measured weekly using a YSI Model No. 30 S-C-T Meter.

### *Data Analysis*

Variations in growth rates and recovery were analyzed using an ANOVA while equality of means was analyzed using an Independent sample t-test. Correlation analyses were also performed with growth and recovery and environmental conditions.

### 3.2.3 Remote Set Study

#### *Experimental Design and Set-up*

This investigation was designed to give preliminary results for remote setting practices with the sea scallop. To do this scallops were set on substrate that was eventually transferred in gear to the farm-based nursery. One type of substrate (6 mm Vexar®) and gear type (3.0 mm mesh pearl nets) were studied.

The largest scallop larvae from a spawn on May 18, 1998, were screened and placed in a 6000-L tank on June 18, 1998 (4 million larvae). Thirty-two pieces of 12.5 cm x 12.5 cm Vexar® were suspended in the tank as settling substrate. The water temperature and food density were maintained at 15-16°C and 15-30 cells/ $\mu$ L. Fifty percent of water in the tank was changed twice a week. On June 29, Vexar® pieces were removed from the tank. Eight pieces were randomly selected and sampled for number of spat present and shell height. The remaining twenty-four were placed four each in six pearl nets. The pearl nets were placed in tanks and taken by boat to the farm-based nursery. Three pearl nets were deployed and the other three were sampled for loss of scallops due to handling.

#### *Sampling Protocol and Environmental Monitoring*

Scallops were retrieved from the study site on July 31, 1998. Vexar® squares were sampled for number of spat present and spat shell height.

Water quality was sampled with a Seabird CTD meter at the onset of the remote set study and when scallops were sampled for final recovery and growth rates. Plankton was also sampled weekly for this study.

### *Data Analysis*

Replicate data were analyzed for differences by ANOVA. Initial, handling and final shell heights and numbers present were tested by ANOVA.

### 3.3 Results

#### 3.3.1 Deployment Date Study

##### *Growth Rates*

Initial shell height replicates for all dates were not significantly different ( $P>0.01$ ) except September 7 (One-way ANOVA;  $F=9.735$ ,  $d.f.=2, 87$ ,  $P<0.001$ ). This was because the scallops in one replicate were leftover from another experiment and they were not randomly chosen for the experiment hence the data were not used for analysis.

Final interval shell heights were significantly different from initial shell heights (Table 3.1; Figure 3.1). Final interval shell heights were significantly different due to deployment date (One-way ANOVA;  $F= 556.621$ ,  $d.f.=4, 445$ ,  $P<0.001$ ). By November 8, mean shell heights for the scallops deployed on August 4 and August 22, had exceeded the 7.0 mm shell height required for transfer to intermediate growout. By June 24 of the following year, the scallops deployed on September 7 and September 26 had passed this shell height. The scallops deployed on October 19 were  $3.3 \pm 0.13$  mm in shell height by June 24.

Growth rates declined over the short-term intervals (Figure 3.2). Significant differences were found between growth rates for the different intervals (One-way ANOVA;  $F= 95.162$ ;  $d.f.=4,11$ ,  $P<0.001$ ). Highest growth rates occurred during the first interval at  $118 \mu\text{m}/\text{d}$  while the lowest growth rates occurred during the last interval at  $3.3 \mu\text{m}/\text{d}$ . The mean growth rate of spat was  $43.2 \mu\text{m}/\text{d}$  from August 4 to November 8, 1997.

Growth rates of scallops over the long term studies showed highest growth in the earliest deployment (Figure 3.3). For scallops deployed on August 4 and 22, growth rates were high until November 8. Growth rates declined over the winter to those of scallops deployed from September 7, 1997, to June 24, 1998, at  $42.4 \mu\text{m/d}$ . Scallops deployed on September 26 and October 19, had lower rates to November ( $11.4$  and  $3.3 \mu\text{m/d}$ , respectively) and to June ( $21.5$  and  $7.2 \mu\text{m/d}$ , respectively).

### *Recovery*

Percent recovery declined over all intervals (Figure 3.2). Variation in recovery rates was also due to deployment interval (One-way ANOVA;  $F=47.129$ ,  $d.f.=4,11$ ,  $P<0.001$ ). Highest recovery was in samples deployed during the first interval while lowest was in the samples deployed on September 26. Recovery on November 8 was not significantly different than after the intervals (Paired t-test;  $t=0.013$ ,  $d.f.=14$ ,  $P=0.990$ ; Figure 3.3).

### *Water Quality*

Temperature declined over the study period (Figure 3.4a). The first interval had the highest temperature at  $15.8^\circ\text{C}$ ; the final interval the lowest at  $7.2^\circ\text{C}$ . There was no significant difference among the three areas of the site (One-way ANOVA;  $F=0.011$ ,  $d.f.=2, 39$ ,  $P=0.989$ ). The mean weekly temperature from July to November was  $11.7^\circ\text{C}$ .

Salinity increased over the study period (Figure 3.4a). Mean weekly salinity was

28.3 while the range was from 26.5 to 31.5 at Ladder Garden. Salinity was similar among the three areas of the site (ANOVA;  $F=0.500$ ,  $d.f.=2, 39$ ,  $P=0.610$ ).

Seston was analyzed for chlorophyll-*a* and phaeopigment concentration, particulate organic matter (POM), and phytoplankton density (cells/L). Significant differences existed for the chlorophyll-*a* (One-way ANOVA;  $F=5.732$ ,  $d.f.=2, 36$ ,  $P=0.009$ ), phaeopigment (One-way ANOVA;  $F=5.555$ ,  $d.f.=2,36$ ,  $P=0.008$ ), and POM (One-way ANOVA;  $F=9.621$ ,  $d.f.=2, 33$ ,  $P=0.001$ ) among the three areas of the farm. Chlorophyll-*a* concentrations (One-way ANOVA;  $F=0.544$ ,  $d.f.=14, 24$ ,  $P=0.881$ ), phaeopigment concentrations (One-way ANOVA;  $F=0.500$ ,  $d.f.=14, 24$ ,  $P=0.910$ ), and POM (One-way ANOVA;  $F=0.715$ ,  $d.f.=14, 21$ ,  $P=0.737$ ) were similar over dates.

Particulate matter remained constant at Ladder Garden. The weekly mean was 5.6 mg TPM/L at Ladder Garden which was the lowest of the three areas of the farm. Particulate organic matter was also constant at Ladder Garden with a mean of 1.9 mg POM/L. This was comparable to Fox Point, but lower than The Run.

Chlorophyll-*a* declined slightly at Ladder Garden while phaeopigments remained fairly constant. Chlorophyll-*a* and phaeopigments averaged 2.4 and 10.1  $\mu\text{g/L}$ , respectively, at Ladder Garden, which was higher than at Fox Point or The Run.

There were no significant differences in the total phytoplankton density among sites (One-way ANOVA;  $F=0.895$ ,  $d.f.=2, 39$ ,  $P=0.417$ ), but there was a significant difference in total phytoplankton density among weeks (One-way ANOVA;  $F=7.084$ ,  $d.f.=13, 28$ ,  $P<0.001$ ; Figure 3.5). The total density peaked around the middle of August followed by a decline which becomes very evident when observing the mean density over the intervals (Figure 3.6). This was the result of auto-nanoflagellates, pelagic pennate

diatoms, and dinoflagellates (Figures 3.7 a and b). The species that contributed to the peak were *Navicula* sp. 1, *Chlamydeomonas* sp., *Ochromonas* sp., *Micromonas* sp., *Rhizosoleni* sp., *Dinophysis* sp., coccolithophore sp., *Prorocentrum* sp., choanoflagellate sp., and *Strobilidium minimum* (Figures 3.8 a and b). Analysis of percent biovolume indicates no distinct pattern (Figure 3.9) although peaks in the different groups were obvious. Percent abundance of phytoplankton size indicated that species  $<5 \mu\text{m}$  were continuously contributing to phytoplankton biovolume (Figure 3.10). Unidentified species increased over time while the abundance of particles 10-20  $\mu\text{m}$  in diameter or length decreased.

Sea star settlement peaked between September 19 and October 23 (Figure 3.11). There were significant differences in sea star settlement among the three areas of the site (Two-way ANOVA;  $F=42.285$ ,  $d.f.=2, 336$ ,  $P<0.001$ ) and over the different sampling dates (Two-way ANOVA;  $F=99.674$ ,  $d.f.=13, 336$ ,  $P<0.001$ ). The highest settlement was at Ladder Garden, the location of the farm-based nursery with an overall average of 79 sea stars per collector per day (ss/coll/d). This was twice the settlement at Fox Point which averaged 39 ss/coll/d and higher than The Run where settlement averaged 62.5 ss/coll/d.

Most of environmental factors were highly correlated to growth and recovery rates (Tables 3.2 and 3.3). TPM and dinoflagellates did not correlate with growth rates.



### 3.3.2 Remote Set Study

#### *Shell Heights*

There were significant differences between shell heights treatments (One-way ANOVA;  $F=933.514$ ,  $d.f.=2,20$ ,  $P<0.001$ ). Tukey's-B test results however, indicate that the initial and handling shell height are statistically similar and that both are lower than the final shell heights. Overall growth rate of scallops in the remote set study was  $31.3 \mu\text{m/d}$  from June 29 to July 31, 1998 (Figure 3.12).

#### *Recovery*

Due to the low numbers of samples, there were significant differences in number of scallops present during the initial, handling and final sampling of scallops (One-way ANOVA;  $F=5.375$ ,  $d.f.=2, 20$ ,  $P=0.014$ ). Final recovery was  $<50\%$  (Figure 3.12). More initial, handling and final sample counts were needed to verify the statistical significance of the relationships between the three sample periods.

#### *Water Quality*

Water temperature and food densities both rose over the deployment period (Figure 3.13). Temperature increased from  $8^{\circ}\text{C}$  on the day of deployment to  $14^{\circ}\text{C}$  on the day scallops were sampled. Chlorophyll-*a* concentration rose from  $2.7$  to  $3.7 \mu\text{g/L}$  over

the study (Figure 3.13). Phytoplankton density had declined over the spring from peak quantities in April. During the remote set trial the phytoplankton densities increased slightly over the June to July period (Figure 3.14). Examination of biovolume contribution by major groups of phytoplankton indicated that microzooplankton had a decreasing abundance during this period while the auto-nanoflagellates had an increasing abundance (Figure 3.15). Size distribution during the month of July was mainly due to phytoplankton less than 10  $\mu\text{m}$  in the greatest dimension (Figure 3.16).

## **3.4 Discussion**

### **3.4.1 Growth Rates**

Growth rates exhibited variation in the present study. Growth rates of scallops deployed in five consecutive intervals from August to November were found to decline. This was expected according to the hypothesis investigated. Growth rates of scallops in remote set on Vexar® were acceptable for commercial practices.

Other observations less pertinent to the factors studied here were made with respect to the growth rates in the previous and other non-related studies. First, growth rates in these studies of 1.4 to 1.9 mm shell height scallops were higher than in the initial size-depth study (Chapter 2) which indicates higher sea scallop growth rates in the summer than the winter. Second, growth rates were lower than June-spawned wild-collected spat cohorts, but comparable to September-spawned wild-collected spat cohorts in Nova Scotia (Dadswell and Parsons 1992a,b). This suggests that growth rates were not maximal in this study. However, they were better than in previous production seasons, are commercially acceptable, and have the potential to improve as will be discussed.

Differences in the mean interval growth rates may be explained by many factors. The most important may be temperature and food availability as well as changes in these factors from the hatchery to the farm-based nursery.

### 3.4.2 Recovery Rates

Recovery is important in maintaining reliable numbers of scallops at time of sale to growers. Remote set practices may utilize less time and resources in a hatchery, however, may have the potential to produce more transferrable scallops to the nursery than practices that grow scallops to a larger size before transfer. For this reason, lower recovery of remote set scallops may be more acceptable than nursery culture of larger scallops depending on the financial obligations involved. The deployment date study indicated that variations in recovery were due to deployment time. The decline over time in recovery was expected. Unfortunately, the lowest recovery rates could have a tremendous impact on a hatchery operation. Recovery of scallops from the remote set study was acceptable, however, initial settlement was low for commercial practices. An improvement in initial numbers of scallops set on remote set equipment is necessary before variation in remote set recovery can be assessed.

Observations of recovery with respect to the other previous nursery studies indicate some findings not relevant to the factors examined here. First, final recovery is higher in these studies than similar sized scallops in the initial size-depth study (Chapter 2). Second, recovery was higher in scallops deployed in August than those deployed in September, October or in June-July (remote set study). These findings suggest that summer deployments have fewer impediments to survival. Third, recovery of bay scallops in similar practices in Nantucket have recovery of approximately 70% (R. Garrison, pers. comm.), and recovery of remote set Japanese scallops is higher than when spat are hatchery reared to a larger size before transfer to the ocean (Bourne and Hodgson

1991). Studies with wild collected sea scallop spat report collected numbers, but not usually an initial and final recovery (Cliche and Guiguère 1994; Parsons et al. 1996). Although the majority of remote set data is for scallop species with different ecology, the higher recovery for these species suggests that there may be opportunity for improved recovery using remote set practices with the sea scallop.

### 3.4.3 Effects of Deployment Date on Growth Rates and Recovery

Temperature, food availability, and sea star settlement exhibited obvious changes throughout the deployment study. In studies of the sea scallop, temperature and food have been the main predictors of growth (Dadswell and Parsons 1991; Côté et al. 1993). Sea stars are the main predator of scallops (Dadswell 1989; Barbeau and Scheibling 1994a). Changes in these parameters may best explain the variation in growth and recovery of the scallops over the different deployment intervals.

Temperature and food availability declined from August to November while sea star settlement increased during the deployment date study. The temperature and food availability increased from June to July in the remote set study. These results are similar with those of Parrish et al. (1995) at South Broad Cove, NF, and Penney and MacKenzie (1996) in Bonavista Bay and Notre Dame Bay, NF, respectively. Variations in temperature and food availability during the deployment date and remote set study were similar to those found in Conception Bay, NF, and Bedford Basin, N.S. (Mayzaud et al. 1989; Navarro and Thompson 1995).

A negative correlation of salinity with growth and recovery rates of scallops in the

deployment study may have been a coincidence as the natural range of tolerance for juvenile sea scallops to salinity is 18 and greater (Bergman et al. 1996) which is lower than the salinity during the present study. The increase in salinity over the study period is representative of the decrease runoff and the increased upwelling that occurs in the autumn. Salinity was adequate for scallops in the remote set study also.

### *Effect of Temperature on Growth Rates and Recovery*

Several metabolic processes of scallops are temperature-dependent thus influence growth rates. Christophersen (1997) found that *Pecten maximus* spat deployed when temperature was  $>10^{\circ}\text{C}$  exhibited up to four fold increase in survival compared to scallops deployed at temperatures  $<10^{\circ}\text{C}$ . Metabolic rates in *Pecten fumatus* decline with decreasing temperature (Cropp and Hurtle 1992). Respiration rates in sea scallops decrease with declining temperature (Shumway et al. 1988). Clearance rates are correlated with ambient temperature in sea scallops as well as in the eastern oyster, *Crassostrea virginica*, and the bay scallop, *Argopecten irradians* (MacDonald and Thompson 1986; Rheault and Rice 1996).

Decreases in metabolic processes due to declining temperature may explain why reduced growth rates were observed in scallops deployed on different dates in this study. Mean temperatures for the five consecutive deployment intervals were  $14.73^{\circ}\text{C}$ ,  $13.57^{\circ}\text{C}$ ,  $11.28^{\circ}\text{C}$ ,  $11.23^{\circ}\text{C}$ , and  $7.90^{\circ}\text{C}$ . Each group of scallops deployed may have exhibited metabolic rates consistent with the ambient temperatures which in turn resulted in lower respiration and feeding rates during each interval. This may have been reflected in

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determined by the type of food particles or species present, not particle flux or quantity (Cranford and Grant 1990; Grant 1996). Sea scallop diet quality is based on several parameters including C:N ratio and presence of specific organic components including the essential fatty acids (FA), docosahexaenoic acid (DHA; 22:6 $\omega$ 3) and eicosapentaenoic acid (EPA; 20:5 $\omega$ 3) for membrane fluidity (Enright et al. 1986; Grant and Cranford 1991; Parrish et al. 1995; Penney and McKenzie 1996).

Sea scallop growth and diet are related. This relationship is based on food availability and quality. Scallops do not necessarily grow when and where food densities are high which is a similar behavior as in the tellinid bivalve *Macoma balthica* (MacDonald and Thompson 1986; Beukema and Cadée 1991; Parrish et al. 1995). Dietary quality, defined as the inversed inorganic content, is the best predictor of absorption (Cranford 1995). When inorganics increase, dietary quality is reduced which causes an exponential decrease in absorption efficiency of POM, particulate organic carbon (POC) and particulate nitrogen (PN; Cranford 1995). This causes sea scallops to maintain clearance rates, but decrease sorting efficiency due to energy requirements which are different from *M. edulis* which under similar conditions would increase gut fullness and absorption efficiency (Newell et al. 1989; MacDonald and Ward 1994). Carbon and nitrogen requirements of sea scallops, which affect somatic and gonadal growth, are determined by temporal changes in food quality (Grant and Cranford 1991). Living phytoplankton also have higher C:N ratios than detritus thus making it a better quality diet (Grant and Cranford 1991; Penney and McKenzie 1996). Certain phytoplankton species tend to dominate in the gut of sea scallop, and thus are assumed to contribute to energy intake of sea scallops (Shumway et al. 1987). Fatty acid profiles are



good for determining egg, larval and somatic tissue conditions which would suggest their importance in the health of bivalves (Napolitano et al. 1992; Farias et al. 1998). Other bivalves including *Ostrea edulis* and *Crassostrea gigas* exhibit the best growth when fed high dietary quantities of 22:6 $\omega$ 3, 20:5 $\omega$ 3, and carbohydrate and low protein, however, little work has been done on the nutritional composition of sea scallop diets (Enright et al. 1986; Thompson and Harrison 1992).

Within different phytoplankton groups variability in biochemistry exists (Enright et al. 1986; Volkman et al. 1989; Viso and Marty 1993). Specific biochemical assays were not performed on the scallops or phytoplankton for this study. However, deductions were made on the biochemical composition (and subsequent influence on growth and recovery of scallops) for plankton groups present at the nursery site from other studies. Further studies should be performed to determine the validity of the following speculations.

*Nitzschia* sp., *Navicula* sp., *Skeletonema costatum*, *Prorocentrum* sp., *Dinophysis* sp., and *Thalassiosira* sp. are six species that Shumway et al. (1987) found in adult sea scallop gut that were found in the water column during July to November of this study. Shumway et al. (1987) considered that many smaller flagellated species were present, but had been digested faster hence specific presence could not be established. Of the species present in Shumway et al. (1987) and present in this study, *Prorocentrum* sp. (30  $\mu$ m in diameter) and *Dinophysis* sp. (44  $\mu$ m in diameter), which are assumed to contribute to the energy intake of sea scallops (Shumway et al. 1987), showed decreasing abundance from August to November in this study. *Navicula* sp. 1 (14  $\mu$ m in length), which was the most abundant species in the scallop gut in Shumway et al. (1987), also exhibited such a

decrease. This suggests that sea scallops deployed in the earliest of the consecutive intervals may have been exposed to diets with a higher measure of energy thus resulting in more energy being used for growth.

Another group exhibiting peak abundance in August was the Cryptophytes. This group of phytoplankton is high in 22:6 $\omega$ 3 and 20:5 $\omega$ 3, which are essential for membrane fluidity in bivalves (Enright et al. 1986; Volkman et al. 1989; Napolitano et al. 1992; Viso and Marty 1993; J. Hall, pers. comm.). Cryptophytes are preferred by sea scallops in mixed species diets and also correlate with sea scallop growth (Shumway et al. 1985; Parrish et al. 1995). High densities of autotrophic nano-flagellates rich in 22:6 $\omega$ 3, including cryptophytes, occur during pre-spawning periods for sea scallops thus may indicate the importance of this fatty acid to the development of eggs (Penney and McKenzie 1996). Mayzaud et al. (1989) found that the dominance of small flagellates in Bedford Basin as the summer progressed (early August) coincided with peaks in protein, carbohydrate and lipids especially the fatty acids, 22:6 $\omega$ 3, 20:5 $\omega$ 3 and 18:5 $\omega$ 3. Healthy phytoplankton populations are associated with essential polyunsaturated fatty acids (PUFA) which are important for proper development (Mayzaud et al. 1989). Phytoplankton populations in Conception Bay, NF, also exhibit dominance of small phytoplankton with high lipids during August (Navarro and Thompson 1995). Higher growth in scallops deployed when cryptophytes were more abundant, specifically in August and early September, was not unexpected.

Recovery of scallops over the different deployment dates correlated with the various densities of food available at the farm-based nursery. It is likely that scallops were able to survive during the lower food densities unless they stopped feeding

altogether. We know, however, that scallops did feed as growth occurred, but not in all scallops, and some scallops even died. This may suggest that although all scallops were feeding some may have been unable to fulfill basal metabolic requirements from food they were eating thus causing them to perish or not grow at all. This will be discussed later in this chapter.

### *Predation Effects on Growth Rates and Recovery*

There was a high negative correlation between recovery and sea star settlement during the short-term intervals. Increasing sea star settlement coupled with declining sea scallop recovery was expected (Dadswell and Parsons 1992a,b; Barbeau and Scheibling 1994a; Gaudet 1994). Successful predation may be due to the similar size of the settling sea stars and scallops as well as debilitation caused by the temperature changes between hatchery and nursery environments (Dickie 1958; Barbeau and Scheibling 1994a).

Because of their small size, all scallops may have been equally vulnerable to predation. Small scallops, although attempted less often are more vulnerable to sea star predation due to their lack of escape mechanisms (Barbeau and Scheibling 1994b). O'Neill et al (1983) found that smaller sea stars were more efficient predators of small mussels. In the 1.4-2.0 mm scallops in the present study, it is expected a swim response would occur when faced with predatory attack, however, this is only speculation based on personal observation. Although sea star settlement coincided with declining growth rates there was no effect on growth as there was no difference in size of empty shells compared to the size of live scallops (pers. obs.). Further research is needed to determine the

response nursery -sized scallops have to predation as well as the size most susceptible to sea star predation.

Dickie (1958) observed a lack of mobility of scallops for about a month when they were exposed to drops of 4-7°C in ambient temperature which he speculated may be detrimental if predators are unaffected. Temperature debilitation may have coincided with highest mortality of scallops in the deployment study which was during the period of peak sea star settlement on the culture gear. High mortality may have resulted from sea star predation on scallops that were unable to escape due to the physiological inhibition caused by the deployment in colder water. Sea star predation was not likely a factor in the remote set study as sea stars were settling in September during the previous year.

Deductions can be made from these findings regarding recovery in the initial-size depth study (Chapter 2) and the importance of timing of deployment. The size-related predation and decreased sea star predation as temperature drops <5°C may explain why recovery was higher in larger scallops as they were less vulnerable to predation as well as why predation leveled off over the winter because temperature became debilitating to sea stars. Predation by bottom-dwelling sea stars has been avoided by using suspension culture, however, sea stars can settle on and penetrate suspended equipment in late summer to early autumn (Dadswell and Parsons 1991; Gaudet 1994). This emphasizes the importance of timing in deployment of nursery-sized scallops due to the vulnerability of scallops to sea star predation (Dadswell 1989; Dadswell and Parsons 1991).

### *Importance of Acclimation on Growth Rates and Recovery*

Deployment of scallops is complicated by the differences in food and temperature between the hatchery and the nursery. All scallops at the hatchery had been held at 15°C and fed a mixed diet of cultured algae, however, each group was deployed at lower temperatures and food densities. Scallops should have been able to succeed in the nursery as they are able to live within -2 to 18°C and control absorption efficiency to acclimate to diet quality (MacDonald and Ward 1994). However, sudden changes in temperature and food densities, such as those between hatchery and nursery environments, may decrease growth rates and recovery due to inhibition of responses which occurs when they can not acclimatize to the changes (Thompson 1984; Cranford and Grant 1991; Côté et al. 1993). Detachment from substrate during temperature shock may cause loss through mesh or crowding in corners which can also reduce recovery (Bourne et al. 1991).

Acclimation of bivalves has been studied to a limited extent. Christophersen (1997) found that *Pecten maximus* spat deployed after temperature acclimation had better growth and survival than non-acclimated spat. Widdows and Bayne (1971) examined oxygen consumption, filtration rate and assimilation efficiency in the blue mussel *Mytilus edulis* with respect to acclimation. They found that mussels transferred from a 10°C environment to a 5°C environment take 14 days to completely acclimate oxygen consumption, filtration rates and assimilation efficiency to those of the 10°C exposure. They found that during the initial acclimation the energy equilibrium becomes imbalanced and to re-establish it energy reserves are mobilized and utilized to balance the

energy. Hall (pers. comm.) found that it takes 15 to 21 days to acclimate sea scallops from 15 to 5°C. During this period of acclimation, lipid profiles are adjusted such that unsaturated fatty acids, particularly 20:5 $\omega$ 3, become more abundant in the membranes. The increase in time necessary to acclimate as indicated by the differences in these studies may be due to greater differences in the temperatures or species requirements.

Observations made in this study support the increase in time necessary to acclimate when temperature differences are increased. Temperature differentials from the hatchery (15°C) to the nursery over these five intervals increased progressively by 0.27°C, 1.43°C, 3.72°C, 3.77°C, and 7.1°C. Growth rates in corresponding intervals were consistently lower suggesting that more time was needed to acclimate.

The purpose of acclimation is not completely understood, however, it appears to relate to maintenance of physiological functioning at the cellular level. Several aspects of metabolism at the cellular level may be affected, however, one aspect that relates to the importance of diet quality is the maintenance of the phospholipid membranes. Ultimately, the alteration of lipids, which are the structural elements at the cellular level, occurs in response to temperature changes, however, the process is not well understood (Hazel 1988; Hazel 1995). This restructuring is necessary because specific fatty acids are necessary in lower temperatures to maintain membrane fluidity. Lower density unsaturated fatty acids have a greater degree of expansion at lower physiological temperatures thus do not solidify as is the case for higher density saturated fatty acids which have a lower degree of expansion at lower temperatures (Lands and Davis 1983). Because temperature modifies the phase of membrane lipids which in turn affects the rate of movement of molecules through cell membranes, it is expected that processes occur

within the membrane during temperature changes to maintain adequate functioning.

Temperature controlled lipid profiles in cell membranes of scallops have been found. Napolitano et al. (1992) found that it is adjustments to compensate for temperature differences which determine lipid profiles rather than actual seston biochemistry. Seston is important in supplying adequate specific fatty acids present when acclimation occurs.

Lipid profiles are used as indicators of nutritional and physiological condition in marine animals (Martinez 1991; Napolitano et al. 1992). Differences exist in lipid profiles of sea scallops at deep or cold sites compared to shallow or warm sites. Scallops in colder environments have higher 22:6 $\omega$ 3 in egg phospholipids and 24-methylene-cholesterol in the adductor muscle than scallops in warmer environments (Napolitano et al. 1992). Bivalves held in colder temperatures also exhibit higher concentrations of 20:5 $\omega$ 3 in their cellular membranes (J. Hall, pers. comm.). Napolitano et al. (1992) observed this in egg phospholipids also, but not to a significant degree.

This suggests that in the present study scallops deployed earlier and later than August, when phytoplankton rich in 22:6 $\omega$ 3 are low in abundance, may take longer to adjust to the temperature because they cannot access the organic compounds they need from their diet. The scallops deployed with little temperature difference and a high quality diet may have been able to acclimate as the temperature decreased because they had access to essential nutrients in their diet.

Sea scallops may require reserves also i.e., in times of food depletion, however, in the hatchery food quality may not have provided the adequate reserves necessary to face a changing environment. At the time of transfer, the phytoplankton species that are high in 22:6 $\omega$ 3 and 20:5 $\omega$ 3, fatty acids presumed to be essential for acclimation, also declined in

abundance during this period. This has implications for scallops newly introduced to this different environment. Scallops that were deployed early were exposed to little temperature difference and high quality food for growth and maintenance. The scallops deployed on all consecutive intervals were exposed to consistently lower temperature and food quantity and quality which would explain consistently lower interval growth rates. The remote set scallops were deployed at low, but increasing temperature and food quality. This may have impacted physiological condition which was reflected in poor recovery and lower growth. As well, the improved growth after longer exposure for the remote set scallops deployed in July, supports the idea that environmental quality is highest in August for nursery culture of sea scallops.

The need for specific dietary components when exposed to a new environment of lower temperature supports the importance of timing of deployment of scallops. In this study, scallops that were deployed earlier than August in another study, exhibited a faster acclimation as indicated by the much higher growth rates of  $156 \mu\text{m}/\text{d}$  as sampled at the end of September which was even higher than those scallops that had gone out in August (C. Couturier, per. comm.). The scallops deployed later in August and September were introduced to a gradually decreasing temperatures and food densities thus adjusted their membranes although slowly, and were not able to get growth rates much over  $40 \mu\text{m}/\text{d}$  over the winter. The scallops deployed in late September and October did acclimate, but poorly as indicated by their lower winter growth rates as compared to those of the scallops deployed earlier. This suggests that deployment until mid-September allows scallops to acclimate and have acceptable winter growth. Scallops deployed later require more time to acclimate thus are not able to attain acceptable winter growth rates to allow



them to reach intermediate size by following June. Christophersen (1997) found that *P. maximus* spat that were temperature acclimated for 1 and 3 weeks before deployment in sub-optimal farm-based nursery conditions exhibited no difference in survival which suggests that more than just acclimation to temperature is required. If overwintering scallops survive, it may not be until the presence of fresh carbon and nitrogen and high temperatures in late spring that high growth rates are stimulated (Shumway et al. 1987).

Physiological shock may have contributed to increased mortality of scallops. If the scallops were not able to attain the organic compounds from their diet or reserves for their cells to function properly they may have become stressed to the point of poor function. This stressed state may increase natural mortality or increased vulnerability to predators as mentioned earlier. When exposed to sudden changes in temperature marginally-sized, e.g., remote set-size, scallops also may debyss and fall out of equipment (Bourne and Hodgson 1991). This indicates the importance of determining the necessary biochemical composition of diet in the hatchery as well as in the farm-based nursery.

The need for acclimation is obvious. Gradual exposure to lower temperatures combined with a diet rich in essential fatty acids may improve growth rates and recovery of scallops transferred to a farm-based nursery during sub-optimal conditions.

### **3.5 Conclusions**

Growth rates and recovery of nursery sized-scallops were influenced by time of deployment at a farm-based nursery during a period that spanned from early summer to late autumn. This was expected. Growth rates and recovery of remote set spat on Vexar® were acceptable also as expected.

Highest growth rates and recovery of nursery-sized scallops were observed during August and early September when the nursery site was characterized by high food densities and temperature and when sea star settlement was low. Remote set scallops deployed in late June were also able to increase growth rates over the summer even though initial growth rates were low. However, scallops deployed in September and October had low recovery as well as low growth rates until the following spring or later.

The ability of nursery-sized scallops to grow and survive may be related to the differences between hatchery and farm-based nursery environments and whether food quality provided at the hatchery is adequate in providing the reserves required to meet the physiological requirements in acclimating to the new environment. There is a need to determine the nutritional requirements of remote set and nursery-sized scallops. As well research is needed to develop acclimation protocols by way of developing a hatchery diet rich in essential fatty acids, and gradually introducing scallops to a changing environment. Improvements in protocol are necessary to increase the settlement densities of remote set scallops on Vexar® before it is used as a commercial practice.

## **Chapter Four:**

### **Toxicity of Ammonia to Sea Scallop, *Placopecten magellanicus*, Spat**

## 4.1 Introduction

Culture of aquatic organisms in closed systems, including hatcheries, requires continuous monitoring of water quality. Many water quality parameters are closely related thus when one becomes problematic, often others are affected. This can create deleterious effects to the animals being cultured. Of interest in this study are the effects of ammonia on sea scallop spat reared under hatchery temperature and pH conditions.

Ammonia is a nitrogenous compound that is present in very low concentrations in the ocean (Carpenter and Capone 1983). In this aqueous form, total ammonia nitrogen (TAN) consists mainly of ionized ammonium,  $\text{NH}_4^+$  (94-98%), and very little is in the un-ionized form,  $\text{NH}_3$  (UAN; 2-6%; Carpenter and Capone 1983). Despite its low concentration, the UAN is toxic due to its permeability across cellular membranes.

The amount of UAN in the water depends on the amount of TAN as well as the temperature and pH (and salinity, to a lesser extent). Emerson et al. (1975) determined the percent UAN of TAN at 0 to 30°C and at pH 6.0 to 10. Percent ionization decreases as temperature and pH increase. Using temperature and pH values and methods by Widdows (1985) to measure TAN concentrations, the percent UAN can be calculated.

In closed culture systems ammonia may be derived from animal wastes and bacterial breakdown of food. Scheller (1997) found dying populations of clams produce ammonia as well as bacterial breakdown, which suggests that mortality events are point sources of ammonia also. Ammonia is the main nitrogenous waste product of sea scallops, however, excretion rates are low such that in culture settings no obvious effects of toxicity occur within a short period of exposure (Strickland 1993). Excretion

combined with high food density and regular mortality of at least a small portion of a tank population suggest that these sources of ammonia under high density situations could potentially create problems especially if tolerance is unknown.

Exposure to ammonia at toxic concentrations can cause serious physiological damage. Ammonia affects behavior, feeding ability and oxygen consumption thus growth of aquatic organisms (Rasmussen and Korsgaard 1996; Harris et al. 1998). In lobsters, ammonia tolerance increases with age due to a reduction in osmoregulation (Young-Lai et al. 1991). It is not known how age affects ammonia tolerance in sea scallops.

Lethal concentrations required to kill fifty percent of the exposed population ( $LC_{50}$ ) have been determined for several species over acute exposures of 96 hours as defined by Epifanio and Srna (1975). They are useful to know when holding animals in a situation in which ammonia concentration may increase.

Culture of sea scallops in a hatchery situation may require holding animals in batches of water for three to four day periods. The purpose of this study was to determine what the lethal ammonia concentrations to scallop spat are under normal hatchery conditions for a four-day exposure period. The hypotheses of this study were:

- (1) For the ammonia concentrations tested, after 96 hours exposure, if ammonia is toxic to scallops, then a higher mortality will occur with increasing dose.
  
- (2) For the two size classes tested, if ammonia toxicity decreases with age, then higher mortality will occur in the small (younger) spat than the larger (older) spat at the same ammonia concentration.

(3) For the ammonia concentrations tested, if increases in ammonia concentration cause decreases in shellfish filtration rates, then scallops fed while exposed to increasing concentrations of ammonia will exhibit a decrease in filtration.

## **4.2 Material and Methods**

### **4.2.1 Study Site**

Experiments were conducted at the Belleoram Sea Scallop Hatchery on the south coast of Newfoundland.

### **4.2.2 Experimental Design and Set-up**

This study was designed to determine the lethal concentrations that kill 50% (LC<sub>50</sub>) of a given population of two size classes of scallops. This was accomplished by holding each size class of scallops in ammonia treatments for 96-hours after which survival was assessed. The effect of ammonia on filtration of food by the smaller scallops was determined by adding the same initial amount of food to each ammonia treatment. A control bucket with no ammonia was used for both size classes as well as a control bucket with food only to determine settlement rates of algae.

Large scallop spat (1.0-2.0 mm shell height) were studied in the autumn of 1997. Small scallop spat (0.5-1.0 mm shell height) were studied in June 1998. A dissecting microscope was used for selection of live animals and measurement of shell height. Mean shell size for the small and large size classes were 640 and 1440  $\mu\text{m}$ , respectively. Twenty and 100 scallops were used for treatments in the large and small size classes, respectively.

A standard ammonia solution of 0.5 g TAN/L was made using reagent grade

ammonium chloride ( $\text{NH}_4\text{Cl}$ ) and diluted to attain ammonia concentrations as needed (Appendix 4.1). The ammonia treatments for the large scallops were 0, 9, 18, 27 and 36 mg TAN/L. For the smaller scallops the ammonia concentrations studied were 0, 6.75, 13.5, 20.25 and 27 mg TAN/L. For molar concentrations see also Appendix 4.1. Treatments were carried out in 5-L buckets which were filled with four liters of the appropriate ammonia concentration. Buckets were placed in a water bath to maintain the temperature at 15°C. Scallops were placed in each bucket. Four replicates per treatment were used for large scallops while three replicates were used for small scallops.

In the experiment that scallops were fed, phytoplankton concentration was initially 40 cells/ $\mu\text{L}$ . Buckets were aerated in the fed scallop treatments, but not in the unfed treatments due to access to airlines.

Hatchery tanks were also sampled for ammonia concentrations to determine the risk for ammonia toxicity in the hatchery. Procedures followed those of Widdows (1985).

#### 4.2.3 Sampling Protocol

Water was checked daily for food densities, temperature and pH. Food densities were measured using a Coulter MultisizerII M/SZER11. Filtration rates were calculated based on decreases in food density (Appendix 4.2).

After 96 hours exposure, each replicate was analyzed for the number of scallops alive. Death was defined as lack of response to mechanical stimulation (gentle tapping on valve with probe) or gaping valves with no or loosely attached viscera. Live scallops were transferred to clean filtered seawater bath and monitored daily for another 48 to 96



hours to determine survival. Survival was calculated as a percentage of live scallop remaining after treating with total initial number.

Concentrations of mg TAN/L in hatchery tanks were sampled in 1997 and 1998. Actual ammonia concentrations were determined using methods by Solarzano (1969) in 1997 and 1998 on Bausch and Lomb Spectronic 20 and Pharmacia Biotech Ultraspec 1000 UV/Invisible Spectrophotometer, respectively.

#### 4.2.4 Data Analysis

Data were analyzed using the SPSS statistical package. For each size class a one-way ANOVA was performed on survival data to determine variation due to the ammonia treatments. Survival data were analyzed using Probit analysis which calculated the 96-h  $LC_{50}$  value. Lethal concentrations were reported as mg UAN/L whenever possible because it is more useful than TAN due its sensitivity to changes in temperature and pH which can fluctuate in a hatchery situation . Percent survival was arcsine-square-root-transformed before a one-way ANOVA was performed to determine differences among concentrations. For the small size class, a one-way ANOVA was performed on the filtration rates to determine variation due to ammonia concentrations.

### 4.3 Results

Survival was significantly different among ammonia concentrations in the large scallops (Figure 4.1; One-way ANOVA;  $F=17.001$ , d.f.=4, 15,  $P<0.001$ ) and the small scallops (Figure 4.1; One-way ANOVA;  $F=54.988$ , d.f.=4, 10,  $P<0.001$ ). The 96-h  $LC_{50}$  for the large scallops was 20.7 mg TAN/L. The 96-h  $LC_{50}$  for the small scallops was 11.8 mg TAN/L. Temperature was maintained between 12.9 and 15.8°C over the study. Treatment pH was maintained between 8.02 and 8.18. The calculated  $LC_{50}$  values for un-ionized ammonia were 0.51 and 0.29 mg/L for the large and small scallops, respectively.

In the small scallop treatments where food was present, as expected no significant differences in initial food density occurred (One-way ANOVA;  $F=0.516$ , d.f.=5, 12,  $P=0.760$ ). Food densities were significantly different due to ammonia concentration (Two-way ANOVA;  $F=6.238$ , d.f.=5, 90,  $P<0.001$ ) and time of sample (Two-way ANOVA;  $F=3.567$ , d.f.=4, 90,  $P=0.011$ ; Figure 4.2). Filtration rates, based on corrected food densities, declined with increasing ammonia concentration (Figure 4.3). Ideally a minimum decrease of 15% in cell count is needed to ensure accurate filtration rates, however, due to the low numbers of scallops in the relatively large volume of water, the decline in cell count was low. Filtration rates may therefore not be confident. The scallops with no ammonia filtered between 0.10 and 0.20 mL/h/animal. All scallops exposed to ammonia had initial filtration rates around 0.05 mL/h/animal. The filtration rates of scallops exposed to 6.75 mg TAN/L dropped in the last 24 hours. Scallops exposed to 13.5 mg TAN/L maintained their filtration rates throughout. Filtration rates of the scallops exposed to 27 mg and 20.25 TAN/L were reduced from around 0.05

mL/h/animal to zero between 24 and 72 hours after initial exposure. Filtration rates were also significantly different due to ammonia concentration (Figure 4.3; Two-way ANOVA;  $F=3.944$ , d.f.=4, 60,  $P=0.009$ ), but not time of sample (Figure 4.3; Two-way ANOVA;  $F=2.402$ , d.f.=3, 60,  $P=0.082$ ). Significant differences in food densities in the survival baths existed due to the ammonia concentration to which scallops had been previously exposed (Two-way ANOVA;  $F=53.563$ , d.f.=4, 75,  $P<0.001$ ) and time (Two-way ANOVA;  $F=22.421$ , d.f.=4, 75,  $P<0.001$ ; Figure 4.4). Filtration rates of scallops in the survival bath were not significantly different due to ammonia concentration scallops had previously been exposed to (Two-way ANOVA;  $F=2.818$ , d.f.=3, 48,  $P=0.055$ ) or time (Two-way ANOVA;  $F=2.403$ , d.f.=3, 48,  $P=0.086$ ; Figure 4.5).

The measured ammonia concentrations in the fed scallop treatment were similar to the desired concentrations of ammonia ( $5.91\pm 4.31$  mg TAN/L,  $13.89\pm 0.6378$  mg TAN/L,  $21.11\pm 0.76$  mg TAN/L and  $28.37\pm 1.27$  mg TAN/L).

Ammonia was detected in hatchery tanks (Table 4.1). Highest concentrations were found in the broodstock tanks (0.116 mg UAN/L). High concentrations in the larval holding units were in the tanks with setting trays (0.03 mg UAN/L) and the buckets in which the scallops were held when tanks were being cleaned (0.047 mg UAN/L).

## 4.4 Discussion

### 4.4.1 Survival after Ammonia Exposure

Ammonia was found to affect adversely nursery-sized sea scallops. As was expected, mortality increased with increased ammonia concentration. Mortality was also higher in the smaller scallops than the larger scallops at the same concentration. This was expected and agrees with other studies. Declining filtration rates with increasing ammonia concentration were also expected.

Ammonia is toxic to sea scallop spat. The small and large spat held at 14.2°C and pH 8 have LC<sub>50</sub>s of 11.8 and 20.4 mg TAN/L. When converted to mg UAN/L these levels are similar to un-ionized ammonia tolerance of other bivalves. Sea scallop spat (0.5 -2 mm shell height) at 14°C and pH 8.0 have 96-h LC<sub>50</sub> between 0.29 and 0.51 mg UAN/L. This was higher than *M. mercenaria* juveniles, but similar to *C. virginica* juveniles and *A. irradians* larval stages (Table 4.2). The LC<sub>50</sub> of sea scallop spat was higher than for sea scallop juveniles (Table 4.2). Tolerance may relate to how well aquatic organisms can avoid exposure to the aqueous environment. Sea scallops follow the trend of other bivalves (clams and oysters) in having a lower tolerance to un-ionized ammonia than crustaceans and higher tolerance than echinoderm embryos (Table 4.2). Differences may be due to experimental design, however, there appears to be a biological trend. Scallops cannot close their valves tightly thus may have a higher tolerance. Crustaceans have little control thus need an even higher tolerance to un-ionized ammonia.

#### 4.4.2 Short-term Effects of Ammonia

Mortality is obviously the most detrimental effect that ammonia exposure can have on sea scallops. Osmoregulation may be the physiological process that fails during ammonia exposure and leads to mortality. The American lobster, *Homarus americanus*, shows reduced osmoregulation in post-larval stages than in adults which may be due to interference with transport mechanisms for sodium across cell membranes (Young-Lai et al. 1991). The fleshy prawn *Penaeus chinensis* exhibits an increase in TAN and decrease in protein nitrogen in hemolymph during exposure to 10 mg TAN/L (Chen et al. 1993). This may be an attempt to balance osmoregulation which is dysfunctioning because of the ammonia exposure. Blood chemistry was not studied in this experiment, however, the production of mucus and closure of valves indicated that the sea scallops were attempting to reduce exposure due to the effect on its osmoregulation.

Less detrimental effects have been caused by ammonia exposure. Behavior is affected by ammonia concentration. Erratic and fast swimming occurs in *Scophthlalmus maximus* when exposed to 11.74 mg TAN/L (Rasmussen and Korsgaard 1996). Reduced activity was observed in juvenile sea bass *Dicentrarchus labrax* at concentrations of >50 mg TAN/L (Tudor et al. 1994). Abraham et al. (1996) found that juvenile sea scallops became less responsive to stimuli as exposure time to ammonia increased. Similar observations of increased gaping, increasingly poor mantle attachment, reduced response and increased mucous production with increasing concentration were made with the sea scallop spat in this study (Table 4.3). Scallops exposed to the highest concentration were found dead with their valves tightly shut, mantles retracted and intact. These scallops

appear to be avoiding exposure to the toxic concentrations while scallops in lower concentrations appear to have produced mucus to reduce exposure. Response to mechanical stimulation seems variable with increasing concentration while excess activity was nonexistent. The behavior of sea scallops was affected by ammonia exposure.

#### 4.4.3 Age-related Effects

Increased tolerance to ammonia with size was found between the two size classes of scallop spat studied here. This contrasts with the much lower tolerance that Abraham et al. (1996) found in juvenile sea scallops, but agrees with the trends found for other aquatics organisms including American oysters, bay scallops, the freshwater mussel, American lobsters and leader shrimp (Epifanio and Srna 1975; Chen et al. 1990; Young-Lai et al. 1991; Lin 1992; Scheller 1997). Decreased tolerance with age occurs in *M. mercenaria* (Epifanio and Srna 1975). Differences in experimental design may explain the high ammonia tolerance of spat in this study in comparison to the lower tolerance in juvenile sea scallop (Abraham et al. 1996). The set-up in this study used 15°C water, as well, one of the treatments in this study was fed and aerated, which may have influenced boundary conditions around scallops ie. ammonia concentrations where there was no aeration. Water also was not changed until the end of the 96 hours bioassay in these experiments. The lack of replenishment of water and aeration may have caused increased levels of ammonia near scallops which would mean that the actual LC<sub>50</sub>s may have been higher than the calculated LC<sub>50</sub>s. Abraham et al. (1996) held scallops at 4 and

10°C, used aeration, did not add food to treatments and changed ammonia treatment water daily to keep ammonia concentrations constant. Also the number of scallops used in treatments differed among sizes studied here and by Abraham et al. (1996). These experimental differences may explain why juvenile had higher tolerance than the spat.

#### 4.4.4. Effects of Ammonia on Filtration Rates

Food consumption of the small scallops decreased with ammonia concentration and time. Filtration rates were highest in the control bucket of scallops with food and without ammonia. The decrease in filtration rates in the last 24 hours may have been due to increased particle loading from decomposition or bacterial presence. Because the concentrations were so lethal to the scallops the feeding may have been reduced due to mortality and not feeding behavior of scallops. The increase in abundance of food size particles and the corresponding increase in negative filtration rates was likely due to mucus production of dying scallops, decomposition of dead scallops or increased bacteria.

#### 4.4.5 Long-term Effect of Ammonia Exposure

Ammonia concentrations in the hatchery rearing tanks are approximately 5% of the 96-hour  $LC_{50}$  for UAN. For short-term exposure this is not a problem, however, for long-term rearing of spat until they reach 1.5 -3.0 mm shell height, this could be a problem.

Ammonia tolerance not only affects survival, but in low concentrations affects feeding and thus growth. This was evident from the low filtration rates of the scallops exposed to 6.75 mg TAN/L. Their filtration rate was 25-50% that of the unexposed scallops. This has implications for long term exposure of scallops to low concentrations of ammonia.

Several studies have investigated effects of long-term chronic exposure to lower concentrations of ammonia. Growth of juvenile turbot *Scophthalmus maximus* is affected by 20 day exposure to concentrations of 0.108 UAN/L (Rasmussen and Korsgaard 1996). Greenlip abalone *Haliotis laevis* exhibits reduced growth when exposed to concentrations of 0.054-0.188 mg UAN/L for 58 days (Harris et al. 1998). Bay scallop larvae also show reduced growth after 12 days when exposed to 4.04 mg TAN/L (Lin 1992). Concentrations >0.110 mg UAN/L reduce feeding of *H. laevis* and for *S. maximus* reduction in feeding begins at about 0.117 mg UAN/L. A concentration of 7.2 mg TAN/L reduces feeding in *C. virginica* and *M. mercenaria* (Epifanio and Srna 1975). Food utilization is also reduced when exposure to low ammonia occurs in *S. maximus* (Rasmussen and Korsgaard 1996). The long-term exposure to low concentrations may not appear obvious due to lack of instantaneous mortality events, however, the prolonged period of reduced feeding creates reduced growth and may stress animals.



## 4.5 Conclusions

Low concentrations of ammonia are toxic to sea scallop spat. Spat exhibited consistently deteriorating behavior from the norm between trials, including decreasing filtration rates and response, and increased mucus production, when exposed to increasing ammonia concentrations with 96-h  $LC_{50}$  values of 11.8 and 20.4 mg TAN/L for 0.5 -1.0 mm shell height and 1.0 - 2.0 mm shell height scallops, respectively. These concentrations were 10-20 times higher than ammonia concentrations measured in hatchery rearing tanks which suggests that there would be no adverse effects over short-term exposure (Figure 4.1). Long term, or chronic, exposure to concentrations up to 5 mg TAN/L, however, may have an effect on the scallops as suggested by the filtration rates measured at 6.75 mg TAN/L. This indicates the need for investigation of chronic exposure to low dosage to determine what effects, if any, may be imposed upon scallops reared in high density situations.

**Chapter Five:**

**Growth Rates of Sea Scallop, *Placopecten magellanicus*, Spat Reared in  
Flow-through Tanks**

## 5.1 Introduction

Flow-through tanks and raceways are commonly used in the nursery culture of bivalves (dePauw 1981). They are a convenient transitional phase from the controlled hatchery to the uncertain farm environment for on-growing to intermediate size. Flow-through systems act as natural seawater columns, but allow the operator to access animals, control water quality, choose substrate, eliminate weather and avoid predators (Rhodes et al. 1981). They deliver natural phytoplankton to cultured organisms to enhance growth and survival of bivalves. This study was designed to assess the feasibility of culturing nursery-sized scallops in a flow-through system at the Belleoram Sea Scallop Hatchery.

Compared to other indoor nursery rearing systems, flow-through systems offer the best growth and survival. Bourne and Hodgson (1991) found *Patinopecten yessoensis* grew better and survival was four times higher in flow-through tanks than in re-circulation tanks. Rhodes et al. (1981) found raceway growth rates of *Argopecten irradians* almost as high as open ocean pens, but much better than re-circulation tanks.

Food availability and quality are the most important factors to consider in land-based nursery culture (Claus et al. 1983). Spat require increased amounts of different quality food than larvae (Claus et al. 1983; Young-Lai and Aiken 1986). The trend is to feed spat partially or solely on natural phytoplankton (Coutteau and Sorgeloos 1991).

Natural food supply, which consists of a complex mixture of organic and inorganic particles, is difficult to mimic in the laboratory situation and requires upkeep (dePauw 1981; MacDonald and Ward 1994). Growth of bivalves, however, is enhanced

when fed enriched natural production (Riva and Lelong 1981) and when cultured diet is supplemented by natural phytoplankton (O'Foighil et al. 1990).

Scallops have a preference for the material on which they settle. Naidu et al. (1981) found that *Placopecten magellanicus* prefers gillnet to flat polyethylene strips for settlement. Tremblay (1988) reported that sea scallops set equally on both tank surface and filaments. Pearce and Bourget (1996) found a preference for polyester filter-wool to nylon monofilament, polyester Astroturf, acrylic plastic, and adult sea scallop shells. Mesh bottom containers were used in the pilot scallop hatchery while solid fiberglass trays are now used. Spat settlement on these two substrata have not previously been compared.

Culture of sea scallops in static (non-flow through) water tanks and fed cultured algae takes at least 40 days (at 30  $\mu\text{m}/\text{d}$ ) after settlement to reach a size at which they are transferred to a farm-based nursery. Flow-through culture may reduce this time. The purpose of this study was to determine the potential of flow-through nursery culture and the suitability of substrate for growing spat to a size to nursery-size. The hypotheses of this study were:

- (1) Growth rates of scallop spat in a flow-through system will be higher than a non-flow-through system.
- (2) Growth rates of scallop spat in flow-through system will be highest on mesh trays compared with other substrates.

## 5.2 Materials and Methods

### 5.2.1 Study Site

This study was carried out at the Belleoram Sea Scallop Hatchery (BSSH), Belleoram, on the south coast of Newfoundland.

### 5.2.2 Experimental Design and Set-up

This study was designed to determine the potential growth rates of scallops grown in a flow-through system. To do this scallops of the same size class were placed in flow-through tanks and growth was monitored periodically. The effect of tray type was also examined by placing the scallops on trays suspended in the tanks.

Scallops were obtained from BSSH on September 11, 1997. Size-grade was 750 to 1000  $\mu\text{m}$  shell height. Initial shell height was measured for thirty scallops.

Four tray treatments were studied. Trays were made of 30 cm diameter PVC pipe about 5 cm high with one of four bottom types: smooth solid fibreglass trays (control), rough fibreglass trays, 290  $\mu\text{m}$  (diagonal) Nitex® mesh or 500  $\mu\text{m}$  (diagonal) Nitex® mesh. The smooth solid trays were considered the control because it was the same type of tray that is routinely used in the spat rearing tanks at BSSH. Three replicates of each tray type were studied. Six trays were tied together in stacks and suspended from a crossbar at the top of the tanks. Two cylindrical 200-L fibreglass tanks (1.0 m high x 0.5 m internal diameter) were used. Two tray types went in each tank with 5000 scallops per

tray initially.

Water flow into the tanks was gravity-fed from a header-tank. Intake water was pumped from 32 m depth, screened using a 20  $\mu\text{m}$  filter bag, collected in a 20-L header-tank and adjusted to flow at 1 L/s into experimental tanks. Food was delivered to tanks daily as needed to maintain densities above 20 cells/ $\mu\text{L}$ .

### 5.2.3 Sampling Protocol

Water temperature and food densities were measured daily. Food density was measured using a Coulter Counter 2F. Particulate matter was measured weekly (Appendix 3.3). Shell height of scallops on each tray type was also measured weekly.

### 5.2.4 Data Analysis

Data were analyzed using the SPSS statistical package for ANOVAs to determine the variation in shell height due to date, tray type and replicate. Paired T-test was used to determine equality of means for temperature, % POM and food densities in the two tanks.

## 5.3 Results

### 5.3.1 Shell Heights

There were no significant differences in the shell heights of scallops used in the different tray types (Two-way ANOVA,  $F=1.043$ ,  $d.f.=3$ ,  $360$ ,  $P=0.374$ ) or replicates (Two-way ANOVA,  $F=0.899$ ,  $d.f.=2$ ,  $360$ ,  $P=0.408$ ). Mean initial shell height was  $922\pm 12\ \mu\text{m}$ .

Shell growth occurred in all treatments (Figure 5.1). Shell heights did not vary due to replicate (Three-way ANOVA;  $F=0.013$ ,  $d.f.=2,1800$ ,  $P=0.987$ ). Significant differences in pooled replicate shell height were due to tray type (Two-way ANOVA;  $F=3.971$ ,  $d.f.=3$ ,  $1800$ ,  $P=0.008$ ) and sample date (Two-way ANOVA;  $F=17.427$ ,  $d.f.=4$ ,  $1800$ ,  $P<0.001$ ). Largest mean final shell height ( $1115\pm 19\ \mu\text{m}$ ) was for the  $500\ \mu\text{m}$  mesh. Smallest mean final shell height ( $1022\pm 23\ \mu\text{m}$ ) was for the solid smooth trays. In all treatments, loss of larger spat in the wash water at sampling may have caused the reduction in shell heights.

Scallop growth rates on the four tray types were low at  $3.45\ \mu\text{m}/\text{d}$  (solid rough),  $3.79\ \mu\text{m}/\text{d}$  ( $290\ \mu\text{m}$  mesh),  $4.79\ \mu\text{m}/\text{d}$  (solid smooth) and  $6.65\ \mu\text{m}/\text{d}$  ( $500\ \mu\text{m}$  mesh).

### 5.3.2 Water Quality

Ambient sea water temperature ranged from  $3.0$  to  $11.2^\circ\text{C}$ . Warming effects in the hatchery raised the tank temperatures slightly. Mean temperature for broodstock

tanks No. 5 and No. 6 were 11.3 and 11.1 °C (Figure 5.2). No significant differences in temperature were found between tanks (Paired t-test;  $t=1.863$ , d.f.=21,  $P=0.076$ ).

Mean food densities for two flow-through tanks were 34.8 ( $\pm 5.9$ ) and 28.4 ( $\pm 4.1$ ) cells/L (Figure 5.3). No significant differences were found between food densities (Paired t-test;  $t=1.698$ , d.f.=21,  $P=0.104$ ) or POM (Paired t-test;  $t=1.318$ , d.f.=5,  $P=0.245$ ) in the two tanks. Mean percent POM was 68% for both tanks (Figure 5.3) while mean food for the two tanks was 36 for tank 5 and 42 for tank 6. There were no significant differences between TPM (Paired t-test;  $t=2.119$ , d.f.=5,  $P=0.088$ ) or PIM (Paired t-test;  $t=0.826$ , d.f.=5,  $P=0.446$ ). The pH in both tanks was also statistically similar (Paired t-test;  $t=1.578$ , d.f.=5,  $P=0.130$ ).

The statistical similarities between the water quality parameters in the two tanks as well as the fact that the tank designs were identical makes the possibilities of pseudoreplication, or tank effect, minimal.



## **5.4 Discussion**

### **5.4.1 Growth Rates**

Growth rates of scallops were influenced by substrate type. This was expected. Growth rates of scallops in the flow-through tanks were, however, not enhanced compared to scallops in the non-flow-through water tanks. This was unexpected. This may indicate that the cultured food quality was not adequate.

### **5.4.2 Effect of Substrate Type on Growth Rates**

Despite the low growth rates, there was variation due to the tray substrate type. Growth rates were highest in the tank with the 500  $\mu\text{m}$  mesh. This may have been due to its micro-environment (continuous flow of water) providing replenishment of food and better removal of fecal wastes. Under steady flow conditions sea scallop growth is higher than in fluctuating flow (Wildish and Kristmanson 1988). As well, at low currents, scallops are limited by the seston depletion effect (Wildish and Kristmanson 1985).

### **5.4.3 Flow-through Protocol**

Growth rates were low and comparable to those of sea scallops deployed on the farm in late September and October for the deployment date study (Chapter 3) whose growth may have been hindered due to lack of acclimation to the new environment.

Scallops in this experiment were also not acclimated to the flow-through tank temperature conditions. Despite the presence of both natural and cultured food, scallops did not show enhanced growth. This indicates that regardless of the presence of high quality food, conditioning to more than food is important. Rodhouse et al. (1981) found that in an onshore nursery using enriched natural phytoplankton, bivalve growth was limited by low temperature. Claus et al. (1983) also found that *Ostrea edulis*, *Crassostrea gigas* and *Venerupis semidecussata* exhibited poor growth under ambient flow-through conditions when cultured food was supplemented, but when transferred to heated conditions, growth rates and survival improved. This indicates the importance of temperature also. Flow-through systems may be more useful when both temperature and food quality are higher.

Food quality is one of the main reasons why exposure to natural diets prior to deployment in the ocean is a common nursery protocol. Bay scallops gradually exposed to partially filtered seawater screened to 25  $\mu\text{m}$ , 50  $\mu\text{m}$  and then 100  $\mu\text{m}$  before transfer to the ocean at 2 mm shell height grew and survived better than scallops fed only cultured diets (R. Garrison, pers. comm.). Japanese scallop spat are reared in outdoor culture tanks using the natural phytoplankton (Couturier 1990). O'Foighil et al. (1990) found that when fed cultured diets Japanese scallops experience a mortality event 4-6 weeks after metamorphosis possibly due to insufficient nutrition as growth remained poor despite additional cultured food. When fed natural phytoplankton, however, growth rates increased. Because of the superior growth many bivalves are fed partially or exclusively natural phytoplankton after they reach the nursery stage (Coutteau and Sorgeloos 1992).

## **5.5 Conclusions**

Growth rates are enhanced by using 500  $\mu\text{m}$  mesh instead of solid smooth or rough fiberglass trays or 290  $\mu\text{m}$  mesh trays. This may be due to the enhanced flow of water thus continuous replenishment of food and removal of fecal wastes to the micro-environment of the scallop.

Poor growth rates were observed in the flow-through system in operation during September and October. Like any nursery culture approach, however, a flow-through system offers less control to the grower. The lack of control over water temperature was manifested in low growth rates of scallops. Because this was a preliminary investigation into the use of flow-through systems, there is much room for improvement. The general protocol implication that this study supports is that intake temperature and diet are important. Future research of nursery culture of sea scallops in a flow-through system is necessary to develop efficient protocols.

## **Chapter Six:**

### **Implications for Hatchery Management**

## **6.1 Introduction**

The energetic basis of bivalve aquaculture is to convert primary production into bivalve tissue with a net result of growth, however, this is poorly understood (Grant 1996). Understanding the importance of food is necessary to predict growth of bivalves under culture conditions for controlling size, density and mortality of animals, as well as developing economic projections and having stability in the industry (Grant 1996). This also applies equally to the selection of sites for growout or nursery culture strategies of sea scallops to an intermediate culture size of 7 mm.

Research was performed from 1996 to 1998 to determine which conditions were better for growth and survival of nursery-sized scallops. The majority of factors studied were related to the food delivered to the scallops hence provided us with a better understanding of the importance of food in nursery culture. The findings of these studies concludes with a list of recommendations for the Belleoram Sea Scallop Hatchery to consider as ways of improving growth rates and recovery of nursery-sized scallops.

## **6.2 Recommendations for Improving Nursery Culture of Sea Scallops**

### **6.2.1 Initial Mesh/Depth/Gear Type/Density Studies**

- 1) Scallops should be transferred when they are large enough ie. 1.4 mm shell height as there were no benefits to growth rates or recovery until shell height was >3.0 mm and earlier deployment may ensure optimal nursery conditions.
  
- 2) Size grading of scallops <3.0 mm shell height needs to be improved to overcome loss of marginally-sized scallops through mesh.
  
- 3) 1.5 mm mesh pearl nets are not recommended for deploying nursery-sized scallops due to flaws in the mesh. Collector bags thus are the only current option for deployment of scallops <3.0 mm shell height, however, due to impediments of flow in the bread tray-collector bag set-up, it is advised that design of the bread tray be altered to allow flow or when scallops reach 3.0 mm they be transferred to pearl nets where flow is more suitable.
  
- 4) Deploy any nursery-sized scallops at 5 m rather than 10 m for enhanced growth. Deployment at greater depths should be investigated for a variety of nursery-sized scallops earlier than October to determine the potential for culture.
  
- 5) Deploy scallops >3.0 mm shell height in pearl nets rather than collector bags for enhanced growth and recovery.

6) Deploy scallops 2.0-3.0 mm shell height at 5200 spat/bag rather than 2600 spat/bag as there was no benefit to growth or recovery at 2600 spat/bag thus more production is gained per unit of equipment at the higher density.

#### 6.2.2 Deployment Date and Remote Set Study

1) Deployment of nursery-sized scallops should occur during July, August and early September to attain maximal growth rates and recovery of scallops. This is due to the provisions of the natural environments to help scallops overcome the change in environment from the hatchery to the farm-based nursery. With respect to deploying scallops, the hatchery should monitor the nursery environment routinely to determine, temperature and food availability as well as settlement of the sea star, the main predator of suspended sea scallops, so as to better predict when deployment will offer high growth and recovery. This should allow the hatchery to better estimate the effect of spatial variability of their product and determine both time of availability as well as numbers for the growers. This may have implications for the hatchery operating its own nursery site rather than one at a local scallop farm.

2) To improve the growth rates and recovery of scallops deployed after early September (thus in essence to widen the window of opportunity for deployment) the hatchery needs to develop a diet that is high in essential fatty acids so that scallops can store adequate reserves so that they are able to physiologically adjust to the nursery environment. As well, developing a protocol for gradual exposure of scallops to the nursery environment

temperatures and food levels may aid scallops in being more resilient when transferred to the actual nursery environment such as a flow-through system.

3) Deployment of scallops through remote set requires the improvement of sea scallop settlement on Vexar® or some other substrate. This may be improved by the use of a downwelling system during settlement as more scallops are concentrated per area of settlement. Deployment of remote set scallops would allow deployment of more spawned batches of scallops due to the faster turnover of tanks (approximately 40 days) compared to growing scallops to a larger size (80 days). The hatchery should consider deploying at least part of their seasonal production using remote set practices to also widen the window of deployment earlier in the year in combination with practices of using a higher quality diet.

### 6.2.3 Toxicity of Ammonia to Sea Scallop Spat

1) Lethal concentrations of ammonia are not found in the hatchery rearing tanks for post-larval scallops, however, because sub-lethal concentrations did cause reduced filtration rates, an investigation should be carried out with respect to chronic exposure to low concentrations of ammonia to assess the risk of mortality or long-term effects on scallops.



#### 6.2.4 Flow-through Culture of Nursery-Sized Sea Scallops

1) If any flow-through culture is performed, trays should be 500  $\mu\text{m}$  mesh rather than solid or smaller mesh trays.

2) Flow-through should be investigated at surface waters where temperature is higher and available food is more diverse, and during warmer months, i.e., July, August and early September, to determine the possibilities of using flow-through as a transitional phase from the hatchery to the farm-based nursery.

### **6.3 Conclusions**

Based on the findings of the sea scallop nursery research and the subsequent recommendations, the Belleoram Sea Scallop Hatchery should have a better understanding of the requirements of the nursery environment. By improving their current practices and implementing new protocol, the length of time for scallops in the nursery stage should be decreased and the total production should be increased thus fulfilling the goals of a typical nursery culture practice.

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## **Tables**

Table 2.1: ANOVA of interval growth rates of scallops in a farm-based nursery at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1996 to July 1997, on the basis of date, depth and mesh size.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	22491.333	29	775.563	7.104	.000
Intercept	80066.732	1	80066.732	733.348	.000
Date	13887.542	3	4629.181	42.400	.000
Equipment	8640.926	3	2880.309	26.381	.000
Depth	1359.512	1	1359.512	12.452	.001
Date * Mesh Size	896.423	8	112.053	1.026	.424
Date * Depth	814.539	3	271.513	2.487	.067
Mesh Size * Depth	601.750	3	200.583	1.837	.148
Date * Mesh Size * Depth	797.666	8	99.708	.913	.510
Error	8079.305	74	109.180		
Total	102368.127	104			
Corrected Total	30570.638	103			

a R Squared = .736 (Adjusted R Squared = .632)

Table 2.2: ANOVA of transformed percent recovery of scallops grown at a farm-based nursery at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1996 to July 1997, on the basis of date, depth, and mesh size factors.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.168	29	$7.477 \times 10^{-2}$	7.190	.000
Intercept	66.908	1	66.908	6434.332	.000
Date	.478	3	.159	15.314	.000
Mesh Size	.754	3	.251	24.158	.000
Depth	$5.888 \times 10^{-3}$	1	$5.888 \times 10^{-3}$	.566	.454
Date * Mesh Size	.605	8	$7.558 \times 10^{-2}$	7.268	.000
Date * Depth	$1.472 \times 10^{-2}$	3	$4.906 \times 10^{-3}$	.472	.703
Mesh Size * Depth	$4.516 \times 10^{-3}$	3	$1.505 \times 10^{-3}$	.145	.933
Date * Mesh Size * Depth	$4.469 \times 10^{-2}$	8	$5.587 \times 10^{-3}$	.537	.825
Error	.770	74	$1.040 \times 10^{-2}$		
Total	72.786	104			
Corrected Total	2.938	103			

a R Squared = .738 (Adjusted R Squared = .635)

Table 2.3: ANOVA of transformed percent recovery of scallops deployed from October 1996 to May 1997, at Shell Fresh Farms Ltd., Pool's Cove, NF, on the basis of mesh size and depth factors.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.456	7	$6.511 \times 10^{-2}$	4.178	.006
Intercept	17.275	1	17.275	1108.345	.000
Mesh Size	.438	3	.146	9.362	.000
Depth	$7.907 \times 10^{-3}$	1	$7.907 \times 10^{-3}$	.507	.485
Mesh Size * Depth	$9.365 \times 10^{-3}$	3	$3.122 \times 10^{-3}$	.200	.895
Error	.312	20	$1.559 \times 10^{-2}$		
Total	18.110	28			
Corrected Total	.767	27			

a R Squared = .594 (Adjusted R Squared = .452)

Table 2.4: Water quality parameters at two depths at Ladder Garden, Shell Fresh Farms Ltd., Pool's Cove, NF, during 1996 and 1997.

Parameter	Depth (m)	Oct 3	Oct 9	Nov 12	Nov 14	Nov 25	Dec 12	Feb 12	Mar 1	Apr 9	May 1	June 5	July 15
Temperature (°C)	5	11.4	11.1	8	8.2	8	5.5	2.7	2.75	1.25	2	1.85	11.1
	10	11.4	11.1	8	8.6	8	5.6	2.75	2.8	1.35	1.9	1.7	9.8
Salinity	5	30.2	28.9	28	28	28	26.8	32.9	31.5	30.5	26.4	31.6	30.5
	10	30.9	28.9	28.1	28.1	28.1	26.9	33.2	31.7	31	26.9	31.6	31.8
Light intensity (microeinsteins)	5	47	-	-	-	-	-	100	110	80	-	-	-
	10	10	-	-	-	-	-	60	70	32	-	-	-
Dissolved Oxygen (% saturation)	5	85	-	-	-	-	-	93	94	94	-	77	83
	10	96	-	-	-	-	-	93	94	94	-	97	89
Dissolved Oxygen (mg/L)	5	5.5	-	-	-	-	-	7.15	7.32	7.49	-	6	5.2
	10	6	-	-	-	-	-	7.2	7.3	7.5	-	7.5	5.75
Chlorophyll- <i>a</i> (µg/L)	5	1	-	-	-	-	-	0.55	0.7	2.9	-	0.6	2.2
	10	0.65	-	-	-	-	-	0.5	0.8	4.1	-	0.5	1.7
Turbidity (formazin turbidity units)	5	8.5	-	-	-	-	-	7	7.7	8.2	-	7.5	7.2
	10	8.5	-	-	-	-	-	7.5	7.7	8.1	-	7.5	7.2



Table 2.5: Pearson correlation coefficients (r) of water quality parameters with interval growth rates and recovery of spat grown at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 9, 1996, to July 3, 1997.

		Salinity	Chlorophyll- <i>a</i> ( $\mu\text{g/L}$ )	DO <sub>2</sub> (mg/L)	DO <sub>2</sub> (%)	Turbidity (formazin turbidity units)	Light intensity (microeinsteins)	Temperature (°C)
Interval growth rates	Pearson Correlation	-.060	.062	-.483	-.473	-.221	.028	.029
	Sig. (1-tailed)	.272	.266	.000	.000	.012	.403	.385
	N	104	104	104	104	104	82	104
Transformed recovery rates	Pearson Correlation	-0.415	-.216	-.035	.147	.288	-.227	0.409
	Sig. (1-tailed)	.000	.014	.361	.068	.002	.020	.000
	N	104	104	104	104	104	82	104







Table 2.6: (cont.) d) Collected on July 3, 1997. (Note: 3.0 mm pearl nets not retrieved)

Equipment type	Pearl Net						Collector Bag						
	1.5		3		1.2		2		5		10		
	5	10	5	10	5	10	5	10	5	10	5	10	
<i>Placopten magellanicus</i>	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Mytilus edulis</i>	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Modiolus modiolus</i>	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Hyatella</i> sp.	x	x	-	-	-	-	x	x	x	x	x	x	x
Cockle	x	x	-	-	-	-	x	x	x	x	x	x	x
Hydrozoan sp.2	x	x	-	-	-	-	x	x	x	x	x	x	x
Copepods	x	x	-	-	-	-	x	x	x	x	x	x	x
Other crustaceans	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Asterias vulgaris</i>	x	x	-	-	-	-	x	x	x	x	x	x	x
Sea urchin larvae	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Ceramium</i> sp.	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Cladophora</i> sp.	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Desmerestia</i> sp.	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Laminaria longicurris</i>	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Polysiphonia</i> sp. 1	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Polysiphonia</i> sp. 2	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Spongomorpha</i> sp.	x	x	-	-	-	-	x	x	x	x	x	x	x
<i>Ulothrix</i> sp.	x	x	-	-	-	-	x	x	x	x	x	x	x
Nudibranch	x	x	-	-	-	-	x	x	x	x	x	x	x
Polychaete worms	x	x	-	-	-	-	x	x	x	x	x	x	x
Mussel seed (unidentified)	x	x	-	-	-	-	x	x	x	x	x	x	x
Sponge	x	x	-	-	-	-	x	x	x	x	x	x	x

Table 2.7: Total dry weight of fouling organisms and description of fouling on top and bottom of 1.5 and pearl nets and 1.2 and 2.0 mm collector bags held at Shell Fresh Farms, Ltd., Pool's Cove, NF, from October 1996 to July\* 1997. (\*3.0 mm mesh fouling was from October 1996 to May 1997)

Mesh Size (mm)	Depth (m)	Fouling mass (mg/cm <sup>2</sup> )	Top coverage	Bottom coverage
1.2	5	1.89	slight algae	light silt and clams
1.2	5	0.93	slight algae	light silt and clams
1.2	5	0.47	sparse algae, clams and worm tubes	moderate silt
1.2	5	0.36	moderate silt and algae, clams and worm tubes	light silt, clams and worm tubes
1.2	10	1.55	light silt, clams, algae	light silt and clams
1.2	10	1.82	light silt, clams, algae	light silt and clams
1.2	10	0.26	light silt, worm tubes and clams	light silt and clams
1.2	10	0.25	light silt, worm tubes and clams	light silt and clams
1.5	5	6.55	algae	light silt and clams
1.5	5	9.52	algae	light silt and clams
1.5	5	7.27	algae	light silt and clams
1.5	10	7.96	slight algae	light silt and clams
1.5	10	0.97	light fouling and silt	light silt and clams
1.5	10	1.08	light fouling	light silt and clams
2	5	0.37	light silt, algae, worm tubes and clams	light silt
2	5	0.28	light silt, worm tubes and clams	light silt and clams
2	5	0.18	light silt, worm tubes, clams, algae and bryozoan	light silt, clams and worm tubes
2	5	0.71	light silt, algae, clams, worm tubes	light silt and clams
2	10	0.30	light silt, clams and worm tubes	light silt and clams
2	10	0.14	light silt, few clams, worm tubes	light silt
2	10	0.15	light silt, clams and worm tubes	light silt
2	10	0.14	light silt, clams and worm tubes	light silt

Table 2.7: (cont.) Total dry weight of fouling organisms and description of fouling on top and bottom of 1.5 and 3.0 mm pearl nets and 1.2 and 2.0 mm collector bags held at Shell Fresh Farms, Ltd., Pool's Cove, NF, from October 1996 to July\* 1997. (\*3.0 mm mesh fouling was from October 1996 to May 1997)

Mesh Size (mm)	Depth (m)	Fouling mass (mg/cm <sup>2</sup> )	Top coverage	Bottom coverage
3	5	4.49	algae and lots of tiny invertebrates	light silt and clams
3	5	1.67	algae	light silt and clams
3	5	1.61	algae	light silt and clams
3	10	0.49	heavy silt	light silt and clams
3	10	0.40	heavy silt- few algae and clams	light silt and clams
3	10	0.28	heavy silt	light silt and clams

Table 2.8: ANOVA of macrofouling accumulation on farm-based nursery equipment at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1996 to July 1997, due to date, depth and mesh size factors.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	265.461	29	9.154	9.633	.000
Intercept	85.939	1	85.939	90.438	.000
Date	75.490	3	25.163	26.481	.000
Mesh size	68.197	3	22.732	23.922	.000
Depth	20.080	1	20.080	21.131	.000
Date * Mesh Size	70.494	8	8.812	9.273	.000
Date * Depth	16.158	3	5.386	5.668	.001
Mesh Size * Depth	20.743	3	6.914	7.276	.000
Date * Mesh Size * Depth	17.114	8	2.139	2.251	.033
Error	70.319	74	.950		
Total	400.575	104			
Corrected Total	335.779	103			

a R Squared = .791 (Adjusted R Squared = .709)

Table 2.9: ANOVA of silt accumulation on farm-based nursery equipment at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1996 to July 1997, due to date, depth and mesh size factors.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	62.496	29	2.155	31.142	.000
Intercept	102.222	1	102.222	1477.2	.000
Date	11.140	3	3.713	53.659	.000
Mesh size	36.551	3	12.184	176.06	.000
Depth	5.734	1	5.734	82.856	.000
Date * Mesh Size	4.711	8	.589	8.510	.000
Date * Depth	1.278	3	.426	6.154	.001
Mesh Size * Depth	4.596	3	1.532	22.139	.000
Date * Mesh Size * Depth	2.251	8	.281	4.067	.000
Error	5.121	74	6.920 x 10 <sup>-2</sup>		
Total	155.740	104			
Corrected Total	67.617	103			

a R Squared = .924 (Adjusted R Squared = .895)



Table 2.10: a) Tukey-B test results for shell height replicates of scallops held in pearl nets held at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1997 to May 1998.

Replicate	N	Subset		
		1	2	3
4.00	60	12.5665		
6.00	60	13.5503	13.5503	
5.00	60	13.6763	13.6763	13.6763
3.00	60	13.7225	13.7225	13.7225
2.00	60		14.6325	14.6325
1.00	60			15.1735

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square (Error) = 9.260.

a Uses Harmonic Mean Sample Size = 60.000.

b Alpha = .05.

b) Tukey-B test results for shell height replicates of scallops held in collector bags at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1997 to May 1998.

Replicate	N	Subset
		1
4.00	30	11.6827
6.00	30	11.7720
5.00	30	12.3257
3.00	30	12.5730
2.00	30	13.1837
1.00	30	13.7410

Means for groups in homogeneous subsets are displayed. Based on Type III Sum of Squares The error term is Mean Square (Error) = 9.452.

a Uses Harmonic Mean Sample Size = 30.000.

b Alpha = .05.

c Equipment = 1.00

Table 2.11: Growth and recovery rates of *Placopecten magellanicus* in cultured and wild situations.

Location	Wild/culture	Age/Size	Growth rate ( $\mu\text{m/d}$ )	Survival (%)	Author
Passamaquoddy Bay	Wild	84 d/ 5 mm	60	--	Black et al. (1993)
	Suspension	Juvenile	120	84.3	Dadswell and Parsons (1991)
Georges Bank	Wild	1 mm spat	4	--	Larson and Lee (1978)
		84 d/ 2.9mm	34.5	--	Black et al. (1993)
Nantucket Shoal	Wild	180 d	27.4/15-73.3	--	Parsons et al. (1993)
N/A	N/A	60 d / 1 mm	25	--	Black et al. (1993)
Logy Bay	Tank	120 d/ 4 mm	33	--	Dabinett and Couturier (1994)
Cape Cod	Suspension	12 mm	24.7	18	Karney (1996); Dutra (1996)
Pool's Cove	Suspension	365 d	10-60	50-60	This study
Mahone Bay	Suspension	Juvenile	140	86.1	Dadswell and Parsons (1991)
Bay of Fundy	Wild	Juvenile	60	--	Dadswell and Parsons (1991)
Passamaquoddy Bay	Suspension	Juvenile	90-120	--	Parsons and Dadswell (1992)
N/A	N/A	160 d/ 10 mm	75	--	Black et al. (1993)

Table 3.1: Independent t-test results between initial and final short-term interval shell heights for the five different deployment intervals at Shell Fresh Farms Ltd., Pool's Cove, NF, beginning in August 1997. (D indicates deployment interval).

		Levene's Test for Equality of Variances			t-test for Equality of Means					
	Equal variance	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
D1	assumed	51.869	.000	-56.927	178	.000	-2.1256	$3.734 \times 10^{-2}$	-2.1992	-2.0519
	not assumed			-56.927	110.527	.000	-2.1256	$3.734 \times 10^{-2}$	-2.1995	-2.0516
D2	assumed	83.884	.000	-23.509	238	.000	-.8292	$3.527 \times 10^{-2}$	-.8986	-.7597
	not assumed			-23.509	160.055	.000	-.8292	$3.527 \times 10^{-2}$	-.8988	-.7595
D3	assumed	48.580	.000	-7.144	118	.000	-.4950	$6.929 \times 10^{-2}$	-.6322	-.3578
	not assumed			-7.144	75.223	.000	-.4950	$6.929 \times 10^{-2}$	-.6330	-.3570
D4	assumed	34.819	.000	-5.308	238	.000	-.1400	$2.637 \times 10^{-2}$	-.1920	$-8.8043 \times 10^{-2}$
	not assumed			-5.308	195.009	.000	-.1400	$2.637 \times 10^{-2}$	-.1920	$-8.7984 \times 10^{-2}$
D5	assumed	2.267	.135	-2.383	118	.019	$-6.6667 \times 10^{-2}$	$2.798 \times 10^{-2}$	-.1221	$-1.1256 \times 10^{-2}$
	not assumed			-2.383	111.301	.019	$-6.6667 \times 10^{-2}$	$2.798 \times 10^{-2}$	-.1221	$-1.1221 \times 10^{-2}$

Table 3.2: Pearson's correlation coefficients of short-term growth and recovery rates of nursery size scallops with mean water quality parameters at a farm-based nursery at Shell Fresh Farms Ltd., Pool's Cove, NF, from August 4 to November 8, 1997. (n=15 for all parameters)

		Temperature	Salinity	Chlorophyll- <i>a</i>	Phaeopigments	TPM	PIM	POM	PIM:POM	% POM	Sea Star Settlement
Growth rate	r -value	0.840	-0.826	0.901	0.940	-0.043	-0.573	.700	-0.702	0.773	-.796
	Sig. (2-tailed)	0.000	0.000	0.001	0.000	0.439	.013	.002	.02	.001	.000
Recovery rate	r -value	0.828	-.698	0.849	0.870	0.233	-.358	.714	-0.610	0.644	-.890
	Sig. (2-tailed)	0.000	0.002	0.000	0.000	0.201	0.095	0.001	0.008	0.005	0.000

Table 3.3: Pearson's correlation coefficients of short-term growth rates of nursery size scallops with mean phytoplankton densities at a farm-based nursery at Shell Fresh Farms Ltd., Pool's Cove, NF, from August 4 to November 8, 1997.

	Total	Microzooplankton	Chaonoflagellates	Dinoflagellates	Prymnesiophytes	Cryptophytes	Auto-nanoflagellates	Centric Diatoms	Pelagic Pennate Diatoms	Unidentified	Rhizoselina sp.	Navicula sp.1	Chlamydas sp.	Ochromonas sp.	Micromonas sp.	Coccolithophore	Prorocentrum sp.	Chaonoflagellate sp.	Strombilitium minimum
r	.994	.558	.772	-.058	.895	.789	.993	-.635	.991	.893	.726	.987	.687	.913	.980	.895	.944	.772	.974
Sig. (2-tailed)	.000	.031	.001	.837	.000	.000	.000	.011	.000	.000	.002	.000	.005	.000	.000	.000	.000	.001	.000
N	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

Table 4.1: Un-ionized ammonia nitrogen (UAN) concentrations measured in hatchery tanks for 1997 and 1998. Calculations are based on regression line equation of the relationship between absorbance and mg total ammonia nitrogen (TAN)/L (Appendix 4.2). Percent ionization is determined from Emerson et al. (1975) using temperature and pH values.

Year	Source	Absorbance	mg TAN/L	Temperature (°C)	pH	% UAN	Concentration (mg UAN/L)
1997	FSW	0.049	0.11	15	8	2.67	>0.01
	Tank 14 (16d)	0.052	0.28	15	8	2.67	0.01
	Broodstock 1	0.112	3.67	12	8	2.13	0.08
	Broodstock 9	0.079	1.81	12	8	2.13	0.04
	Tank 9 (46 d- 2d)	0.039	-0.45	16	8	2.87	-0.01
	Tank 17 (67 d- 2d)	0.049	0.12	17.7	8.08	3.31	>0.01
	Tank 17 (wash ie algae and waste)	0.041	-0.34	16.2	8.06	2.87	-0.01
	Larvae in small bucket- Tank 22	0.072	1.41	17.7	8.02	3.31	0.05
	Larvae in large bucket- Tank 22	0.048	0.058	17.7	7.99	3.31	>0.01
	1998	Broodstock tank 1	0.0725	6.28	10	8	1.83
Broodstock tank 10		0.073	6.33	10	8	1.83	0.12
Tank 19 (larvae)		0.003	0.18	16	8	2.87	0.01
Tank 20 (larvae)		0.002	0.09	16	8	2.87	>0.01
Tank 6 (trays; oldest)		0.003	0.18	16	8	2.87	0.01
Tank 13 (setting trays)		0.013	1.06	16	8	2.87	0.03
Tank 17(setting trays)		0.0085	0.66	16	8	2.87	0.02
Tank 15 (setting trays)		0.005	0.35	16	8	2.87	0.01
Tank 16 (Downwellers)		0.004	0.27	16	8	2.87	0

Table 4.2: Estimated toxicity of un-ionized ammonia nitrogen (UAN) concentrations to different shellfish based on total ammonia nitrogen (TAN), temperature, pH and calculated LC<sub>50</sub> values. Percent un-ionized ammonia is estimated from Emerson et al. (1975). All bioassays were performed over 96 hours except \* which is 72 hours. Where no pH value was given, it was assumed to be 8.0.

Species	Size/Age	Rearing temperature (°C)	pH	LC <sub>50</sub> (mg TAN/L)	% UAN	Concentration (mg UAN/L)	Reference
<i>Crassostrea virginica</i>	Juvenile	20	7.70-8.23	6	3.82	0.23	Epifanio and Srma 1975
<i>Mercenaria mercenaria</i>	Juvenile	20	7.70-8.23	3.3	3.82	0.13	Epifanio and Srma 1975
<i>Argopecten irradians</i>	Veliger Pedi-larvae	23	8.1	5.25 7.84	4.7	0.25 0.37	Lin 1992
<i>Placopecten magellanicus</i>	Juvenile	4 10	- -	1.8 1.0	1.14 1.83	0.03 0.02	Abraham et al. 1996
	0.5-1.0 mm 1.0-2.0 mm	1414	8.0 8.0	11.8 20.4	2.67 2.67	0.29 0.51	This study
<i>Strongylocentrotus purpuratus</i>	Embryos	15	-	3.8*	2.67	0.1	Greenstein et al. 1995
<i>Peneaus monodon</i>	Juvenile	25	8	37.4	5.38	2.01	Allan et al. 1990
<i>Metapeneaus macleayi</i>	Juvenile	27	8	26.3	6.15	1.62	Allan et al. 1990

Table 4.3: Behavior of scallop spat (1.0- 2.0 mm shell height) after 96-hour exposure to different total ammonia nitrogen (TAN) concentrations. Response includes the retracting of mantles and closing of valves. Similar behavior was observed in scallop spat (0.5 to 1.0 mm shell height) over the ammonia concentration range 0 to 27 mg TAN/L.

Ammonia concentration (mg TAN/L)	Trial 1-4
0	Gaping, most with tentacles fully (some partially) extended; immediate response; some swimming, moving with foot or clapping valves.
9	Gaping, most with tentacles partially or not extended; none to immediate response; few moving; few swimming.
18	Majority gaping with mantle retracted and/or irregularly attached or gone or not gaping at all; few with tentacle partially extended; none to immediate response; mucous production, in some cases extensive.
27	Majority gaping with mantle retracted and/or irregularly attached or gone; none with tentacles extended; none to immediate response; mucous production, in some cases extensive.
36	Few gaping, most closed with mantle retracted; none with tentacles extended; majority had no response; mucous production in a few.



## Figures

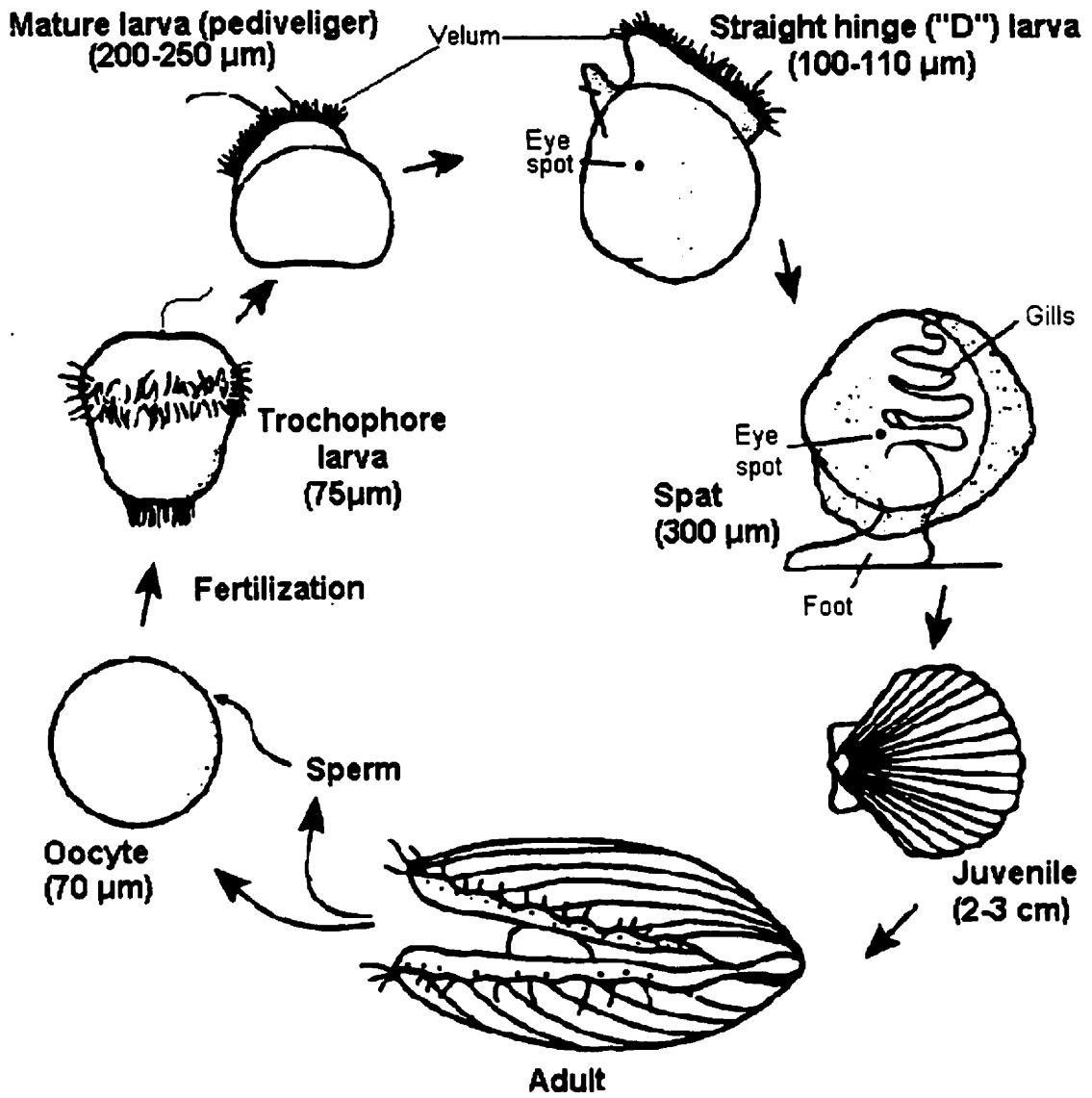


Figure 1.1: Life cycle of the scallop (adapted from Bourne et al. 1989).

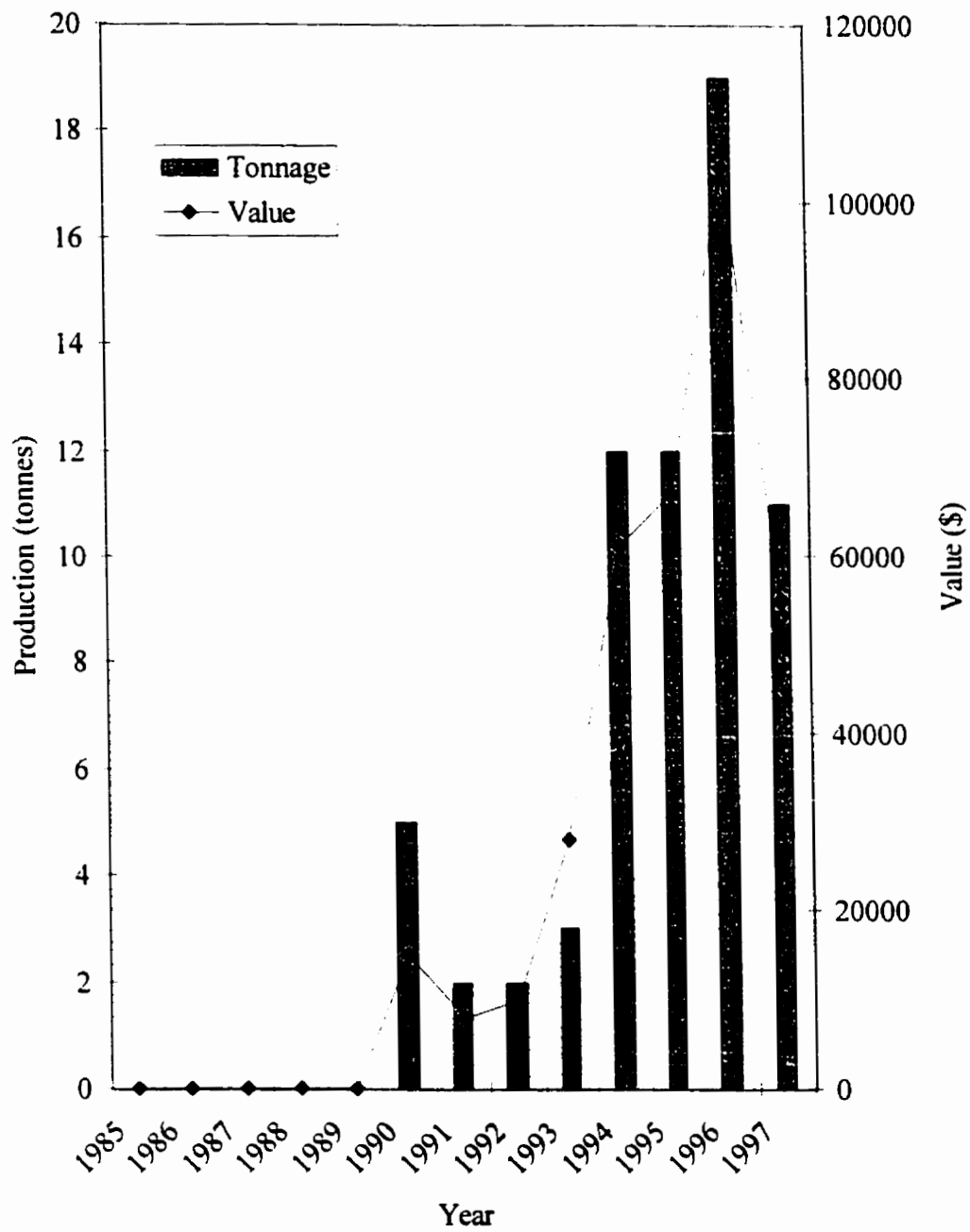


Figure 1.2: Production and value of cultured sea scallops in Newfoundland from 1985 to 1997 (Source: Department of Fisheries and Oceans Statistics Board 1999).

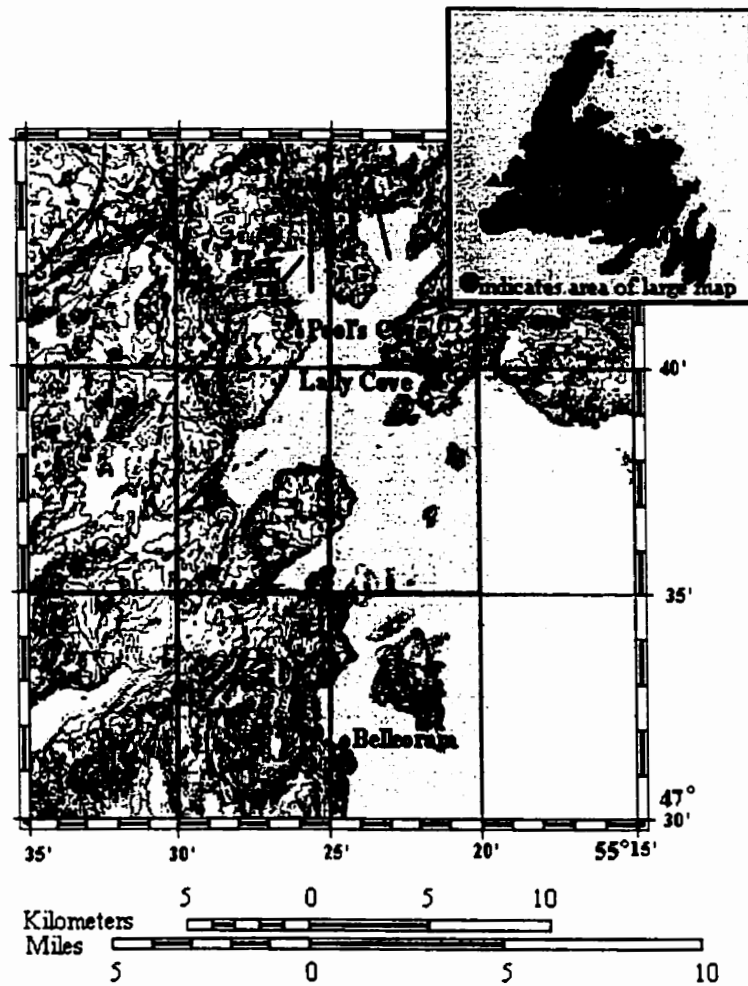


Figure 2.1: Location of Shell Fresh Farms Ltd., Pool's Cove, NF, showing the three main areas of the farm; The Run (TR), Fox Point (FP) and Ladder Garden (LG). Ladder Garden is the site of the farm-based nursery. (adapted from Department of Environment and Lands 1993)

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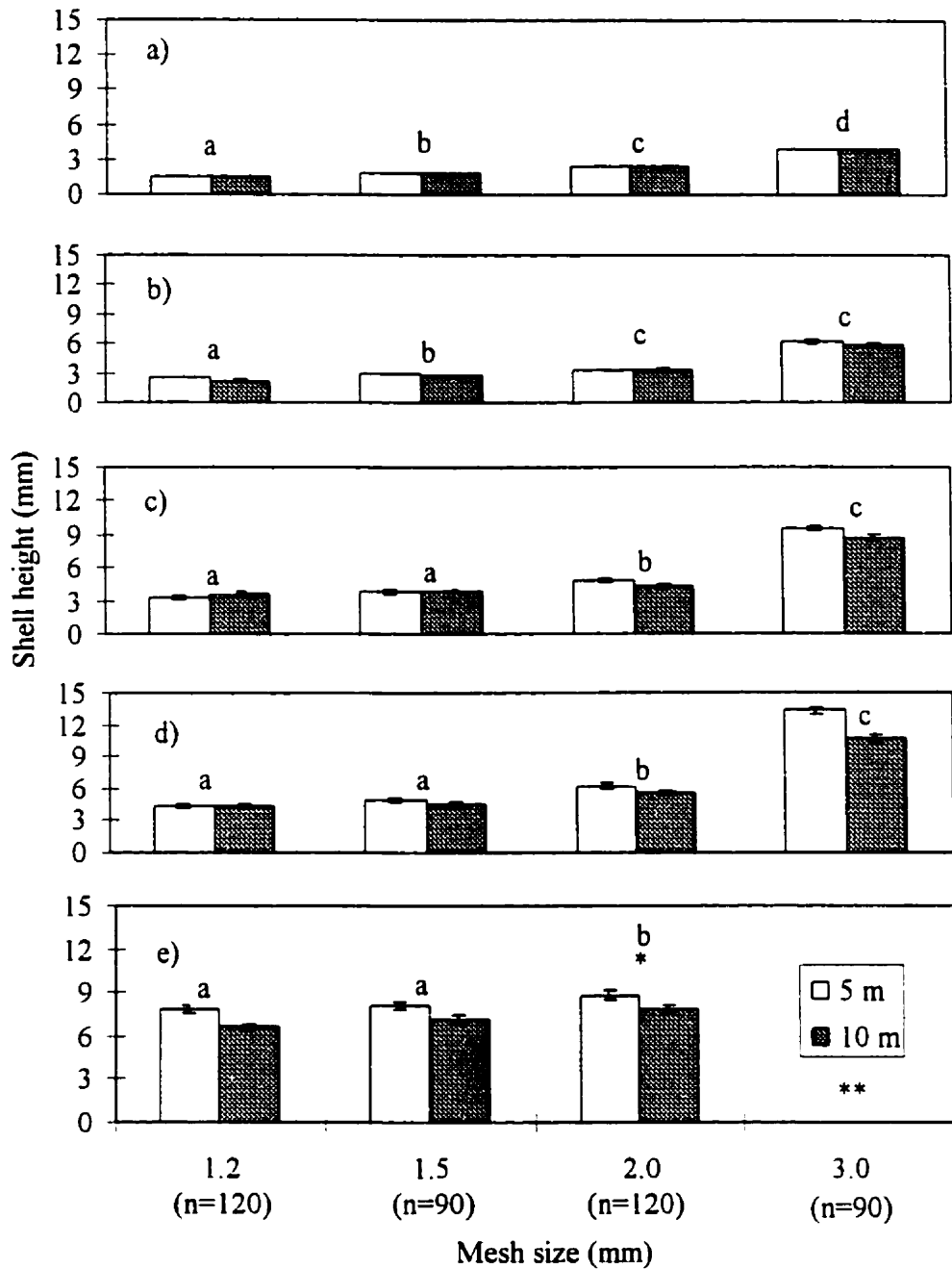


Figure 2.2: Mean shell height ( $\pm$ S.E.) of hatchery-reared scallop spat grown in four mesh sizes at two depths at Shell Fresh Farms Ltd., Pool's Cove, NF, starting on a) October 9, 1996 (n=30 for all mesh sizes) and as sampled on b) November 25, 1996, c) March 1, 1997 (\* n=90), d) May 1, 1997, and e) July 3, 1997 (\*\*n=0). Common letter denotes no significant difference among shell heights for equipments on each sample date (Tukey's-B test).

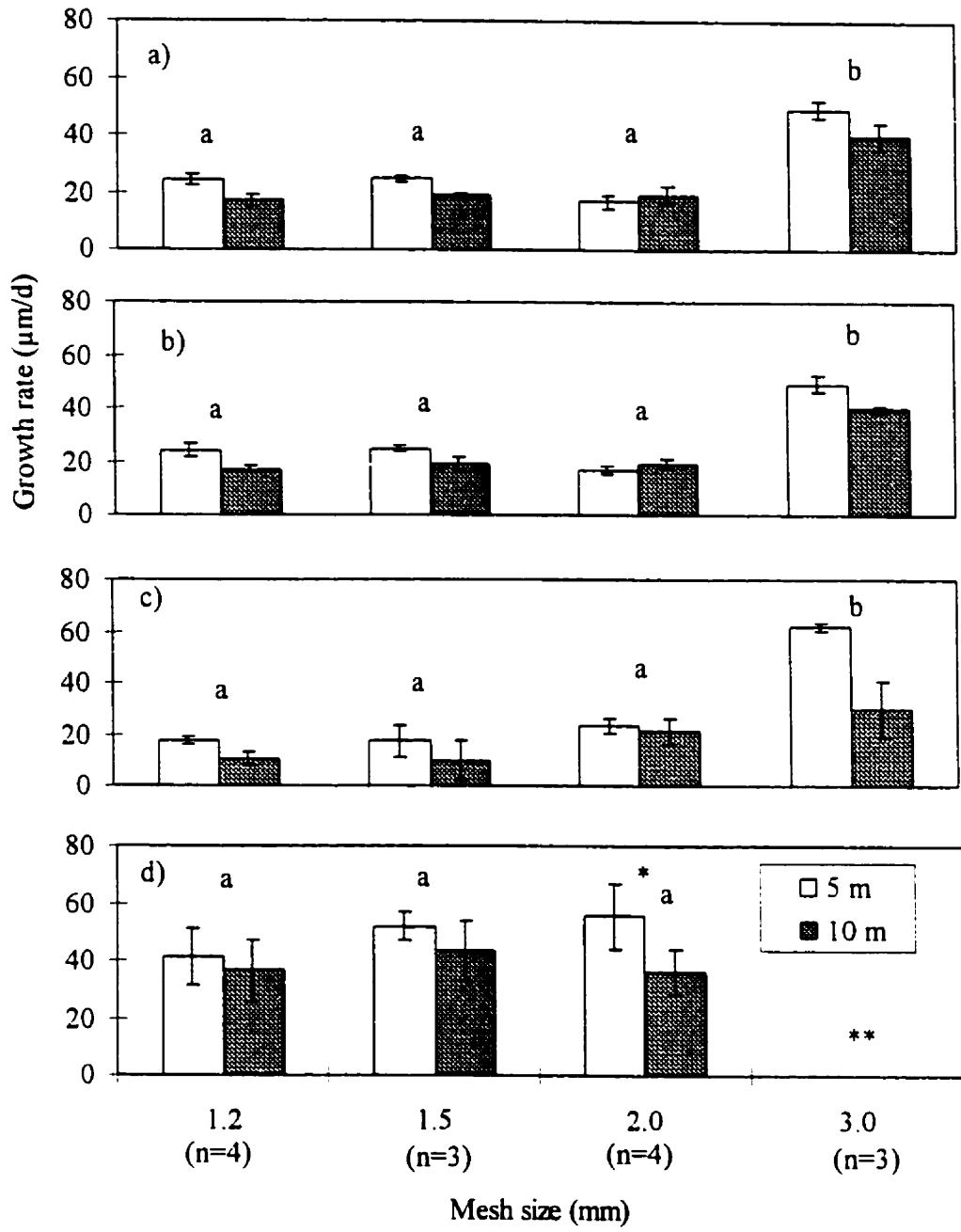


Figure 2.3: Mean interval growth rates ( $\pm$ S.E) of scallops held in four mesh sizes at two depths over four time intervals at Shell Fresh Farm Ltd., Pool's Cove, NF, from a) October 9 to November 25, 1996, b) November 25, 1996 to March 1, 1997 (\*n=3), c) March 1 to May 1, 1997, and d) May 1 to July 3, 1997 (\*\*n=0). Common letters denote no significant differences among recovery for mesh sizes on each sample date (Tukey's-B test).

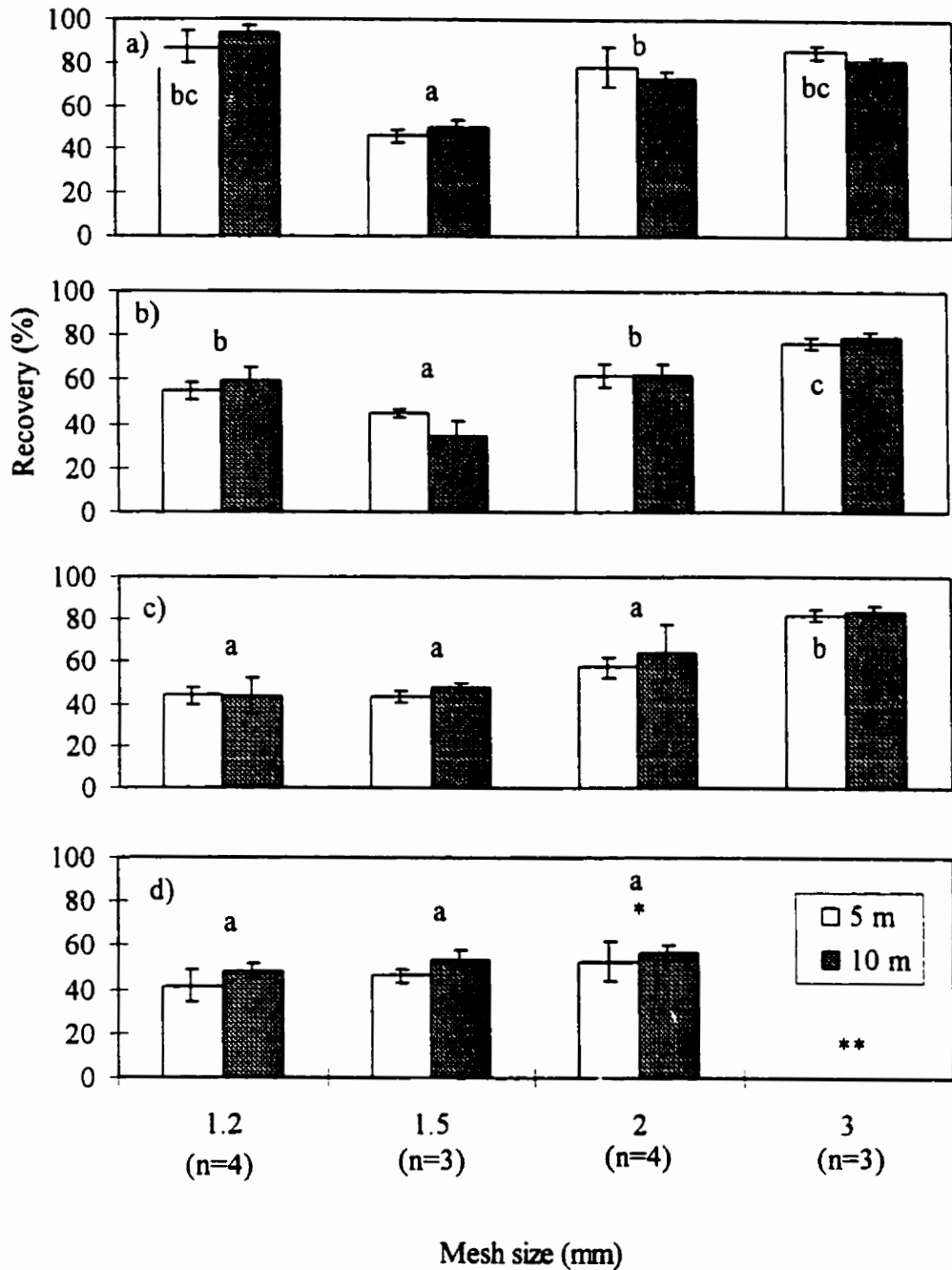


Figure 2.4: Mean recovery ( $\pm$ S.E.) of scallops deployed on October 9, 1996, in four mesh sizes at two depths at Shell Fresh Farms Ltd., Pool's Cove, NF, as sampled on a) November 25, 1996, b) March 1, 1997 (\*n=3), c) May 1, 1997, and d) July 3, 1997 (\*\*n=0). Common letters denotes no significant difference among recovery for each mesh size on each sample date (Tukey's-B test).



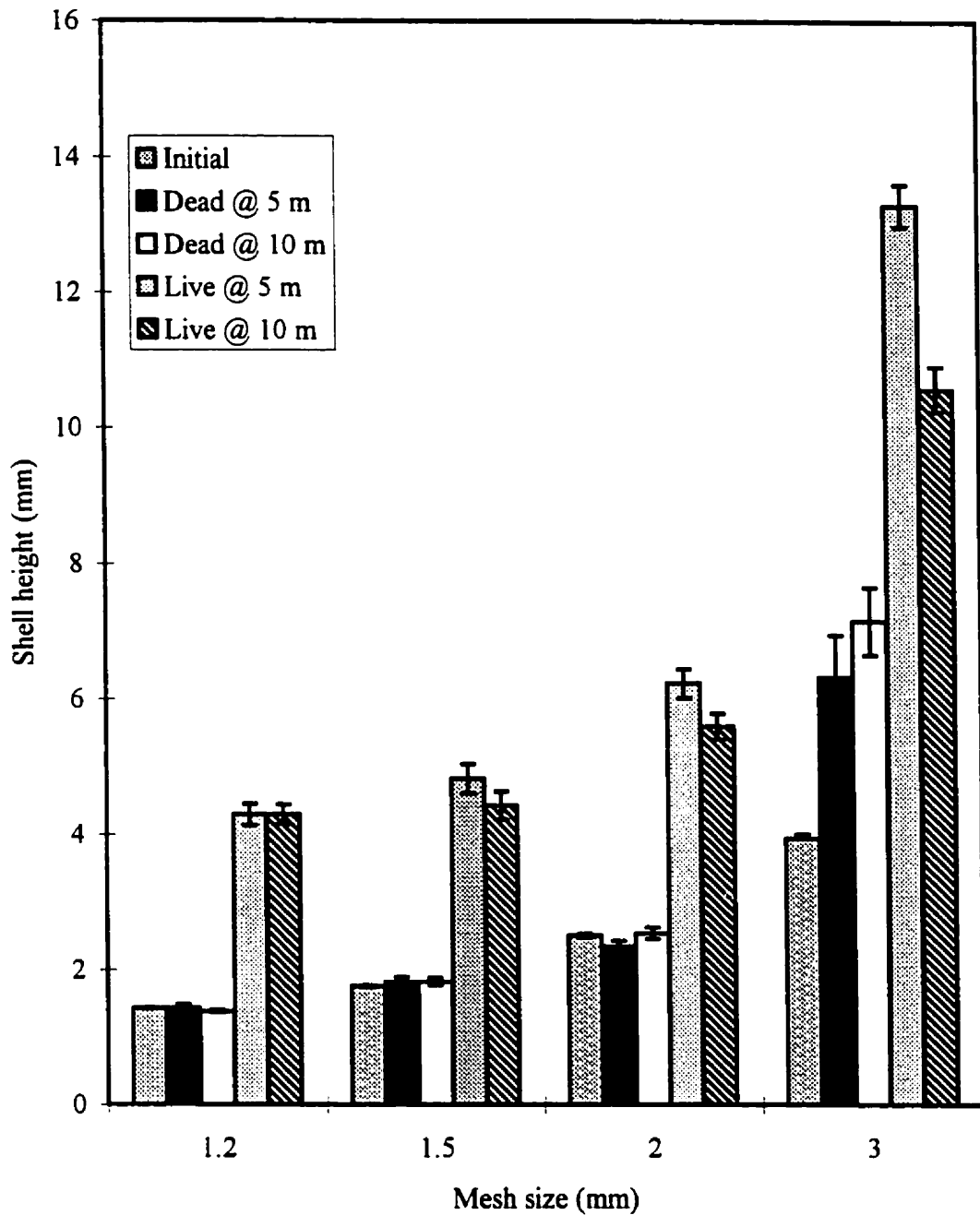


Figure 2.5: Mean initial (live) and final (live and dead) shell heights ( $\pm$  S.E.) of scallops held in 1.2 and 2.0 mm mesh collector bags and 1.5 and 3.0 mm pearl nets on a farm-based nursery at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 9, 1996 to May 1, 1997.

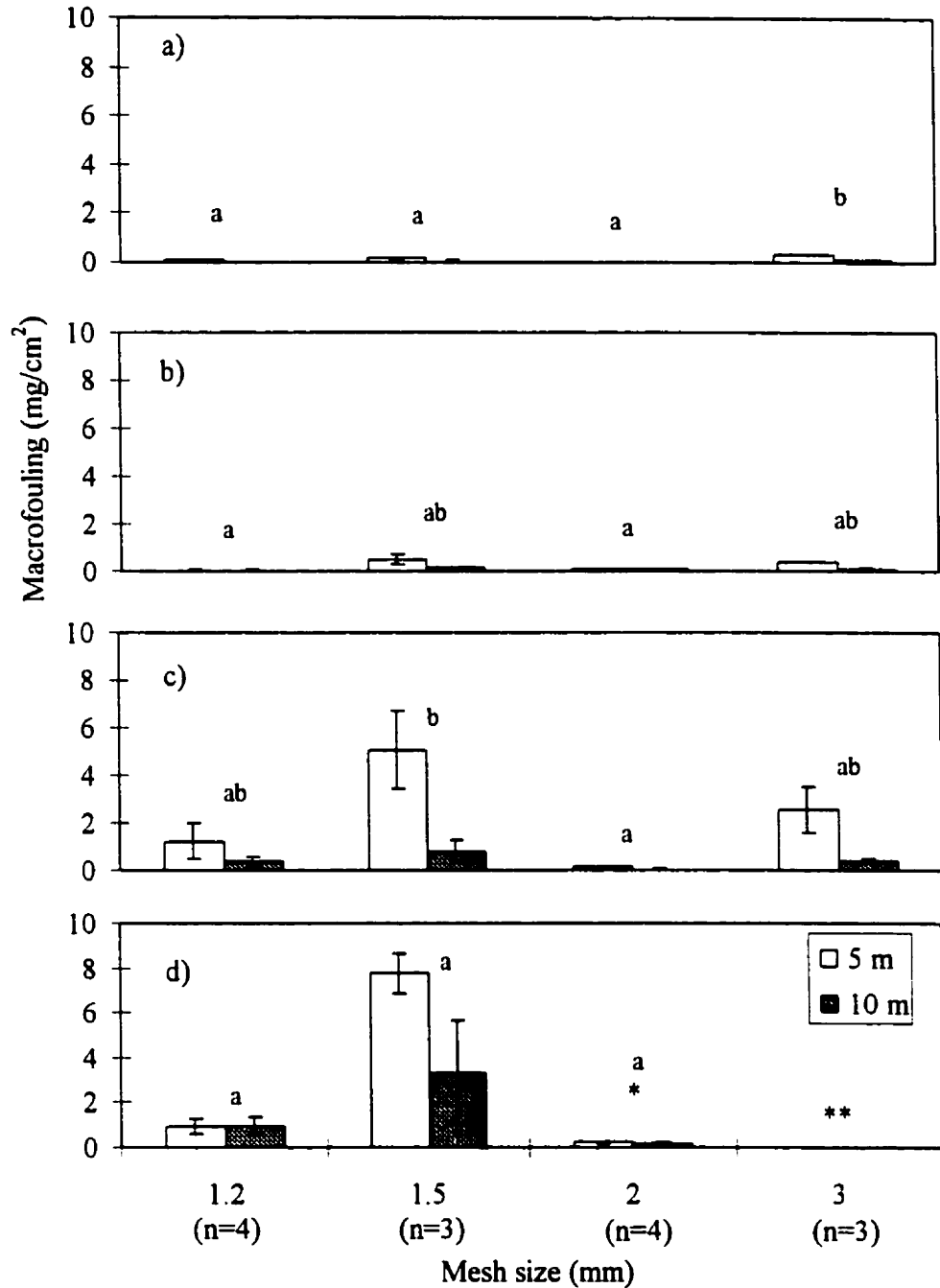


Figure 2.6: Mean macrofouling accumulation ( $\pm$ S.E.) on four mesh sizes of equipment suspended at two depths on October 9, 1996, at Shell Fresh Farms Ltd., Pool's Cove, NF, as sampled on a) November 25, 1996, b) March 1, 1997 (\*n=3), c) May 1, 1997, and d) July 3, 1997 (\*\*n=0). Common letter denotes no significant difference among macrofouling on mesh sizes for each sample date (Tukey's-B test)

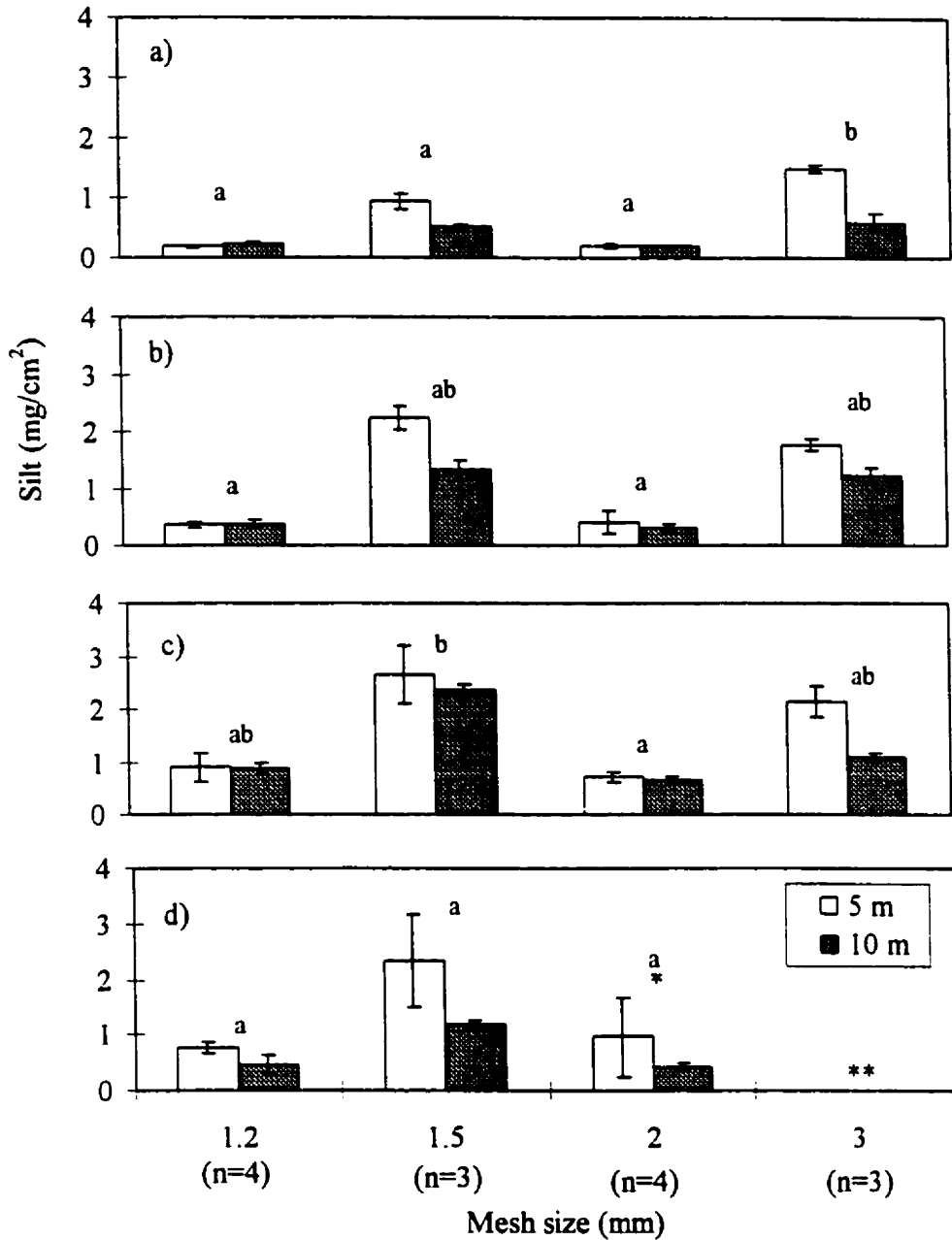


Figure 2.7: Mean silt accumulation ( $\pm$ S.E.) on four mesh sizes of equipment suspended at two depths on October 9, 1996, at Shell Fresh Farms Ltd., Pool's Cove, NF, as sampled on a) November 25, 1996, b) March 1, 1997 (\*n=3), c) May 1, 1997, and July 3, 1997 (\*\*n=0). Common letter denotes no significant difference among silt accumulation on mesh sizes for each sample date (Tukey's-B test).

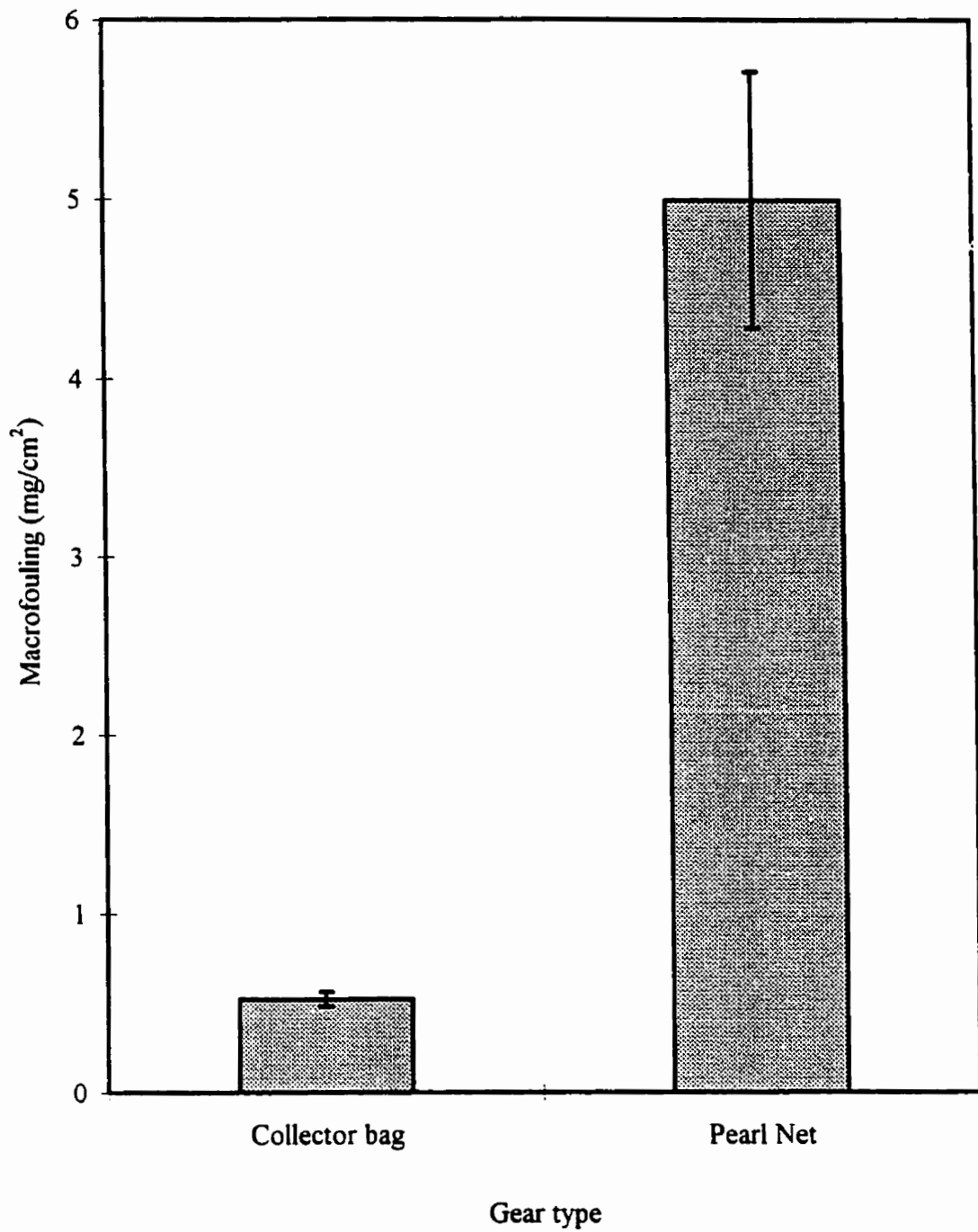


Figure 2.8: Mean macrofouling accumulation ( $\pm$  S.E.) on 3.0 mm mesh gear held at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1997 to May 1998 (n=3).

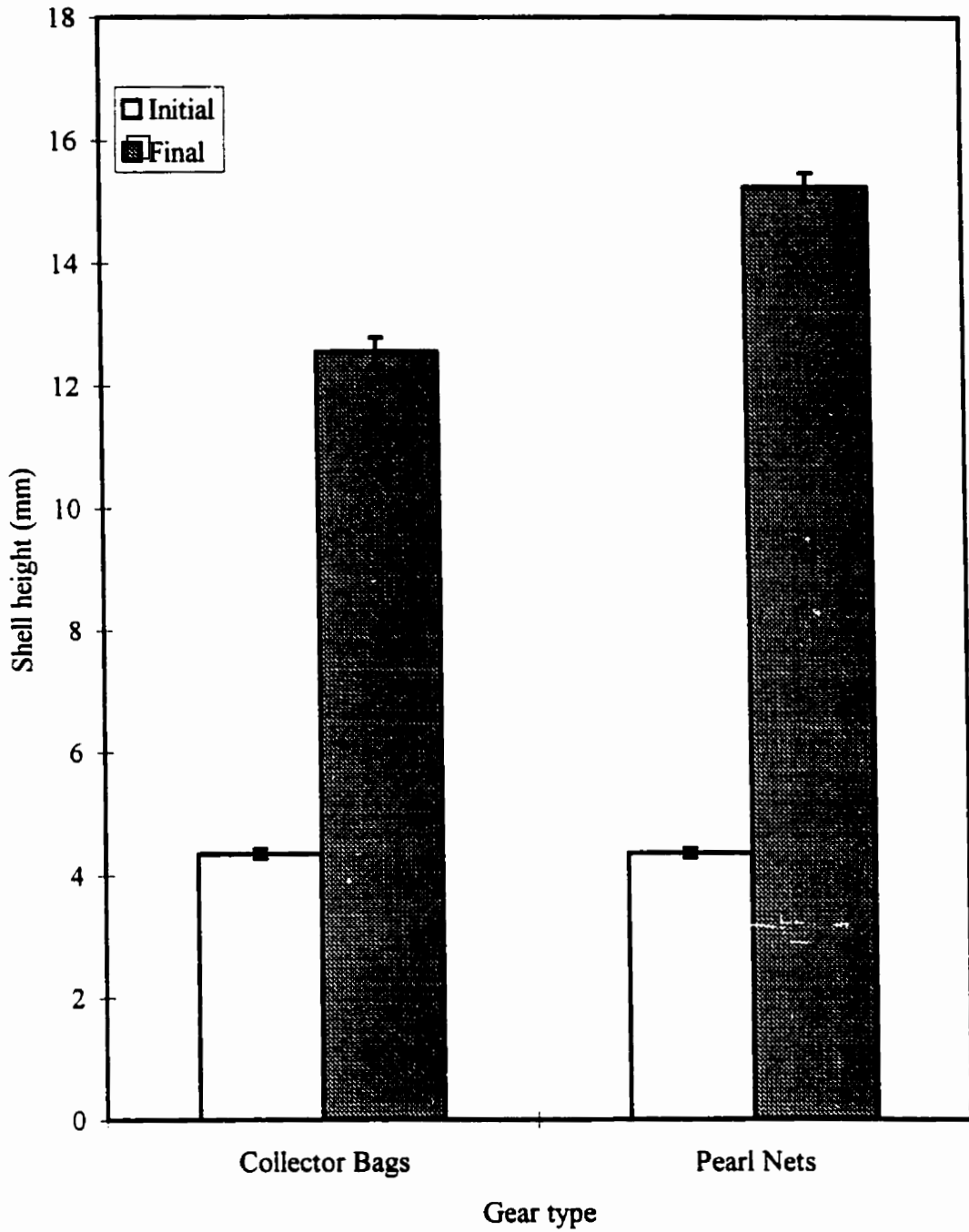


Figure 2.9: Mean initial and final shell heights ( $\pm$  S.E.) of scallops grown in 3.0 mm gear at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1997 to May 1998 (n=90).

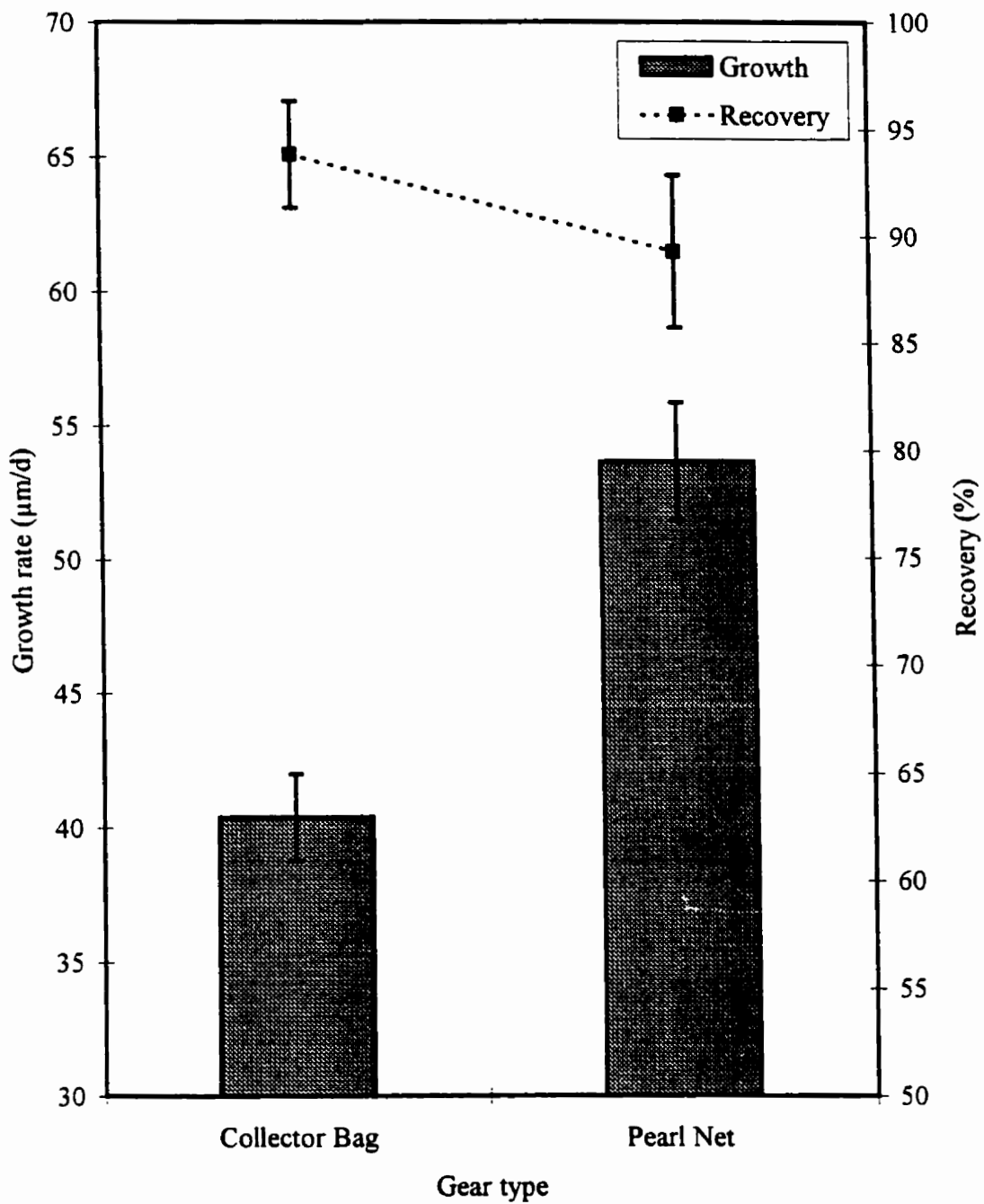


Figure 2.10: Mean growth rates and recovery ( $\pm$  S.E.) of scallops grown in 3.0 mm gear at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1997 to May 1998 (n=3).

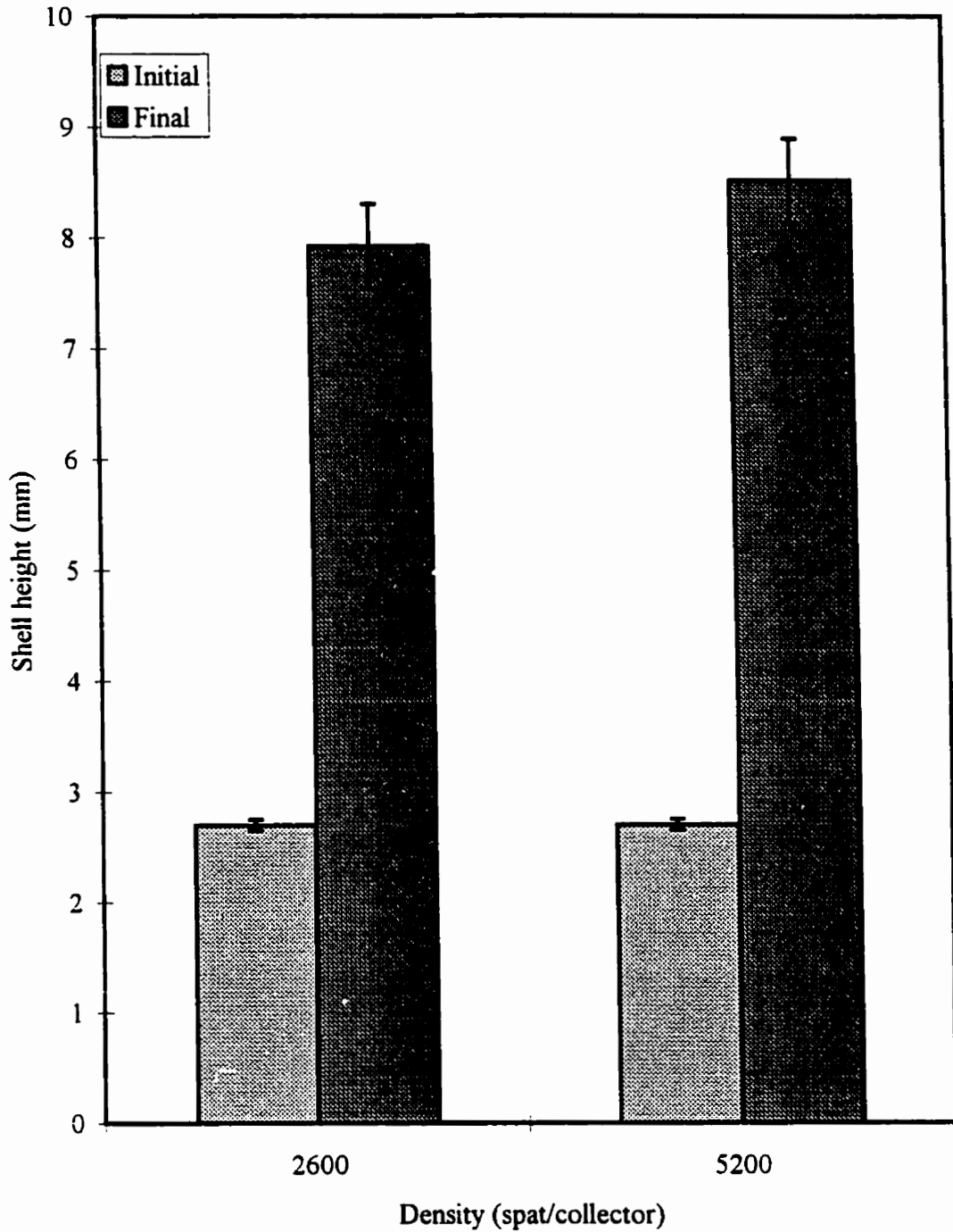


Figure 2.11: Mean initial and final shell heights ( $\pm$  S.E.;  $n=30$ ) of scallops in 2.0 mm collector bags grown at two densities at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1997 to June 1998.

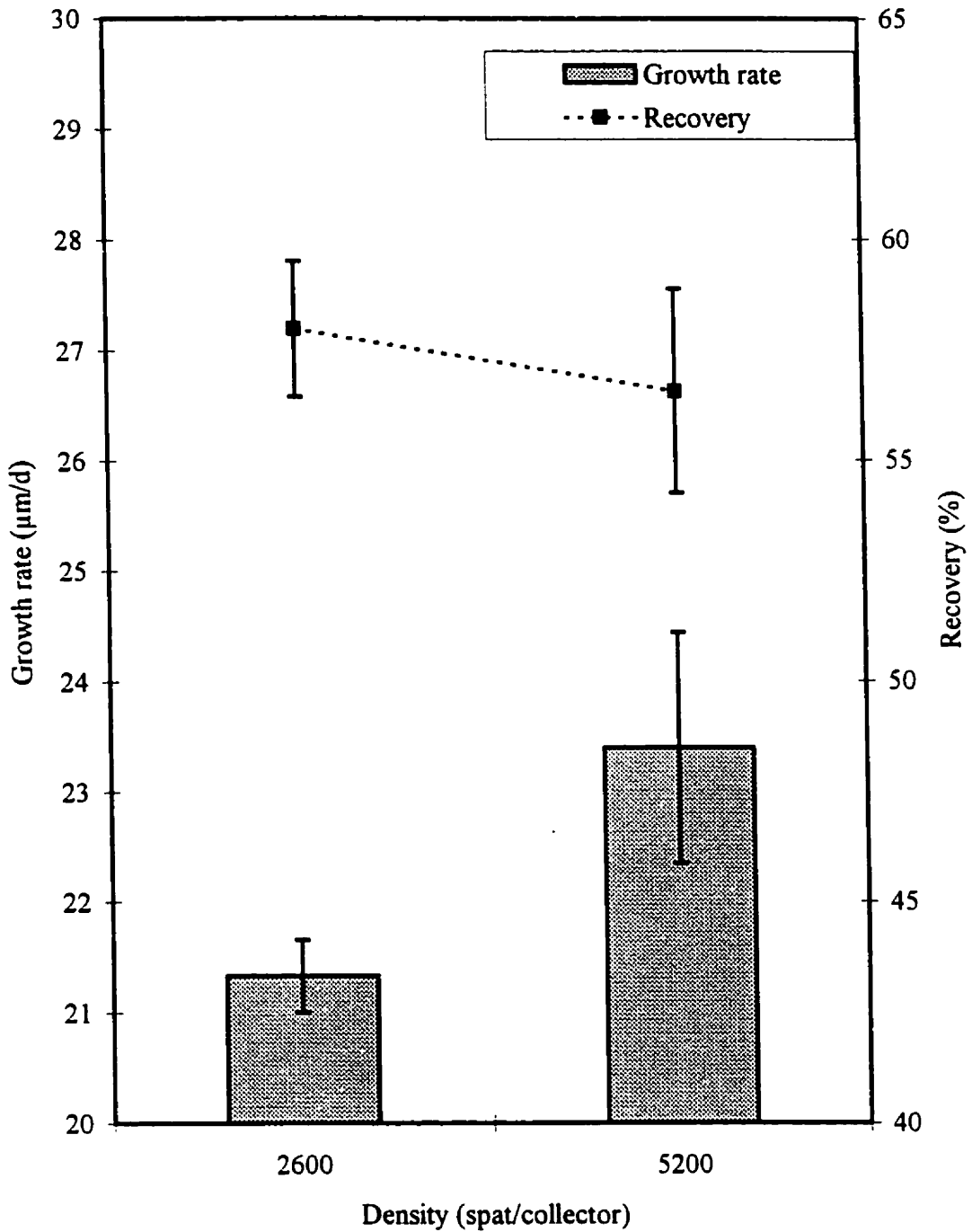


Figure 2.12: Mean growth rates and recovery ( $\pm$  S.E.) of scallops in 2.0 mm collector bags at two densities grown at Shell Fresh Farms Ltd., Pool's Cove, NF, from October 1997 to June 1998 (n=3).



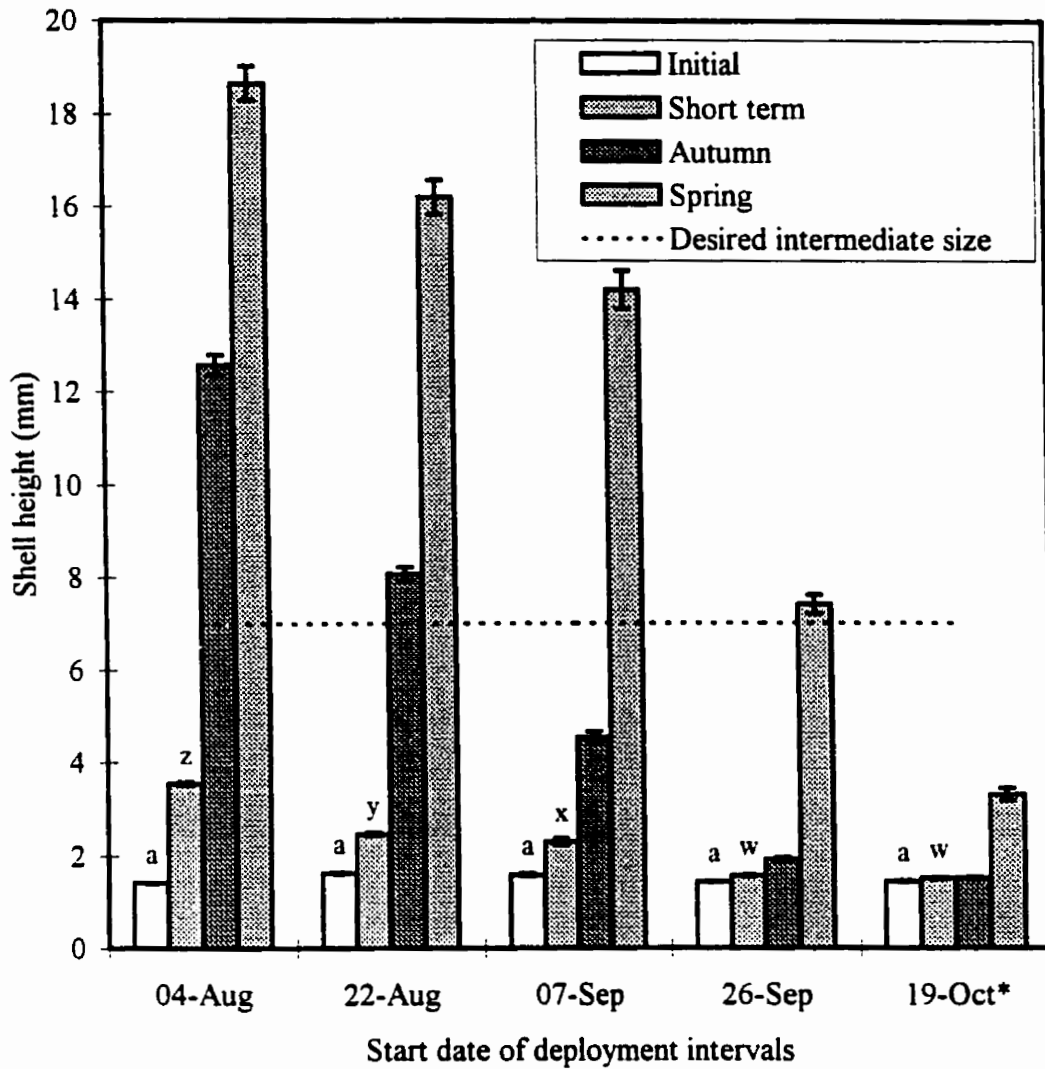


Figure 3.1: Mean shell height ( $\pm$  S.E.) of scallops at the end of deployment over five consecutive two week intervals in 1997, and on November 8, 1997, and June 24, 1998, at Shell Fresh Farms Ltd., Pool's Cove, NF. The start date of an interval was the end date of the previous short-term interval. Common letter denotes no significant difference among mean shell heights for each sample period (Tukey's B test). [\*long-term equals short-term shell height for this date]

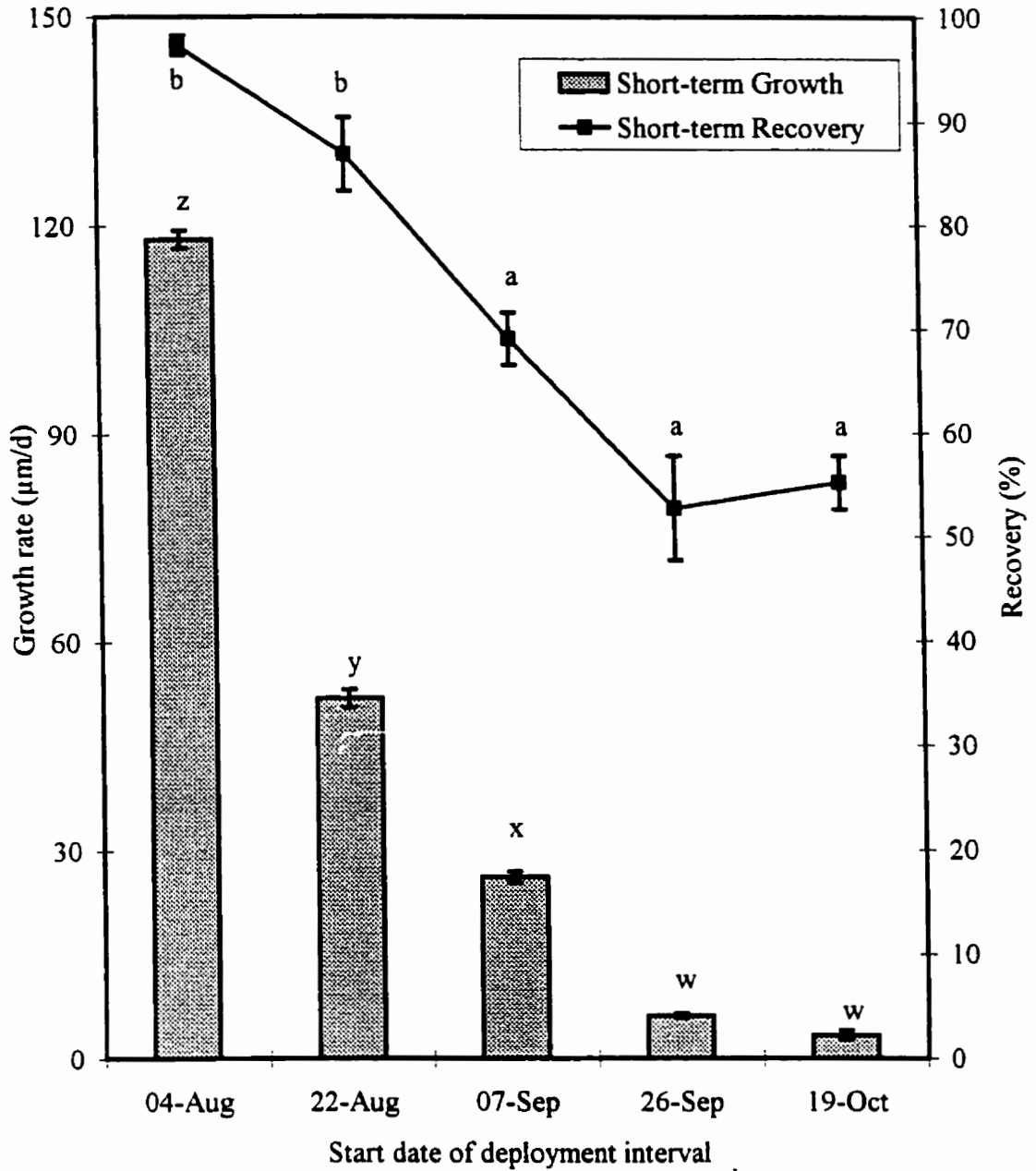


Figure 3.2: Mean growth rates and recovery ( $\pm$  S.E.) of scallops over consecutive deployment intervals at Shell Fresh Farms Ltd., Pool's Cove, NF. The start date of an interval is the end date of the previous interval. Common letter denotes no significant difference in growth rates or recovery rates among intervals (Tukey's B test).

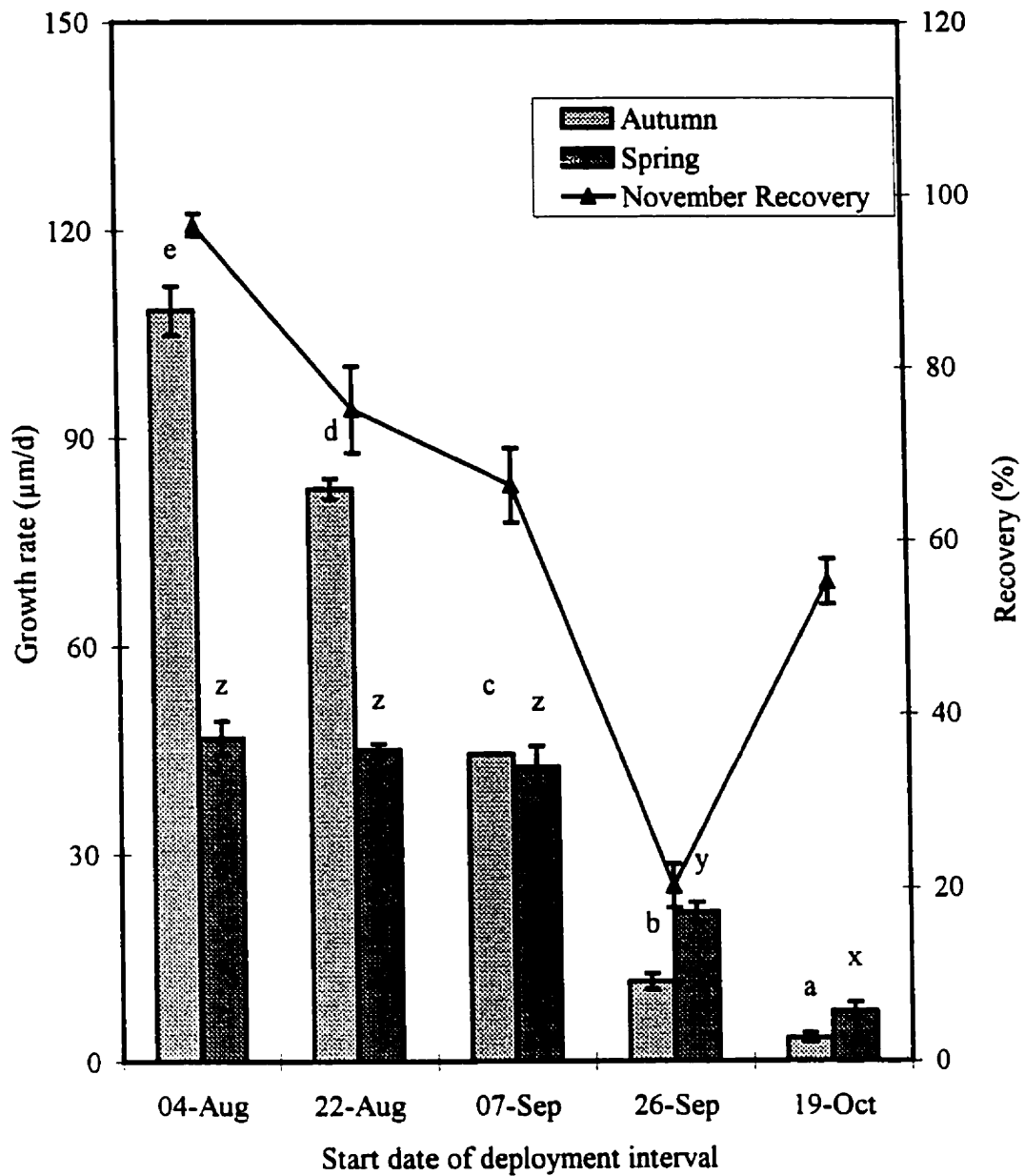


Figure 3.3: Mean growth rates and recovery ( $\pm$  S.E.) of scallops deployed at a farm-based nursery at Shell Fresh Farms Ltd., Pool's Cove, NF, on five dates in 1997 and sampled on November 8, 1997, and June 24, 1998. Common letter denotes no significant difference in growth rates or recovery rates among intervals (Tukey's B test).

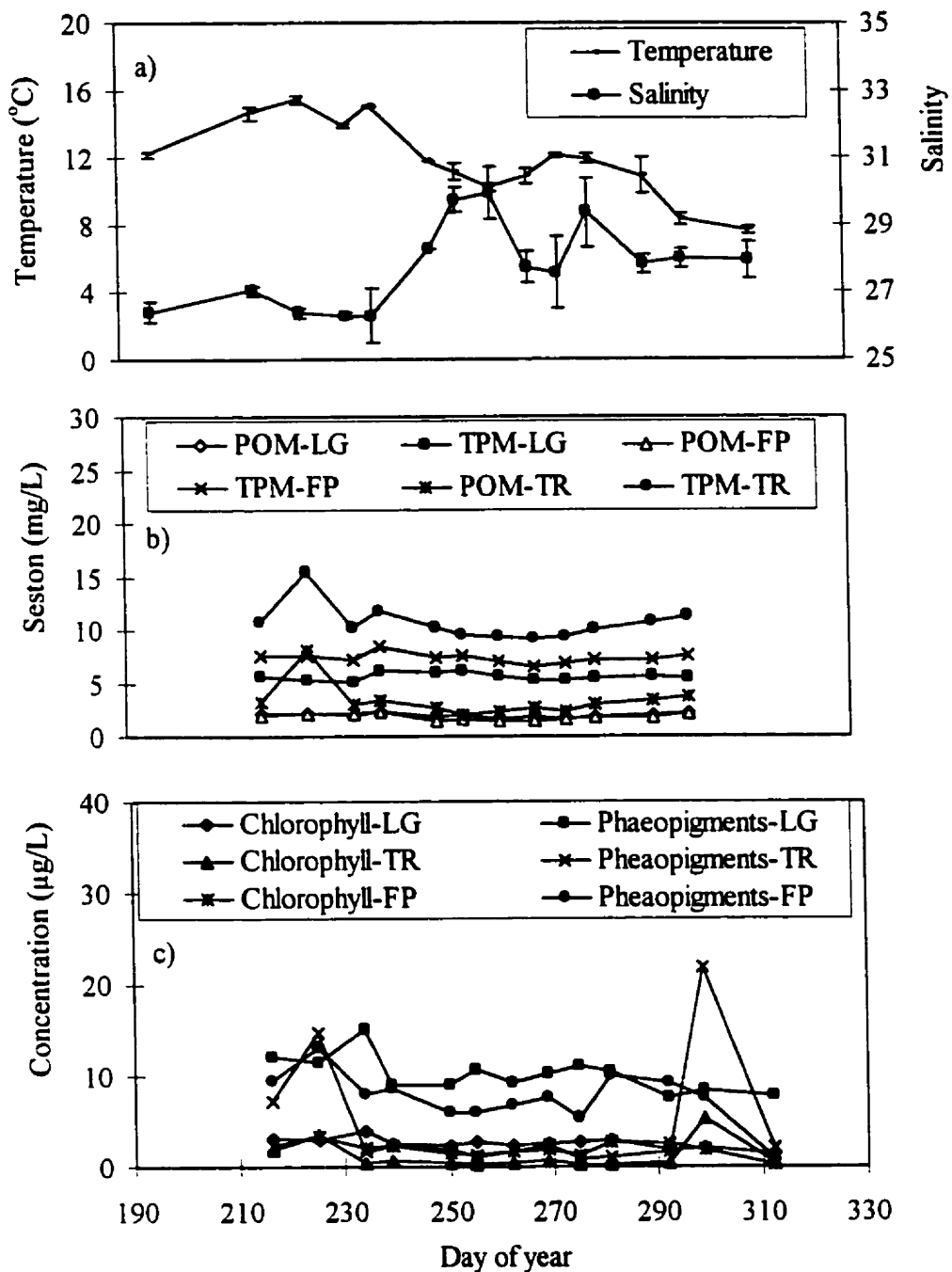


Figure 3.4: Water quality at Shell Fresh Farms Ltd., Pool's Cove, NF, from July 15 to November 22, 1997. a) Temperature and salinity ( $\pm$  S.E.;  $n=3$ ), b) seston, c) chlorophyll and phaeopigments at 5 m. (TPM-total particulate matter; POM-particulate organic matter; LG-Ladder Garden; FP- Fox Point; TR- The Run)

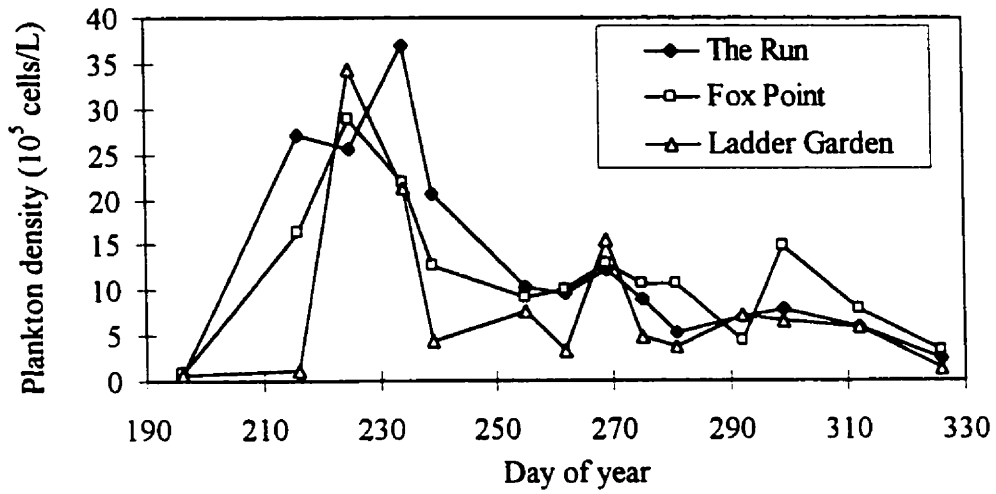


Figure 3.5: Total plankton densities at three areas of Shell Fresh Farms Ltd., Pool's Cove, NF, from July 15 to November 22, 1997.

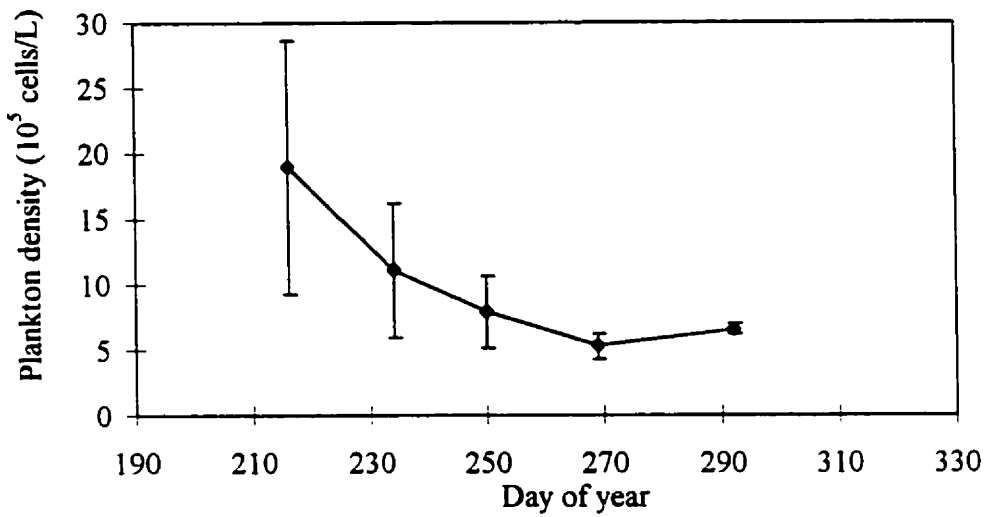


Figure 3.6: Mean density ( $\pm$  S.E.) of total plankton density over five intervals of scallop deployment on a farm-based nursery at Shell Fresh Farms Ltd., Pool's Cove, NF ( $n=3$ ). Intervals began on August 4 (day 216) and ended on November 8, 1997 (day 312).

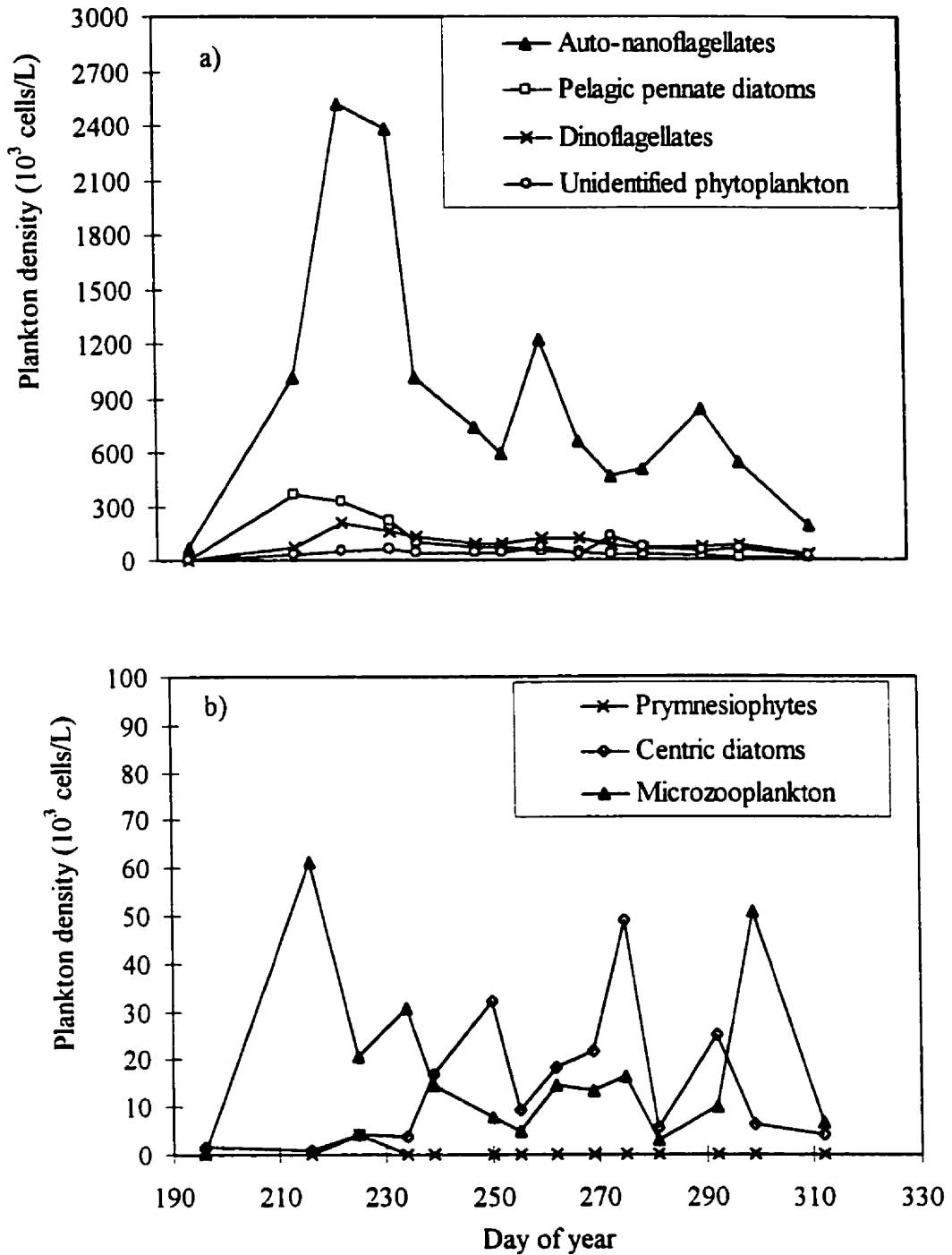


Figure 3.7: Mean density of a) four dominant and b) three less dominant groups of major plankton at Shell Fresh Farms Ltd., Pool's Cove, NF, from July 15 to November 22, 1997.

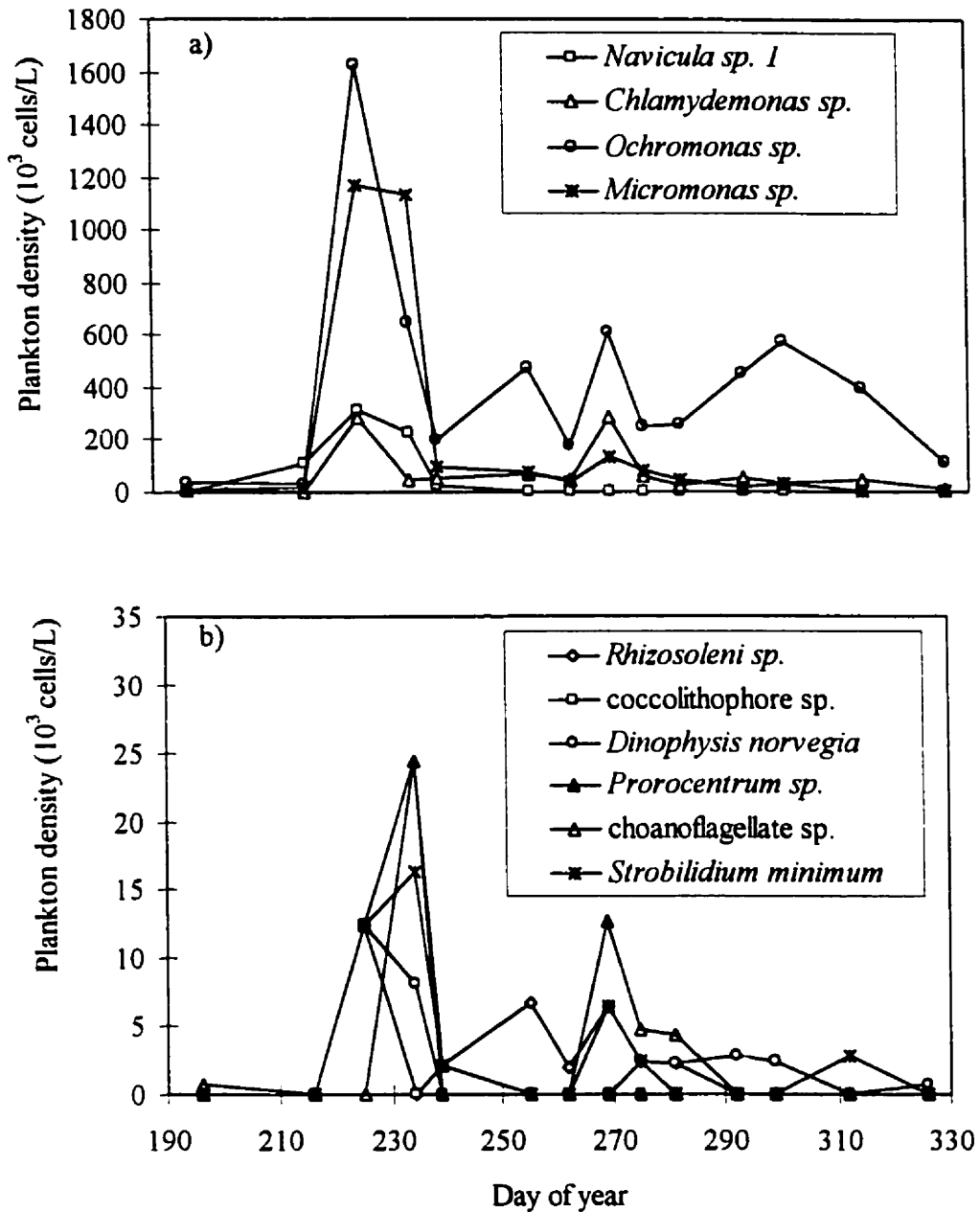


Figure 3.8: Mean density of a) dominant and b) less dominant plankton species that showed a declining trend over intervals of scallop deployment at a farm-based nursery at Shell Fresh Farms Ltd., Pool's Cove, NF, from July 15 to November 22, 1997.

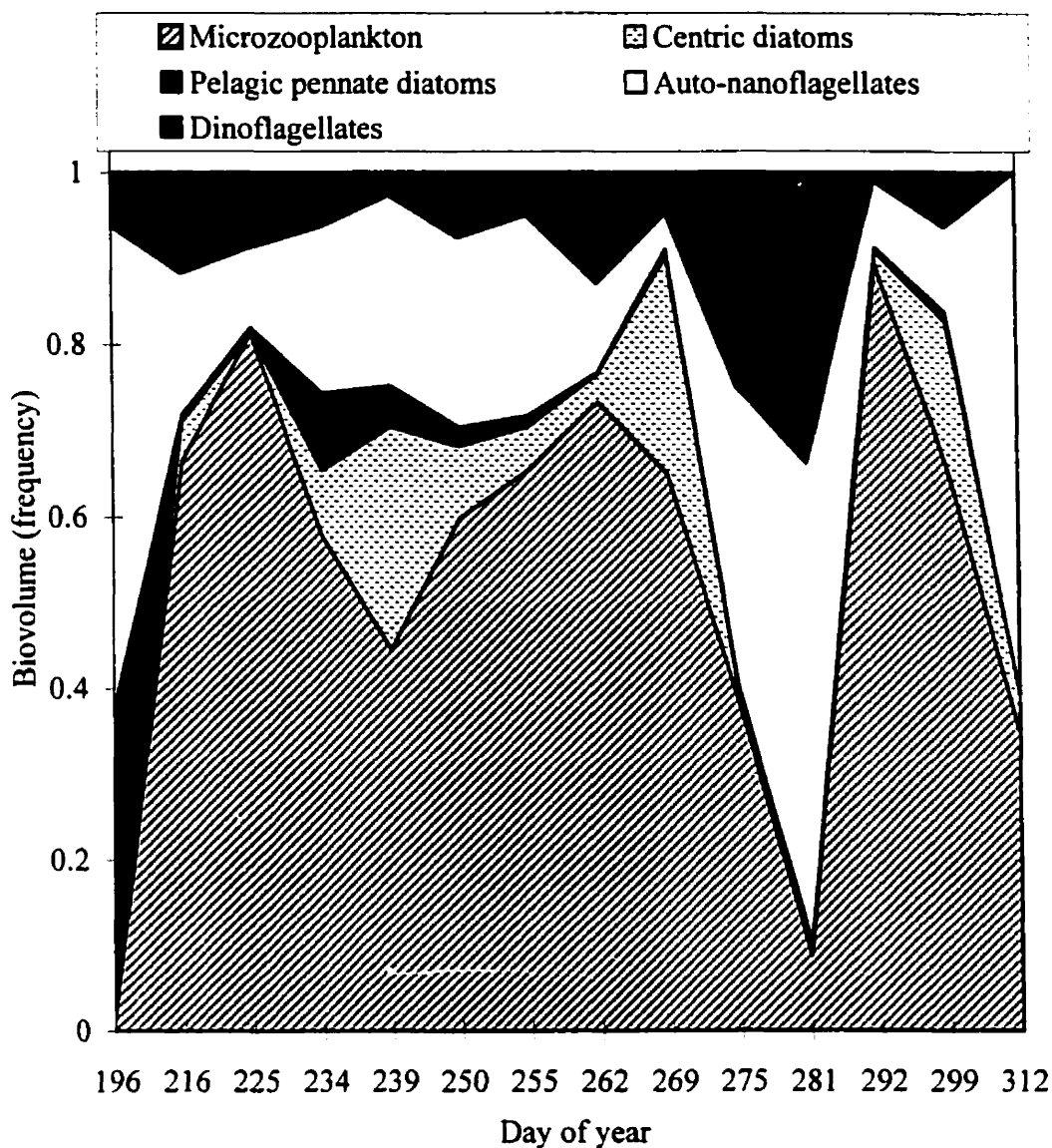


Figure 3.9: Biovolume frequency of different plankton groups at 5 m at Shell Fresh Farms Ltd., Pool's Cove, NF, from July 15 to November 8, 1997. Microzooplankton: ciliates, tintinnids and choanoflagellates. Centric diatoms: long chained species. Pennate diatoms: single-celled species. Auto-nanoflagellates: all 2 to 20  $\mu\text{m}$  flagellates. Dinoflagellates: autotrophic and heterotrophic species.



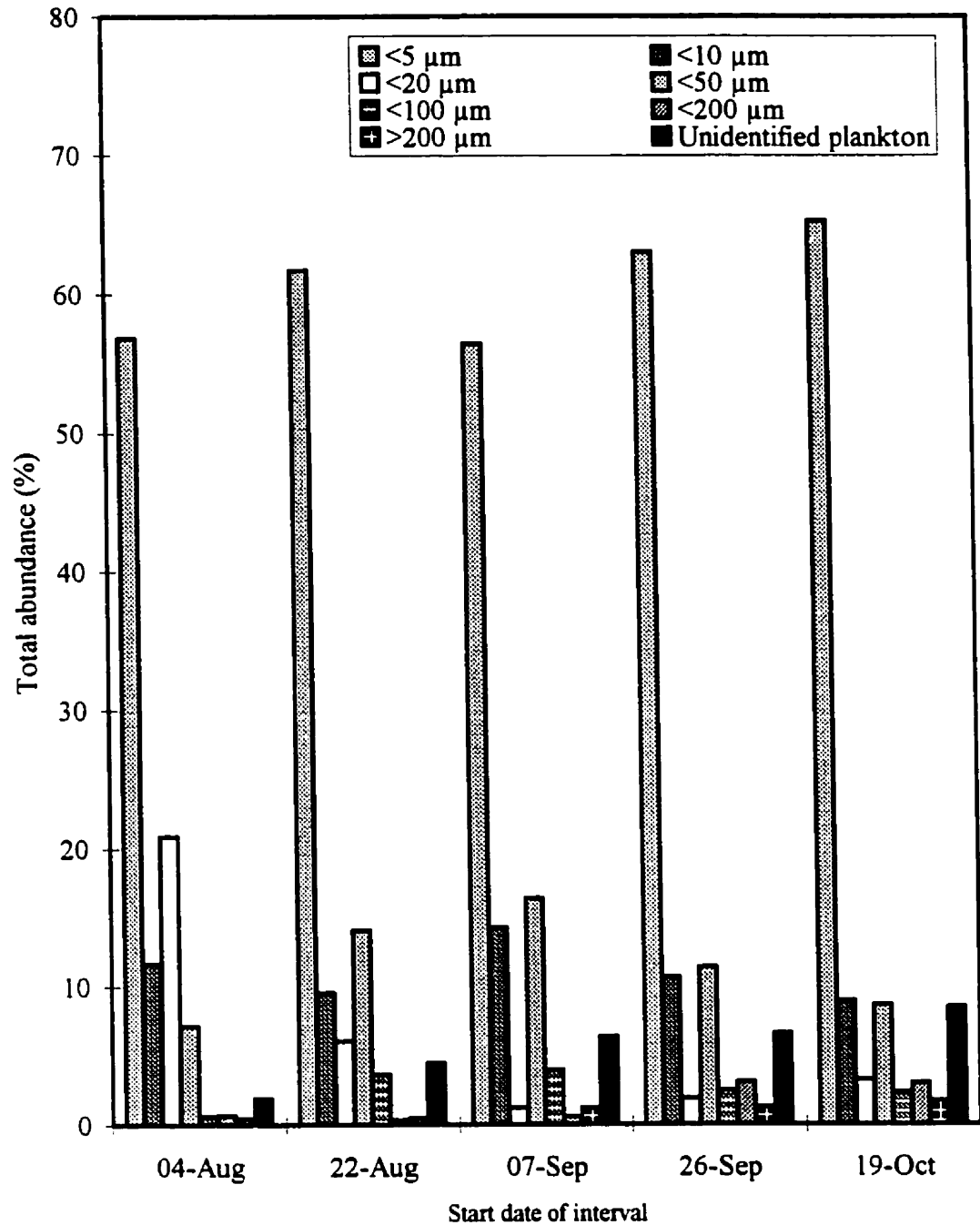


Figure 3.10: Particle size frequency distribution of plankton at Ladder Garden, Shell Fresh Farms Ltd., Pool's Cove, NF, over five consecutive deployment intervals of scallops at a farm-based nursery.

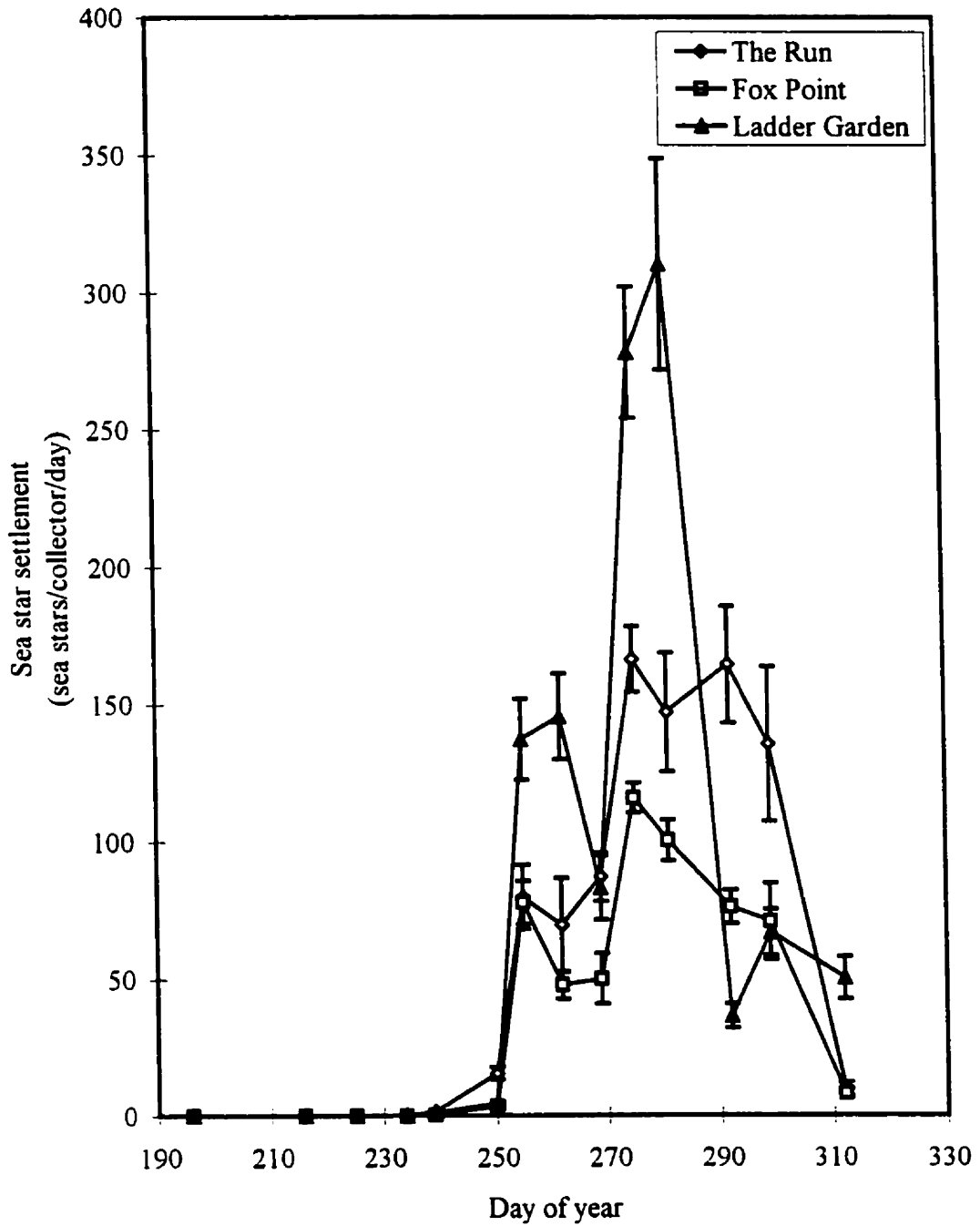


Figure 3.11: Mean sea star settlement ( $\pm$  S.E.) at three areas of Shell Fresh Farms Ltd., Pool's Cove, NF, from July 15 to November 22, 1997 (n=8).

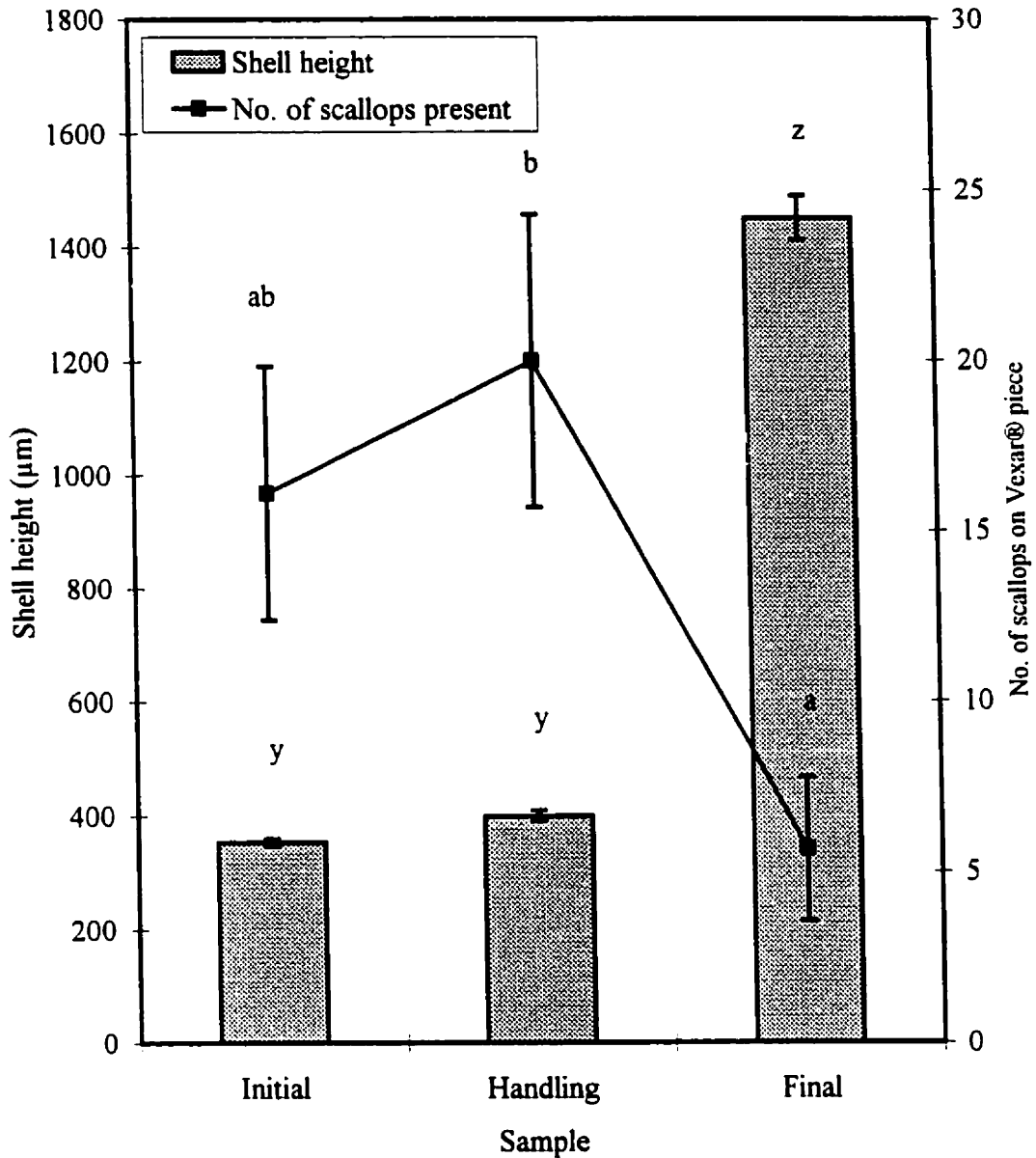


Figure 3.12: Mean shell height and number ( $\pm$  S.E.) of scallops set on 6 mm Vexar® before and after transfer and after deployment at a farm-based nursery at Shell Fresh Farm, Pool's Cove, NF, from June 29 to July 31, 1998. Common letter denotes no significant difference among shell heights or number of scallops present for the sample times (Tukey's B test).

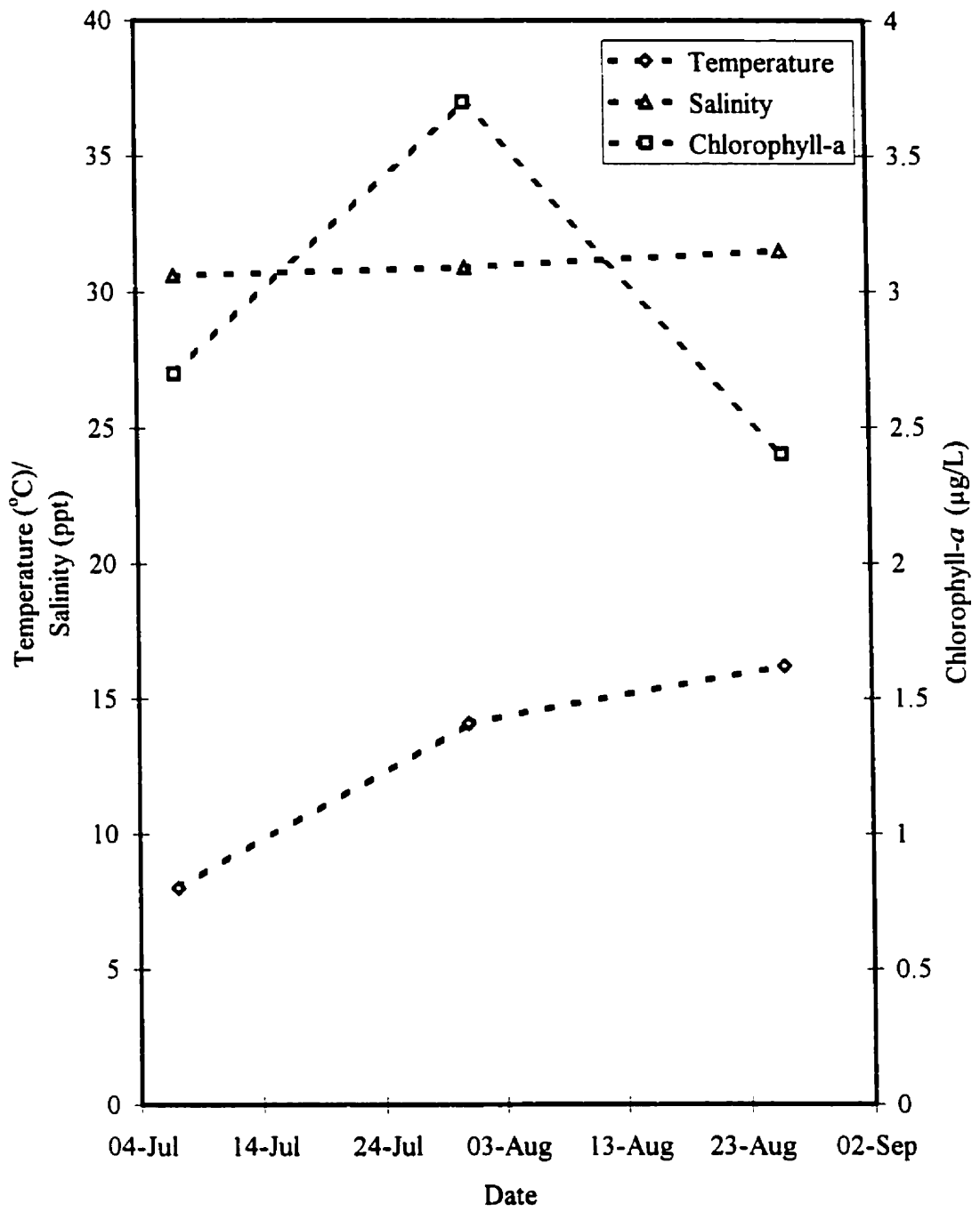


Figure 3.13: Temperature, salinity and chlorophyll-*a* levels at Ladder Garden, Shell Fresh Farms Ltd., Pool's Cove, NF, from July 7 to August 28, 1998.

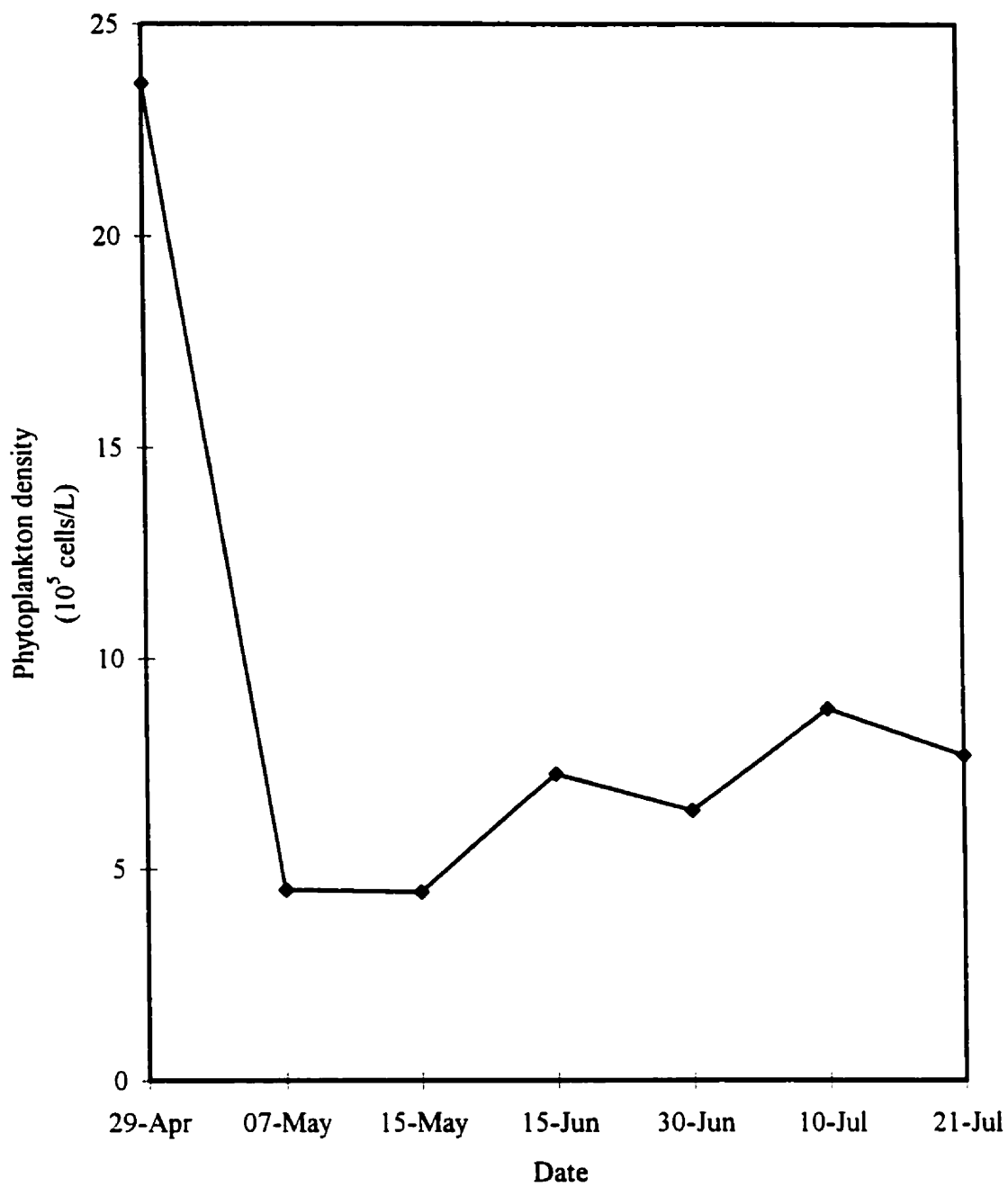


Figure 3.14: Total phytoplankton density at 5 m at Ladder Garden, Shell Fresh Farms Ltd., Pool's Cove, NF, from April 29 to July 21, 1998.

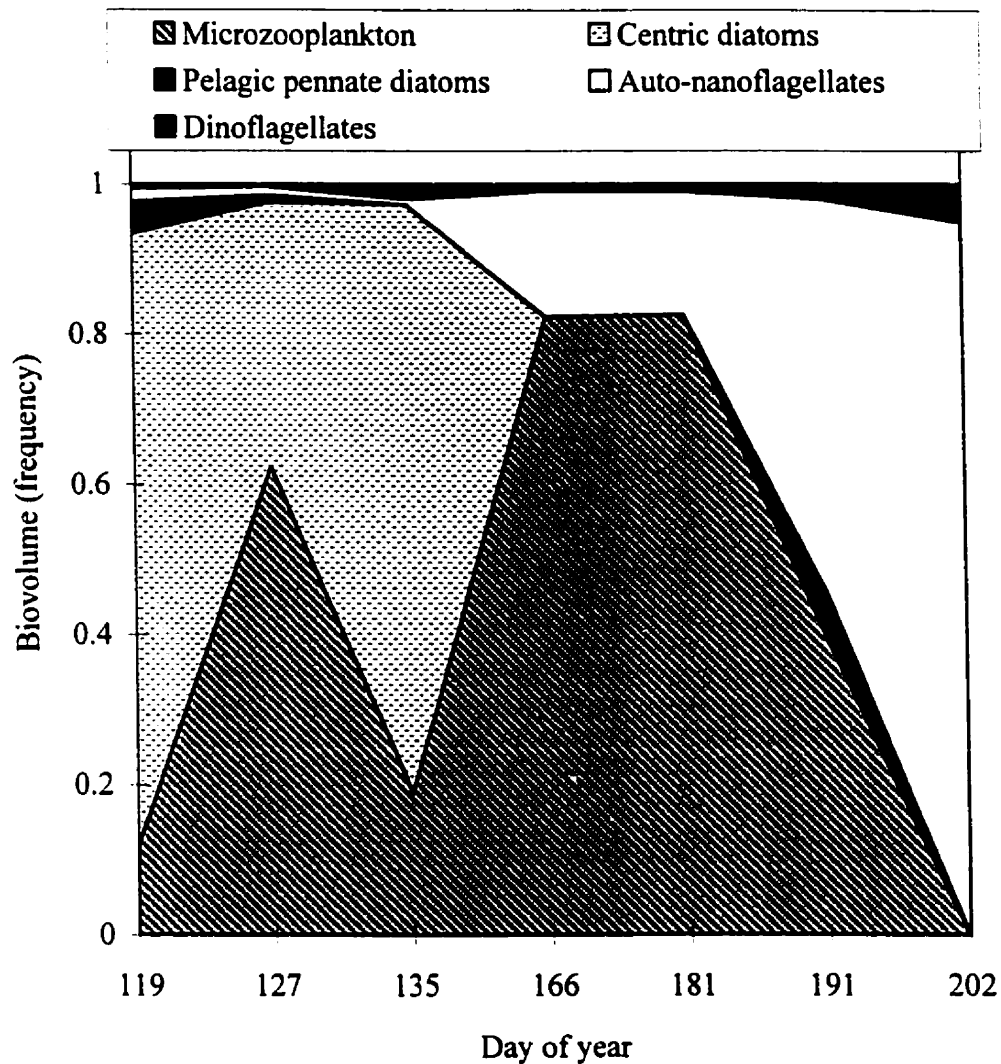


Figure 3.15: Biovolume contributions of different groups of plankton at 5 m depth at Shell Fresh Farms Ltd., Pool's Cove, NF, from April 29 to July 21, 1998. Microzooplankton: ciliates, tintinnids and choanoflagellates. Centric diatoms: long chained species. Pennate diatoms: single-celled species. Auto-nanoflagellates: all 2 to 20  $\mu\text{m}$  flagellates. Dinoflagellates: autotrophic and heterotrophic species.

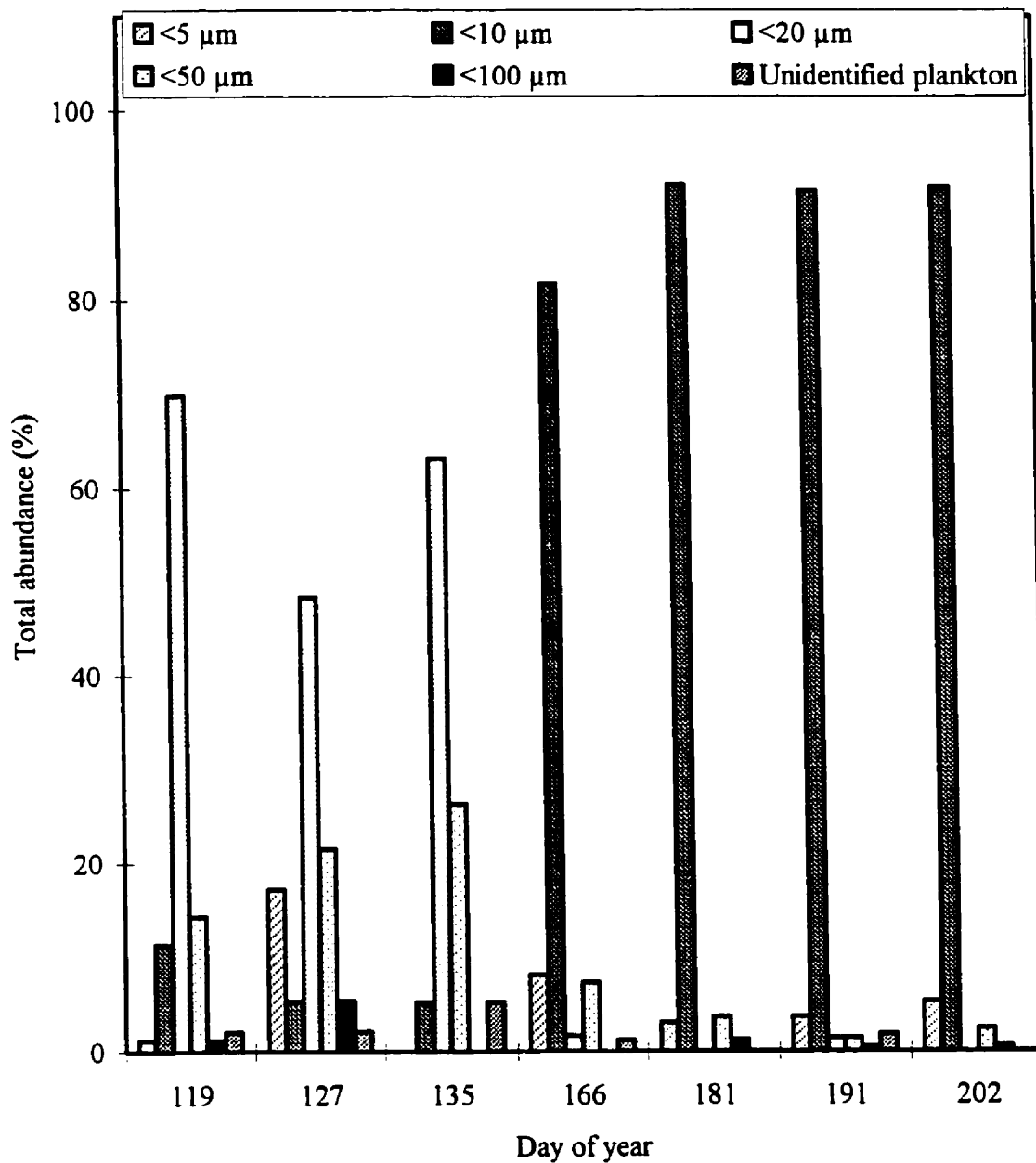


Figure 3.16: Particle size frequency distribution of plankton at Shell Fresh Farms Ltd., Pool's Cove, NF, from April 29 to July 21, 1998.

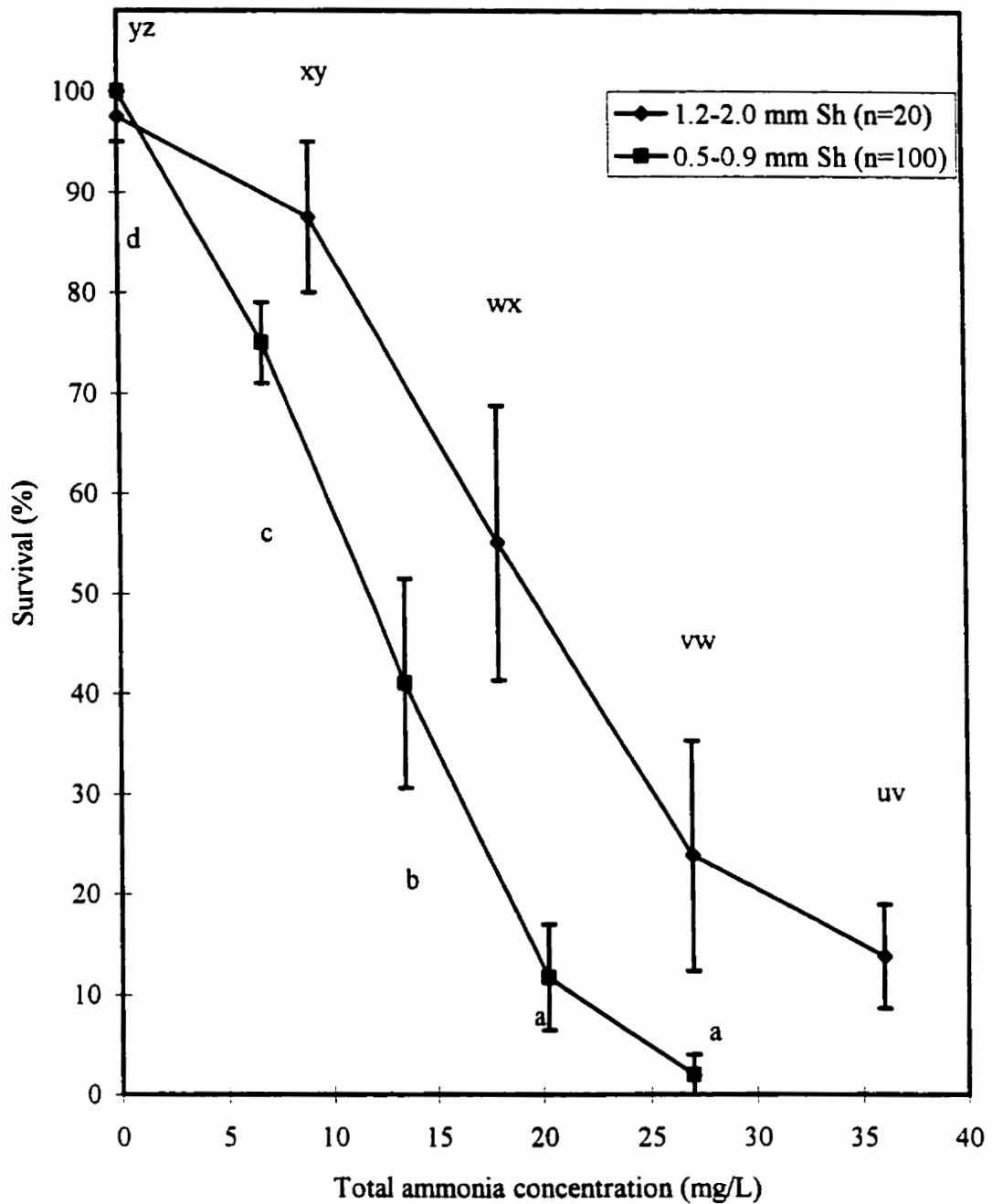


Figure 4.1: Mean percent survival ( $\pm$  S.E.) of two size classes of scallops exposed to five concentrations of total ammonia ( $n=3$ ) for a 96-hour period. Common letter denotes no significant difference in survival among the different ammonia concentrations (Tukey's B test).



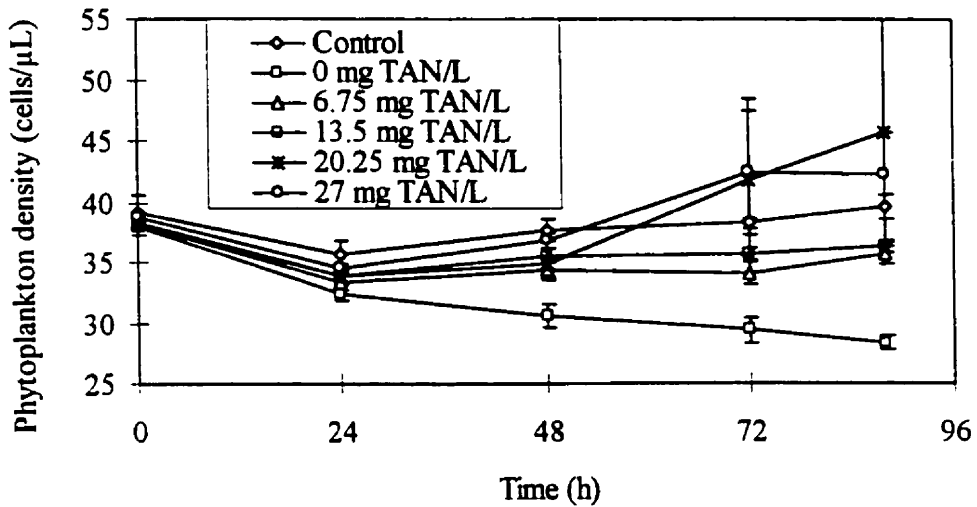


Figure 4.2: Mean densities of cultured phytoplankton ( $\pm$  S.E.) over a 96-hour period in five total ammonia nitrogen concentrations (mg TAN/L).

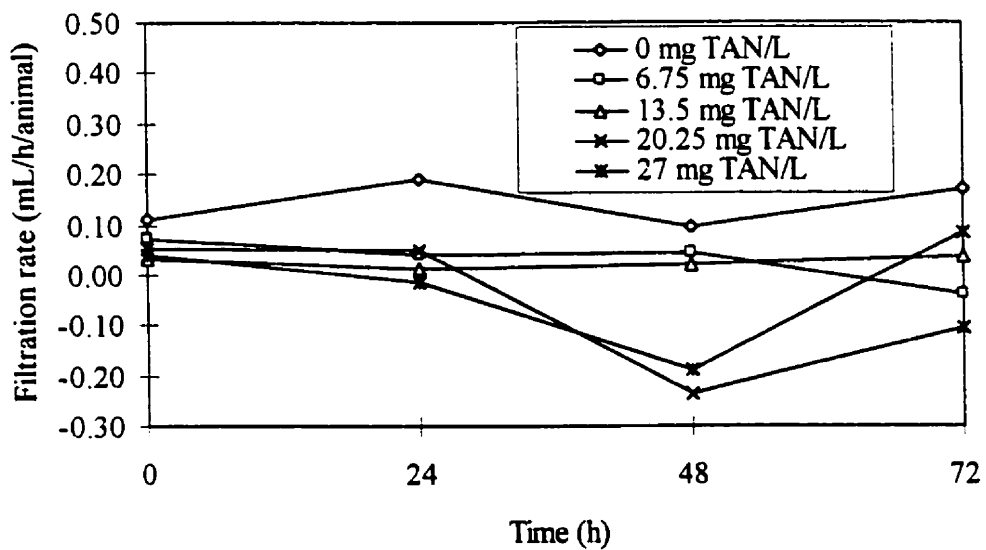


Figure 4.3: Mean filtration rate of scallop spat (640  $\mu$ m shell height) held in five ammonia nitrogen concentrations (mg TAN/L) for a 96-hour period. [Tukey's B test: Filtration for different concentrations (20.25=27=13.5=6.75) < (13.75=6.75=0); Filtration rates over time (48=72=24=0)]

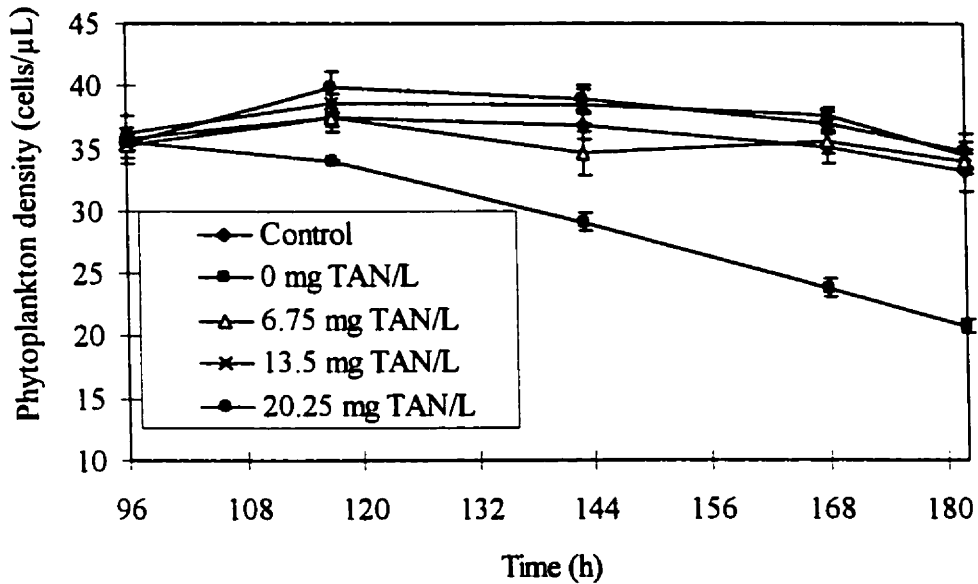


Figure 4.4: Mean concentration of phytoplankton ( $\pm$  S.E.) in recovery baths of scallops held in different total ammonia nitrogen concentrations (mg TAN/L).

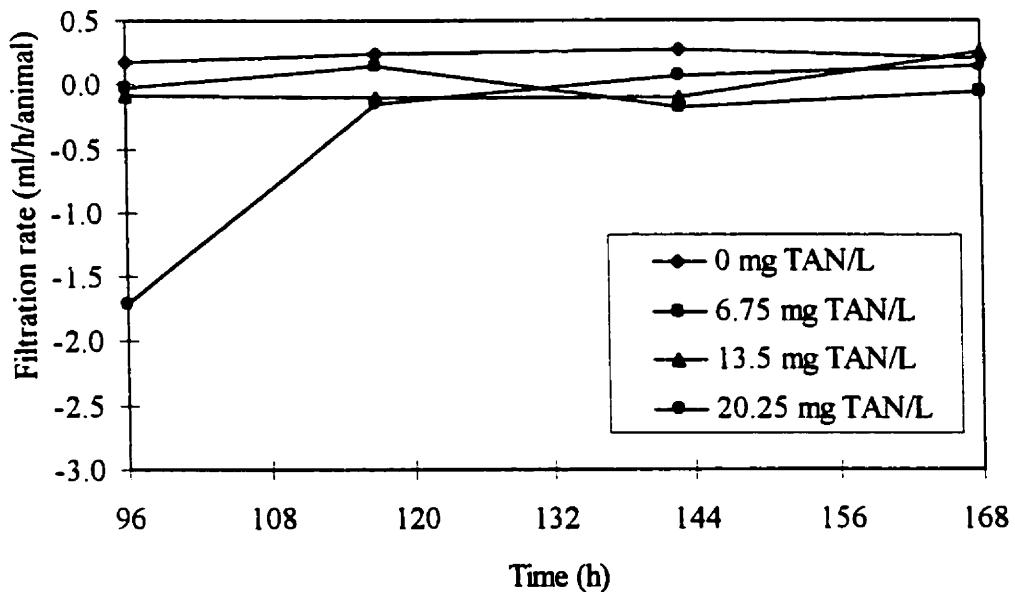


Figure 4.5: Mean filtration rates of scallops held in recovery bath after 96-hour exposure to four concentrations of total ammonia nitrogen (mg TAN/L). The highest concentration of 27 mg TAN/L killed all scallops hence a recovery bath was not necessary.

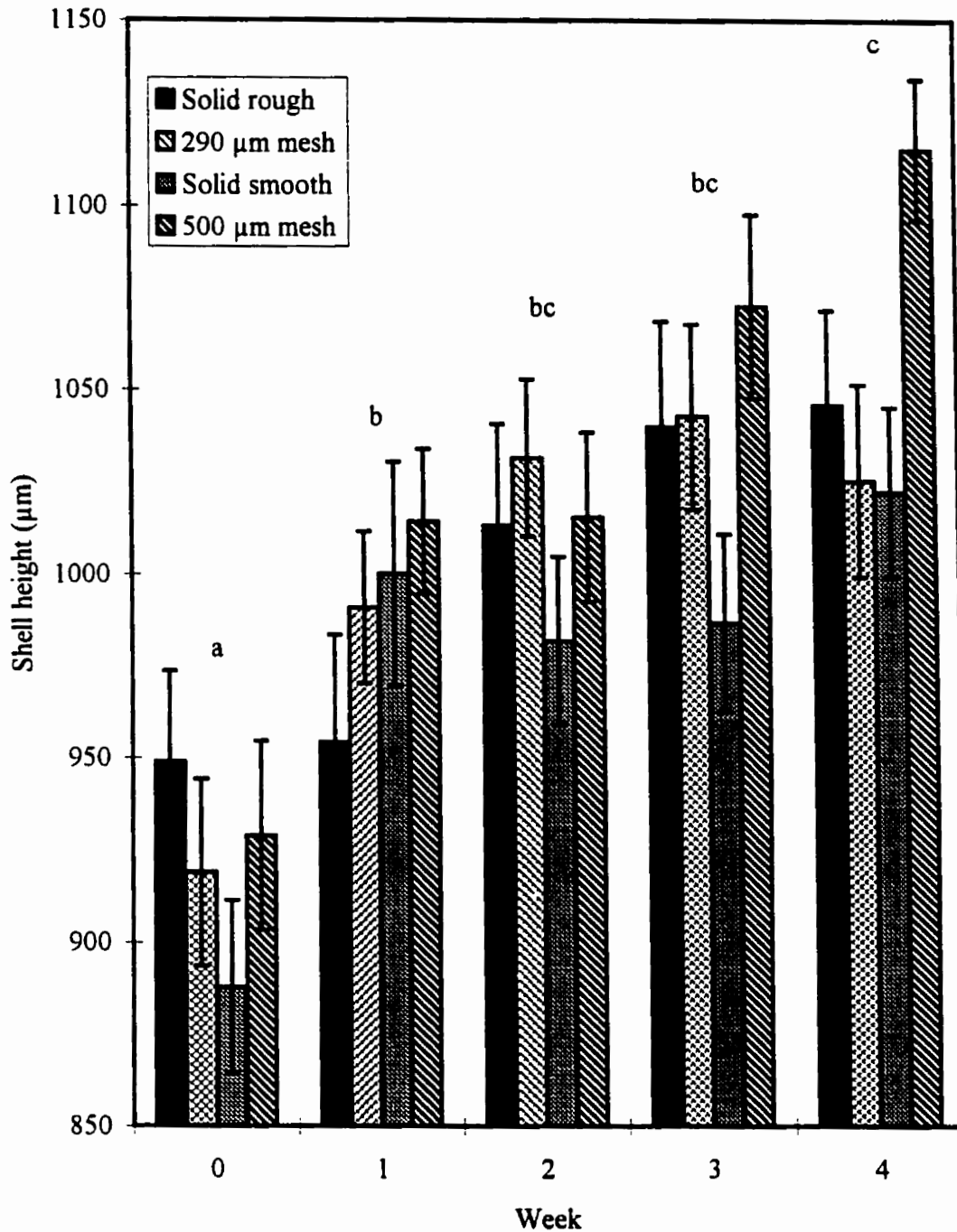


Figure 5.1: Mean shell height ( $\pm$  S.E.) of scallops grown in a flow-through tank on four tray types ( $n=90$ ). Common letter denotes no significant difference among shell heights for different dates (Tukey's B test).

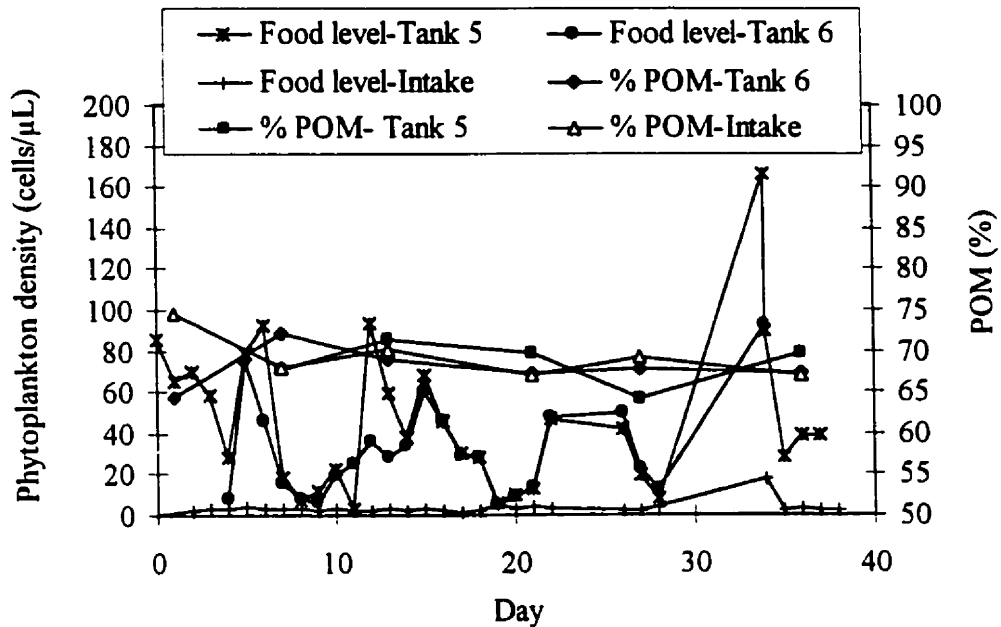


Figure 5.2: Total (cultured + natural) and natural food densities and percent particulate organic matter (POM) in flow-through tanks. (Tank No. 6 had Solid Smooth/ 290 μm trays and Tank No. 5 had Solid Rough/ 500 μm trays)

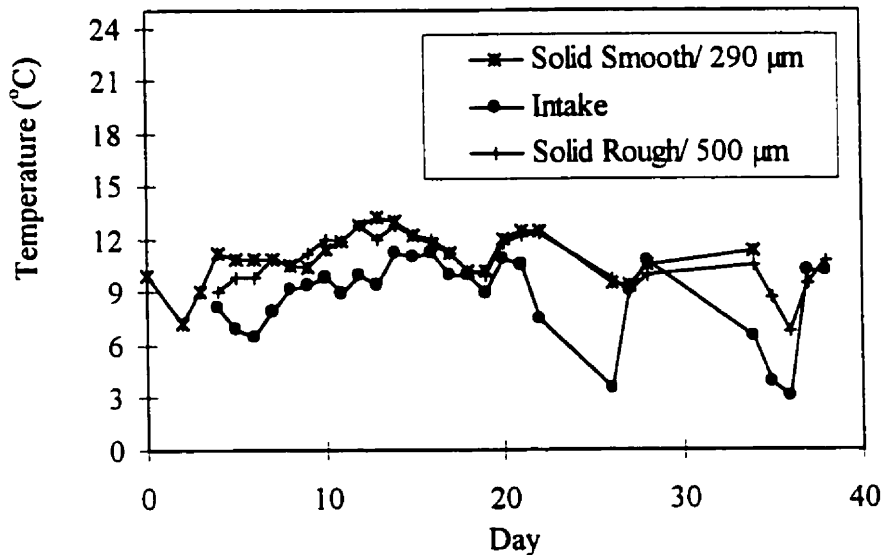


Figure 5.3: Daily water temperature in flow-through tanks and intake line. Both experiments ran for 28 days (Solid Rough/ 500 μm was started four days later than Solid Smooth/ 290 μm).

## **Appendices**

Appendix 1.1: Classification of the sea scallop (Brusca and Brusca 1990; Waller 1991).

Kingdom Animalia

Phylum Mollusca

Class Bivalvia

SubClass Pteriomorpha or Lamellibranchia

Superorder Filibranchia or Pteriomorpha

Superfamily Pectinacea

Family Pectinidae

Supragenera *Palliolum*

Genus and Species *Placopecten magellanicus* (Gmelin, 1791)

Appendix 2.1: Mesh sizes of equipment and the dimensions of the mesh used for size grading.

Mesh Type	Length (mm)	Width (mm)	Diagonal (mm)
3.0 mm pearl net	2.40	1.10	2.64
	2.30	1.30	2.64
	2.30	1.20	2.59
Average	2.33	1.20	2.62
1.5 mm pearl net	1.20	0.40	1.26
	1.20	0.40	1.26
	1.20	0.50	1.30
Average	1.20	0.43	1.28
1.2 mm collector bag	1.00	0.60	1.17
	1.10	0.70	1.30
	1.20	0.80	1.44
Average	1.10	0.70	1.30
2.0 mm collector bag	1.50	1.30	1.98
	1.70	1.30	2.14
	1.60	1.30	2.06
Average	1.60	1.30	2.06
3.0 mm Vexar	2.20	1.80	3.00
	2.10	1.90	3.20
	2.10	2.00	2.80
Average	2.13	1.90	3.00
2.0 mm Vexar	1.80	1.10	1.80
	1.70	1.10	2.00
	1.60	1.10	1.90
Average	1.70	1.10	1.90
1.4 mm Nitex	1.00	1.00	1.41
	1.00	0.90	1.35
	1.00	1.00	1.41
Average	1.00	0.97	1.39
1.7 mm Nitex	1.20	1.10	1.63
	1.20	1.10	1.63
	1.20	1.20	1.70
Average	1.20	1.13	1.65

Appendix 2.2: Size differential between scallop shell height after screening and maximum mesh dimension (diagonal).

Holding unit	Mesh Size (mm)	Pre-Screen (mm)	Size differential (mm)	Size Differential %
3.0 mm Pearl Net	2.8	3	0.2	7.14
1.5 mm Pearl Net	1.5	1.7	0.2	13.33
2.0 mm Collector Bag	2	2	0	0.00
1.2 mm Collector Bag	1.2	1.4	0.2	16.67
		Average*	0.2	12.38

\* not including 2.0 mm Collector bag

The size differential is the difference between the maximum dimension of the pre-screen mesh and the mesh the scallops will be held in on the farm-based nursery. Because there was no difference between the 2.0 mm mesh and its pre-screen mesh dimensions, there was no size differential. Based on the average percent size differential of 12.38% for the other three holding units, the 2.0 mm mesh should have had a maximum mesh dimension of 2.24 mm mesh to retain 100% of its scallops. The similarities in pre-screen and holding mesh sizes allows for marginally sized scallops to fall through equipment. In the case of the 2.0 mm collector bags, any scallop less than 2.2 mm may have fallen through, that is if the mean size differential is actually adequate enough and size grading methods are efficient.



Appendix 2.3: a) Required spat volume for desired stocking densities based on pre-determined volumes of known counts.

Shell Height (mm)	Density (spat/mL)	Density (spat/unit)	Volume required (mL)
>3.0	55.2	500*	9.1
2.0-2.9	176	5000*	28
1.7-1.9	360.4	1000@	2.8
1.4-1.6	626.6	5000 <sup>s</sup>	8

\* 3.0 mm pearl net, 2.0 mm collector bag, @1.5 mm pearl net, <sup>s</sup>1.2 mm collector bag

b) Sample counts for determined volume to estimate actual loading density.

Shell Height (mm)	Volume req'd (mL)	Sample 1			Sample 2			Average (spat/unit)
		Volume (mL)	Spat Count per Unit	Density (spat/mL)	Volume (mL)	Spat Count per Unit	Density (spat/ mL)	
>3.0	9.1	5.6	539	96.25	9.1	723	79.45	799
2.0-2.9	28	8	2105	263.125	-	-	-	7367.5
1.7-1.9	2.8	2.8	1454	519.286	2.8	1471	525.357	1462.5
1.4-1.6	8	8	5999	749.875	8	6081	760.125	6040

Appendix 2.4: Floor coverage of scallops in the four equipment types based on size and density.

Equipment type	Mean SH (mm)	Density (spat/unit)	Area (mm <sup>2</sup> )/unit	Scallop area (mm <sup>2</sup> )	% Coverage
1.5 mm pearl net	1.75	1463	122,500	2.41	2.88
3.0 mm pearl net	3.94	799	122,500	12.19	7.94
1.2 mm collector bag	1.43	6040	320,000	1.61	3.22
2.0 mm collector bag	2.50	7394	320,000	4.91	12.07
3.0 mm pearl net	4.35	500	122,500	14.86	6.07
3.0 mm collector bag	4.35	1200	320,000	14.86	5.57
2.0 mm collector bag	2.70	2630	320,000	5.73	4.71
	2.70	5260	320,000	5.73	9.42

Appendix 2.5: Length and weight of Netron used in collector bags.

Sample	Length (cm)	Mass (g)
1	88.50	34.96
2	91.60	34.48
3	89.30	34.56
Average	89.80	34.67

Appendix 2.6: Sample calculations for measurements made throughout the study.

a) Fouling and siltation calculations:

Weigh numbered aluminum dish and record.

To obtain dry weight, oven dry at 80°C, for 24 hours or until constant weight.

Weigh the dried dish plus fouling.

$$\text{Fouling weight (g)} = [\text{Dish weight + fouling (g)}] - \text{Dish weight (g)}$$

Example:

Sample: Bottom 1.5 mm Pearl net on string at 10 m on July 3

$$\text{Dish weight (g)} = 2.6130 \quad \text{Dried [Dish weight + fouling (g)]} = 5.2558 \text{ g}$$

$$\text{Fouling weight (g)} = 5.2558 \text{ g} - 2.6130 \text{ g} = 2.6428 \text{ g or } 2642.8 \text{ mg}$$

Pre-weigh an ash-free glass fibre filter. Filter a sub-sample known volume from the water used to wash the silt from the equipment. Weigh an aluminum dish. Place filter in dish and in oven at 80°C for 24 hours or until constant weight.

$$\text{Siltation} = [\text{Dish wt + filter wt + silt wt (g)}] - [\text{dish wt + filter wt(g)}] \times \frac{\text{Total volume (L)}}{\text{subsample volume (L)}}$$

Sample: Bottom 1.5 mm Pearl net on string at 10 m on July 3

$$\text{Volume of water} = 0.05 \text{ L of } 15.5 \text{ L} \quad \text{Filter weight (g)} = 0.0889 \text{ g}$$

$$\text{Dish weight (g)} = 1.0145 \text{ g} \quad \text{Dish weight + filter + silt (g)} = 1.1135 \text{ g}$$

$$\text{Total Silt (g)} = (1.1135 - 1.0145 - 0.0889) \times 15.5/0.05 = 3.131 \text{ g or } 3131 \text{ mg}$$

Standardization of siltation and fouling (for comparing collector bags and pearl nets):

$$\text{Silt or fouling mg/cm}^2 = \frac{\text{Total dry weight (siltation or fouling in mg)}}{\text{total surface area (top and bottom; in cm}^2\text{)}}$$

Based on previous example:

$$\text{Fouling mg/cm}^2 = 2642.8 \text{ mg}/2450 \text{ cm}^2 = 1.08 \text{ mg/cm}^2.$$

$$\text{Siltation mg/cm}^2 = 3131 \text{ mg}/2450 \text{ cm}^2 = 1.27 \text{ mg/cm}^2.$$

#### b) Growth rates of scallops

Growth rates are dependent on an initial and final  $S_h$  over a known time period.

$$\text{Growth rate } (\mu\text{m/d}) = \frac{\text{Mean Final } S_h (\mu\text{m}) - \text{Mean Initial } S_h (\mu\text{m})}{\text{Number of days}}$$

Sample growth rate calculation:

For scallops grown in 1.5 mm pearl net at 10 m.

$$\text{Growth rate } (\mu\text{m/d}) = \frac{7210 \mu\text{m} - 1753 \mu\text{m}}{267 \text{ d}} = 20.4 \mu\text{m/d}$$

#### c) Recovery calculations

Recovery rates are dependent on an initial and final numbers of live scallops in equipment. An actual total count was taken for the scallops in pearl nets, however, because the density in the collector bags was higher total spat volume was measured.

This was sub-sampled for number alive. This value was then used to back-calculate the mean final total spat live.

$$\text{Percent recovery (\%)} = 100 \times \frac{\text{Mean Final Total Live Scallops}}{\text{Mean Initial Count Scallops}}$$

Sample percent recovery (%) calculation:

For scallops grown in 1.5 mm pearl net\* at 10 m.

$$\text{Percent recovery (\%)} = 100 \times \frac{787 \text{ scallops}}{1463 \text{ scallops}} = 53.8\%$$

\*total number of scallops were counted in the pearl nets

Appendix 2.7: Estimated counts and loss in nursery units according to estimated % undersized spat loaded into each unit.

Shell Height (mm)	Stock Density (spat/ unit)	Estimated % undersize	# undersized per unit	Expected # in each unit*	Estimated loss**
>3.0	799	6.67	53	746	1272
2.0-2.9	7368	6.67 <sup>#</sup>	491	6877	15726
1.7-1.9	1463	30	439	1024	10534
1.4-1.6	6040	20	1208	4832	38,656
				Total	66,188

# this does not account for the lack of a size differential (Appendix 1.2)

\* after loss through mesh has occurred

\*\*for total number of replicates in experiment (n=24 for 1.2 and 3.0 mm pearl nets; n=32 and 30 for 1.2 and 2.0 mm collector bag, respectively)

Appendix 3.1: Fixation of phytoplankton samples with Lugol's iodine.

Lugol's Iodine:        10 g KI (Potassium Iodide)  
                              20 mL water  
                              5 g I<sub>2</sub> (Iodine)  
                              50 mL water  
                              5 g NaC<sub>2</sub>H<sub>3</sub>O<sub>3</sub>·3H<sub>2</sub>O (Sodium acetate trihydrate)

Mix the ingredients in the order given and stir it well to dissolve the iodine chips.

Store the solution in a Nalgene bottle.

Filter 1 L of sea water with 290  $\mu$ m mesh. For fixing the sea water samples, add 10 mL Lugol's Iodine (about 1% of the 1 L seawater) and 10 mL 37% formaldehyde (about 1% of 1 L sea water).



Appendix 3.2: Calculation for phytoplankton densities.

Before phytoplankton density calculations can be made measurements of total area of 10 mL settling chamber and grid on the Zeiss Axiovert 35 (West Germany) microscope eyepiece lens were made. (diameter of the settling chamber = 25390  $\mu\text{m}$ )

$$\begin{aligned} \text{Area of settling chamber} &= \pi(0.5 \times d)^2 \text{ where } d = \text{diameter.} \\ &= \pi(0.5 \times 25390 \mu\text{m})^2 \\ &= 506308575 \mu\text{m}^2 \end{aligned}$$

Dimensions of the grid were determined using eyepiece unit equivalents to micrometers. At 40X, 20 epu = 25  $\mu\text{m}$ , and grid length and width is 200 epu or 250  $\mu\text{m}$ .

$$\begin{aligned} \text{Grid Area} &= \text{length} \times \text{width} \\ &= 250 \mu\text{m} \times 250 \mu\text{m} \\ &= 62500 \mu\text{m}^2 \end{aligned}$$

Phytoplankton density is calculated as follows:

$$\frac{\text{Sample Count (cells)} \times \frac{\text{Total Settling Area } (\mu\text{m}^2) \times \text{Concentrated + Wash Volume (mL)}}{\text{Total Volume (mL)}}}{\frac{\# \text{ of grids} \times \text{area of grid } (\mu\text{m}^2)}{\text{Total Volume (mL)}} \times \text{Count Volume (mL)}}$$

where total settling area = 506308575  $\mu\text{m}^2$

concentrated + wash volume = volume left after decanting and any rinse water

# of grids = grids which phytoplankton were counted in

count volume = volume of concentrated sample in which algae were counted

total volume = decanted volume + concentrated volume

Sample phytoplankton density calculation for Ladder Garden total count on September 7,

1997:

$$\text{Total Phytoplankton Density} = \frac{303 \text{ cells} \times \frac{506308575 \mu\text{m}^2}{27 \times 62500 \mu\text{m}^2} \times \frac{112 \text{ mL}}{10 \text{ mL}}}{1.006 \text{ L}}$$

$$= 1012125 \text{ cells/L}$$

Sample count may be either a total, species, genus or other group count.

Appendix 3.3: Calculation of total particulate and organic and inorganic matter in a sea water sample.

Pre-weigh an ash-free glass fibre filter. Filter a known volume of sea water.

Weigh an aluminum dish. Place filter in dish and in oven at 80°C for 24 hours or until constant weight.

$$\text{TPM} = \frac{[\text{Dish wt} + \text{filter wt} + \text{TPM (g)}] - [\text{dish wt} + \text{filter wt(g)}]}{\text{Sample volume (L)}}$$

Transfer the filter to a muffle oven for 24 hours at 500°C to remove POM. Weigh again.

$$\text{PIM} = \frac{[\text{Dish wt} + \text{filter wt} + (\text{TPM-POM}) \text{ wt (g)}] - [\text{dish wt} + \text{filter wt(g)}]}{\text{Sample volume (L)}}$$

$$\text{POM} = \text{TPM} - \text{PIM}$$

Sample: August 4, 1997, Ladder Garden

Volume of water	= 4 L	Filter weight (g)	= 0.0891 g
Dish weight (g)	= 1.0009 g	Dish weight + filter +TPM (g)	= 1.1128 g
TPM (g/L)	= [1.1128 - (1.0009- 0.0891)]/4 L = 0.0057 g/L or 57 mg/L		
PIM (g/L)	= [1.1043 - (1.0009- 0.0891)]/4 L = 0.0036 g/L or 36 mg/L		
POM (g/L)	= 0.0057 g/L - 0.0036 g/L =0.0021g/L or 21 mg/L		

Appendix 3.4: Calculation of chlorophyll-*a* and phaeopigments in seawater samples.

Calibration equations                      Chlorophyll-*a* ( $\mu\text{g/L}$ ) = 0.0425 ((1.7701)( $F_b - F_a$ ))

Phaeopigment ( $\mu\text{g/L}$ ) = 0.0425 ((1.7701) ((2.303 x  $F_a$ )- $F_b$ )))

where  $F_a$  and  $F_b$  are readings from the fluorometer before and after HCl have been added to the prepared sample. These equations must be multiplied by the appropriate dilution factors to determine the sea water concentrations of chlorophyll-*a* and phaeopigments.

Sample calculations: September 7, 1997- original sample size was 4-L. The filter was dissolved in 7.2 mL of 90% acetone which was further diluted by 10 to get the fluorometric readings.

$$\begin{aligned} \text{Chlorophyll-}a \text{ } (\mu\text{g/L}) &= 0.0425 ((1.7701)(4.00-2.26)) \times 10 \div 7.2 \times 4 \\ &= 0.727 \mu\text{g/L} \end{aligned}$$

$$\begin{aligned} \text{Phaeopigment } (\mu\text{g/L}) &= 0.0425 ((1.7701) ((2.303 \times 2.26)-4.00))) \times 10 \div 7.2 \times 4 \\ &= 0.504 \mu\text{g/L} \end{aligned}$$

Appendix 4.1: Preparation of ammonia concentrations to be tested for ammonia toxicity.

Ammonium chloride (NH<sub>4</sub>Cl) was the standard reagent for attaining NH<sub>3</sub>, the following assumptions are made based on the molecular weight of NH<sub>4</sub>Cl:

NH <sub>4</sub> Cl (g)	NH <sub>4</sub> Cl (mg)	NH <sub>3</sub> (g)	NH <sub>3</sub> (mg)	moles (M)	micromoles (μM)
53.5	53500	18	18000	1	1000000
5.35	5350	1.8	1800	0.1	100000
0.535	535	0.18	180	0.01	10000
<b>0.0535</b>	53.5	0.018	<b>18*</b>	0.001	<b>1000</b>
*The values to be tested fall within this range					
<b>Test levels for larger scallops (1.2-2.0 mm)</b>					
0.107	107	0.036	36	0.0020	2000
0.08025	80.25	0.027	27	0.0015	1500
0.0535	53.5	0.018	18	0.0010	1000
0.02675	26.75	0.009	9	0.0005	500
<b>Test levels for smaller scallops (0.5-1.0 mm)</b>					
0.08025	80.25	0.027	27	0.0015	1500
0.060187	60.187	0.02025	20.25	0.001125	1125
0.040125	40.125	0.0135	13.5	0.000750	750
0.020063	20.063	0.00675	6.75	0.000375	375

For large scallops, 3L of 0.18 g NH<sub>3</sub>/L were diluted in FSW to make these test solutions:

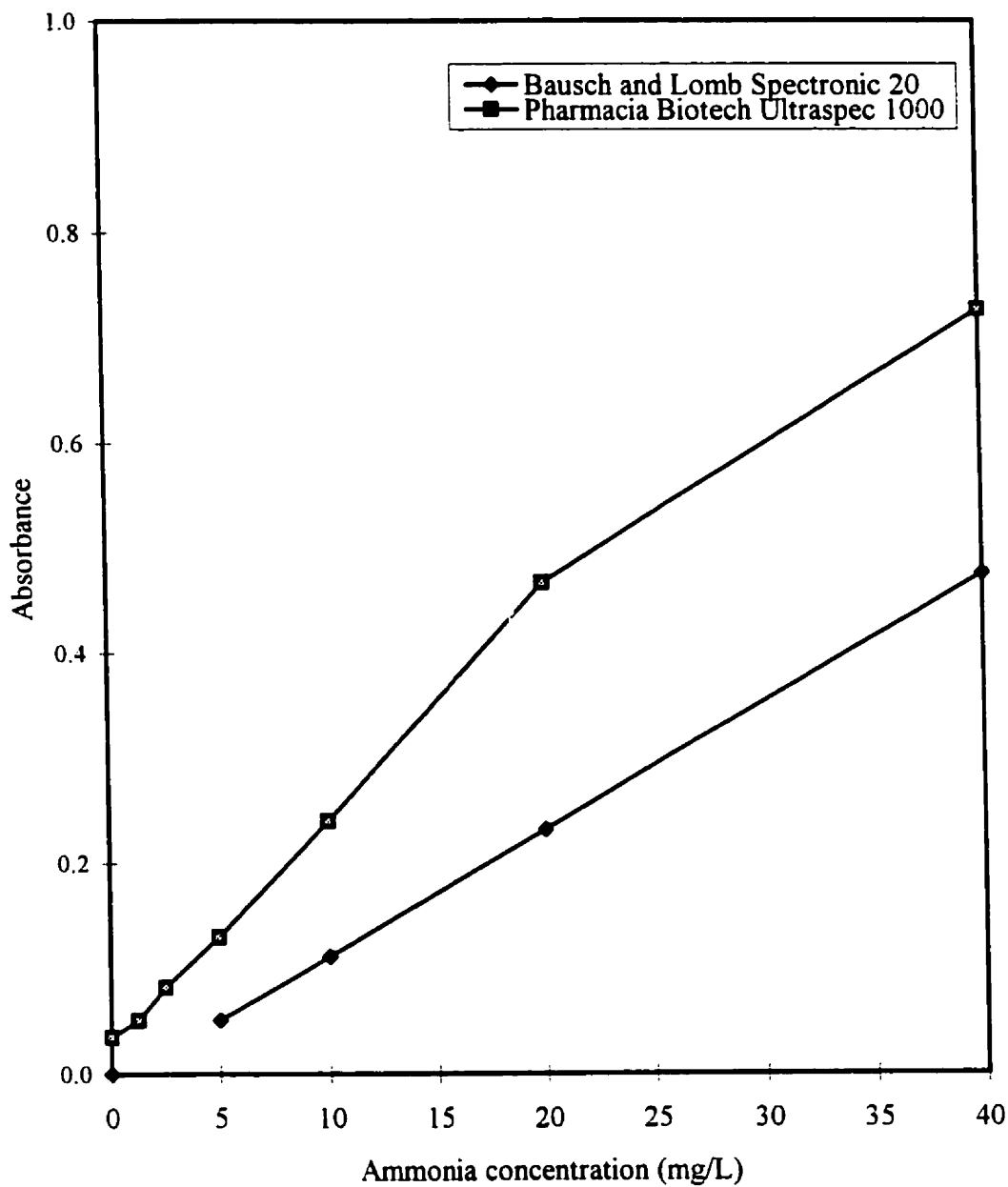
$$0.8 \text{ L } (0.18 \text{ g NH}_3/\text{L})/4\text{L} = 0.036 \text{ g NH}_3/\text{L} = 36 \text{ mg NH}_3/\text{L}$$

$$0.6 \text{ L } (0.18\text{g NH}_3/\text{L})/4\text{L} = 0.027 \text{ g NH}_3/\text{L} = 27 \text{ mg NH}_3/\text{L}$$

$$0.7 \text{ L } (0.18 \text{ g NH}_3/\text{L})/7\text{L} = 0.018 \text{ g NH}_3/\text{L} = 18 \text{ mg NH}_3/\text{L}$$

$$2.0 \text{ L } (0.018 \text{ g NH}_3/\text{L})/4\text{L} = 0.009 \text{ g NH}_3/\text{L} = 9 \text{ mg NH}_3/\text{L}$$

For smaller scallops, the mass of NH<sub>4</sub>Cl needed to make 4L of each solution was measured using a precision balance and added to FSW (see table for quantities for 1L).



Appendix 4.2 : Standard curve for ammonia absorbance on a Bausch and Lomb Spectronic 20 (1997) and Pharmacia Biotech Ultraspec 1000 (1998). Regression line equations for the new and old spectrophotometer are  $Y=1.39 \times 10^{-2}X+ 9.571 \times 10^{-4}$  (n=6) and  $Y=1.778 \times 10^{-2}X + 4.697 \times 10^{-4}$  (n=7).

Appendix 4.3: Calculation of filtration rates of scallops based on food densities (Coughlan 1969).

Feeding chambers were 5-L buckets of 4-L of food at an food density of approximately 40 cells/L. One hundred scallops were present in each container. Food density was measured daily. A control bucket was used to compare the gravitational settling of food particles. Filtration rates (mL/h/animal) were based on the following equation:

$$F = \frac{(\text{Volume of water (mL)} \times \ln C_o/C_t) - (\text{Volume of water (mL)} \times \ln C_o'/C_t')}{\text{Time (hours)} \quad \text{Time (hours)}} \div \text{Number of scallops per tank}$$

where  $C_o$  = initial particle concentration,  $C_o'$  = initial concentration in control chamber  
 $C_t$  = final particle concentration and  $C_t'$  = final concentration in control chamber

Sample Calculation: Scallops in 0 mg TAN/L from 24-48 hours.

$$F = \frac{(\text{4000 mL}) \times \ln 3307/2724) - (\text{4000mL}) \times \ln 3661/3855)}{\text{24 hours} \quad \text{24 hours}} \div 100 \text{ scallops}$$

=0.409 mL/hour/scallop