TRACKING AND GROWTH OF LARVAE OF THE GIANT SCALLOP, Placopecten magellanicus (GMELIN, 1791) ON A SCALLOP FARM IN NOTRE DAME BAY, NEWFOUNDLAND

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Tracking and Growth of Larvae of the Giant Scallop. *Placopecten magellanicus* (Gmelin.1791) on a Scallop Farm in Notre Dame Bay. Newfoundland

by

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ABSTRACT

Culture of the giant scallop began in Newfoundland in 1986 after research indicated a supply of wild spat was possible. The failure of wild seed production in sufficient quantities had negative consequences, and since 1991 wild scallop spatfall has been very low. It was proposed that the release of millions of D-veliger larvae, raised by hatchery techniques, on a site may increase annual spatfall and collection. Enhancement was attempted in 1993 on a scallop farm in Newfoundland but no increase in spat collection was realized. This study was initiated to investigate the possible fate of these released larvae.

Three-day-old scallop veligers were stained with calcein (a non-toxic fluorescent dye) by immersion in a solution of 150 mg/L calcein for 16 h at densities of 40 larvae/mL. High mortalities were observed at densities of 250 larvae/mL. Satisfactory fluorescence was observed in preserved samples which subsequently retained the stain for at least two years.

Two batches of calcein stained scallop larvae were released on a shellfish farm located in Charles Arm, Notre Dame Bay, Newfoundland during the summers of 1994 and 1995. Upon recapture of these larvae by plankton tows, growth rates of 3.57 and 3.85 µm/d in 1994, and 9.72 and 2.52 µm/d, in 1995, were observed. Differences in the growth rates varied seasonally and annually (1994 & 1995), and were related to water temperature and food concentration (chlorophyll-*a*). Size frequency distribution, current meter data, and current directional maps created by drift bottle drogue surveys showed evidence of possible entrainment of larvae within Charles Arm. As well, evidence was also reported suggesting that bivalve larvae were also transported out of the system.

Larval abundance and shell height distribution varied with tidal state. Higher numbers and larger size bivalve veligers were observed during the mid to late flood and ebb tides. Mean size of bivalve larvae at four stations sampled on the site differed

significantly over the tidal cycle. Variation in larval abundance was also greater during neap tides as compared to spring tides. Diel differences in larval abundance were also observed. This has important implications for any sampling regime directed to measuring size and numbers of bivalve larvae over time, for example, larval monitoring programs used in the aquaculture industry.

An enhancement project designed to increase the subsequent spatfall of giant scallop veligers is possible but should be very intensive. Hundreds of millions of greater than 200µm scallop veligers may have to be released within the site to result in an observed increase in spatfall and to counteract losses due to natural mortality and net outward transport from the system.

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INTRODUCTION

Biology

Sea or giant scallops, *Placopecten magellanicus* (Gmelin, 1791), occur on the Atlantic coast of North America, ranging from Cape Hatteras, North Carolina, to Labrador (Posgay, 1957). Most wild beds occur at depths of 10 m to 100 m in the central and northern parts of the species distribution. In the southern part of their range, the majority of giant scallops are found below 50 m, presumably because of their inability to tolerate water temperatures above 20°C. The giant scallop is a benthic, subtidal, active suspension-feeder ingesting phytoplankton, small zooplankton, spores and detrital particles (Shumway *et al.*, 1987). It can live up to and exceeding 20 yr with growth rates depending on local environmental conditions.

The sexes are generally separate, and can be identified by gonad color, with males being cream-colored and females being bright orange. These are easily distinguishable when gonads are ripe. Hermaphrodites are found occasionally. Naidu (1970) reported a 1.3% occurrence of hermaphroditism in scallops of the Port au Port area in Newfoundland.

Scallops are highly fecund with females producing over one hundred million eggs and males several billion sperm (C. Couturier, pers. comm.). Spawning is normally synchronous within a locality and gametes released into the surrounding water (Parsons, 1994). The likelihood of fertilization success is very low in this species as it is for other broadcast spawners (e.g., mussels). Most populations display a single annual spawning

period extending over one or two months between July and October, although there are reports of semiannual spawning (DuPaul *et al.*, 1989; Dadswell and Parsons, 1991; Andrews, 1992).

Fertilized eggs (65-70 µm) develop in about 3 d at 15°C into planktonic D-shaped (straight-hinged) veliger larvae which begin to lay down a calcareous (calcium carbonate) shell (Couturier *et al.*, 1995). The veligers spend four to six weeks in the water column (Culliney, 1974) where they feed mostly on phytoplankton. When larvae reach about 220 µm they develop paired eyespots and a foot (pediveliger stage) and eventually settle on the bottom (when appropriate cues are present), where they attach to a variety of substrates and undergo metamorphosis. Once settled they remain attached by byssal threads, although they are capable of detachment and rapid swimming until they are approximately 15 mm in length (Manuel and Dadswell, 1991).

Scallop aquaculture industry: Newfoundland

Large-scale scallop (*Patinopecten yessoenssis*) aquaculture has been practiced in Japan for forty years (Ventilla, 1982). Annual production exceeding 100 000 metric tonnes (mt) of whole animal and exports to the US market exceeding 10 000 mt per year have made the Japanese a major competitor with giant scallops. Scallops make up 20% of world mollusc production, yielding approximately 500 000 mt per year. Total Canadian aquaculture production (shellfish and finfish) is less than 0.3% of the world

production, which reflects the slow development of techniques for rearing the giant scallop (Dadswell and Parsons, 1991).

Reports from the Newfoundland Aquaculture Industry Association indicate that in 1995, giant scallop culture was 1% of Newfoundland's total aquacultural production with 12 tonnes produced at a value of $66 945 which was 14% of the shellfish value. This was an increase of 400% from 1993, when only 3 tonnes were produced. Giant scallops now make up 3% of shellfish production with blue mussels accounting for the other 97%.

The first field-culture trials with sea scallops were initiated in the late 1960's by researchers at Memorial University's Marine Sciences Research Laboratory (MSRL) (Couturier *et al.*, 1995). At that time attempts were made to collect juvenile scallops (spat) on artificial substrates at various sites along the coast of Newfoundland and Labrador (Couturier, 1990). An experimental scallop spat collection program began in 1971 as a response to dwindling natural stocks. This was the first of its kind in North America (Naidu *et al.*, 1989). By the late 1970's the biological feasibility of sea scallop culture had been demonstrated but seed supply remained unreliable (Couturier *et al.*, 1995). Early commercial work was done in Newfoundland in 1986 (Naidu and Cahill, 1986) when commercial farms were established at Piccadilly, Port au Port Bay and Charles Arm, Notre Dame Bay. Another farm was established at Pool's Cove, Fortune Bay in 1987.

Placopecten magellanicus has a number of features that make it an excellent candidate for culture in Atlantic Canada (Caddy, 1989; Dadswell, 1989), for example the

large natural populations in many regions which could provide the necessary spat. One of the cornerstones of the security of existing enterprises and future expansion and diversification in the shellfish sector is the reliability of seed supply (Helm, 1995), and at present in Newfoundland the bivalve culture industry relies on wild-caught seed-stock. Even though the giant scallop has been grown commercially in suspended culture in this province for the past decade (Dabinett and Couturier, 1994), there are still only a few active growers, and this fact may be attributable to unreliable seed supply. Both researchers and farmers have identified that either the lack of spat or lack of knowledge of spat settlement is a limiting factor for aquaculture operations. At all events, the greatest biological constraint is now seed supply (Dabinett and Couturier, 1994).

Although *Placopecten magellanicus* has been grown commercially in Newfoundland for the past decade, it was not until 1989 that the provincial Department of Fisheries and the Port au Port Economic Development Association began a program to monitor biological, chemical and physical parameters at the Piccadilly spat collection site (Andrews, 1992). It was observed that spawning, indicated by a drop in the gonadosomatic index, usually occurred in late August with highest larval concentrations (85 µm shell height) recorded in the first week of September. By early October larvae were greater than 200 µm in shell height. These dates vary within about two weeks inter-annually. During this study, Port au Port in 1991 had a record year for spatfall, when the mean yield was 6 379 spat per collector (> 20 million seed from 6 000 collectors). Yields then continued to drop each year with densities 5-10 times lower in 1992 (mean yield of 1

573 per collector) and even lower yields in 1993 and 1994, with 1994 having a record low with fewer than 300 000 spat recovered from 6 000 collectors (Dabinett and Couturier, 1994). The causes for the decline are unclear, but a cooling trend in the environment and/or overfishing of the broodstock populations have been proposed (Dabinett and Couturier, 1994). In spite of the monitoring program, spat collection at the other farms has not been successful nor dependable, indicating the need for some other method to increase and/or ensure spat collection.

Larval dispersal

Prior to the early 1970's, research on the giant scallop was confined to the adult stage, but since that time there have been descriptions of larval development (Culliney, 1974), larval distributions (Tremblay and Sinclair, 1988; Tremblay *et al.*, 1993) and settling behaviour (Parsons *et al.*, 1993). This research on larval dispersion is of the utmost importance when considering scallop recruitment.

Literature relevant to the distribution and dispersal of bivalve veligers was divided into three groups by Mann (1986). The first of these groups treats larvae as purely passive particles for which dispersal is determined by the physical elements. The second, and by far the largest group, consists of field observations of horizontal and/or vertical distribution of the veliger larvae. Behavioural data characterize the third group. Data are obtained from laboratory experiments measuring rates of swimming velocity and

threshold limits to environmental cues, through the manipulation of a stimulus such as temperature, light, salinity or pressure while all other variables are maintained at a constant level.

Problems concerning the dispersion of the larvae of benthic marine invertebrates have been addressed from a number of different perspectives and at a variety of spatial and temporal scales (Scheltema, 1986). Some of these include wind-induced distribution (Hudon and Fradette, 1993), the relationship between feeding and vertical distribution (Raby *et al.*, 1994), vertical distribution in relation to water column stratification (Tremblay and Sinclair, 1990a), seasonal and depth characteristics (Robins-Troeger and Dredge, 1993), influence of tides (Levin, 1986), drifting (Tremblay *et al.*, 1993) and broad scale distribution in relation to physical oceanography (Tremblay and Sinclair, 1992). These studies reported larval dispersal ranging from a few centimeters to thousands of kilometers across wide expanses of ocean. Temporal intervals over which dispersal has been reported range from a few minutes, to over a tidal cycle, to months. Distribution data are usually collected and reported in conjunction with various biological (e.g., chlorophyll and phytoplankton cell concentrations) and physical (e.g., temperature, salinity, density) functions. Inference is then made of the cause or causes of the observed distributions from a synthesis of all data.

The maximum potential distance that a larva can be dispersed and the likelihood that it survives to settlement are related to (a) the length of its planktonic life, (b) the rate and direction of the currents that transport it (Scheltema, 1986) and c) its behaviour in

relation to environmental conditions (Gallager *et al.*,1996; Manuel *et al.*, 1996). Two principal characteristics of the neritic environment that have can significantly modify the duration of planktonic development are temperature and the 'quality' and quantity of food (i.e., food abundance) (Couturier *et al.*, 1995). These characteristics have mainly been studied in the laboratory while very few studies correlating these characters with larval development under field conditions have been attempted.

Adequate knowledge of larval behaviour during planktonic life and investigation of larval dispersal require sufficiently detailed information on circulation patterns. Currents and eddy diffusion affect the distribution of larvae. Currents (i.e., advection) transport larvae horizontally in the direction of flow while eddy diffusion is the random dispersion of suspended particles that results from turbulent flow. The horizontal component of eddy diffusion can be measured along a two-dimensional sea surface by the change in mean distance between freely drifting objects. Consequently, eddy diffusion accounts for horizontal dispersion (spatial displacement) of planktonic larvae with respect to one another. The dispersal of larvae, as well as the variance in duration of planktonic development, determines how many larvae of the same cohort will remain together in the plankton and subsequently settle together in one place (Strathmann, 1974).

Mark and recapture techniques

Nielsen (1992) reviewed methods for marking fish and shellfish. Marking of animals provides three broad categories of information: 1) to label individual animals for specific study; 2) to identify animals as they move and intermingle with others; and 3) to provide a method for population study. The data collected from these studies can then be used for stock contribution analysis, population abundance estimates, age and growth studies and distribution and movement studies. Distribution and movement studies have become increasingly important in the shellfish aquaculture sector.

Larval tracking

There are several difficulties in studying larval dispersal in the marine environment. These include: extreme dilution of larvae, particularly if breeding periods are extended; unpredictable timing of egg or larval release; and small size and inconspicuous coloring of larvae which limit the potential for directly tracking their movements (Willis and Oliver, 1990). As a result there are very few instances in which invertebrate larvae have been tracked directly in the field. Most of the studies, as mentioned earlier, combine information about larval distributions in the field with knowledge of larval sources, behaviour, recruitment patterns, and relevant hydrodynamic properties. Often, hydrodynamic models are constructed or simulations of larval transport

are performed to explore probable larval trajectories (Levin, 1990). Direct tracking has also been attempted using aerial surveys and satellite imagery (Willis and Oliver, 1990), and have provided an important perspective for identifying the physical processes and meteorological and hydrodynamical conditions that affect larval dispersal.

A different approach to the study of larval movements in the water column is to release and recover labelled larvae. The earliest reported attempts to mark invertebrate larvae were made over four decades ago when Loosanoff and Davis (1947) successfully stained oyster (*Crassostrea virginica*) eggs, trochophores and veligers by immersion in neutral red. Other stains were also tested but these either killed eggs and larvae or stained too lightly. Since that time others like Hidu and Hanks (1968) marked the shells of larval hard-shell clams (*Mercenaria mercenaria*) and postlarval hard-shell, soft-shell (*Mya arenaria*) and coot clams (*Mulinia lateralis*) with alizarin sodium monosulfonate by immersion of actively growing individuals in the stain for 2-7 d. Manzi and Donnelly (1971) further developed techniques for staining large numbers of *C. virginica* and *M. mercenaria* larvae with alizarin red and neutral red. In most cases these chemicals were not used in the tracking of stained animals but rather for the specific staining of selective parts so that other research could be undertaken. Nile Red has been used to stain intracellular lipid droplets, for detecting neutral lipid deposits in tissue sections, permitting study of metabolic pathways and lipid reserves (Fowler and Greenspan, 1985; Greenspan *et al.*,1985; Castell and Mann, 1994), and to monitor physiological conditioning (Jackson, 1993).

Despite the availability of appropriate stains, there are very few reports that document the use of these techniques to mark shellfish for dispersal studies in the field. Only one successful mark, release, and recapture *in situ* study of stained larval movements in marine invertebrates has been described. Millar (1961) released European oyster larvae stained with neutral red at two points in a Scottish loch. He successfully recaptured 103 larvae 12 to 18 h later, over 1 mile (2.2 km) from the release site. Mark, release and recapture methods such as these are among the most direct means of studying dispersal, but are rarely attempted. This project represents one such attempt.

Project Objectives

The larval phase of the giant scallop is important to the aquaculture industry because high retention or survival of larvae has been linked to successful recruitment to fishable sizes in scallops (Caddy, 1989). Dickie (1955) suggested that years of poor recruitment in scallops were due to advection of larvae from favourable settlement areas. A trial enhancement experiment to increase recruitment was previously performed by researchers at a mussel/scallop aquaculture site in Charles Arm, Newfoundland whereby millions of 3-day-old veliger larvae were produced using hatchery methods and released on site. This did not result in increased spat collection 2 months later (Dabinett and Couturier, 1994). The experiment raised two important questions: 1) were the released larvae retained within the site?; and 2) was the method used for spat collection

appropriate?

This project was designed to investigated the retention of released giant scallop larvae within an inlet in Notre Dame Bay, Newfoundland. Incorporation of larval marking with a non-toxic, fluorescent dye (calcein) and tracking (release and recapture), will be used to determine if released *P. magellanicus* larvae are entrained within the site.

Although the staining method was adapted from Rowley and Mackinnon (1995), specific procedures for *P. magellanicus* had first to be developed. Furthermore, the efficacy of this stain on newly hatched D-veligers had to be determined and the procedure adapted for field conditions.

The objectives of this study were:

1. To describe a protocol for staining scallop larvae with the fluorescent stain calcein, which binds irreversibly to calcium as it is deposited in the shell.

2. To determine the efficacy of this staining procedure on newly developed D-veligers.

3. To conduct mark, release and recapture trials with stained scallop larvae in Charles Arm, NF.

4. To determine growth rates of calcein-stained scallop larvae under natural conditions with temperature and chlorophyll-*a* measurements as variables of the "natural conditions".

5. To study the spatial and temporal distributions of stained scallop and wild bivalve larvae within Charles Arm.

6. To measure current speed and direction within Charles Arm using moored current meters, and surface drift patterns using bottle drogues and present these data in relation to larval distribution.

7. To determine the abundance and size frequency distribution of bivalve larvae at three sampling sites over time to give an indication of spawning episodes and wild growth rates in Charles Arm.

8. To estimate the effects of tidal cycles on larval distribution by sampling the distribution and transport of mesozooplankton throughout tidal cycles in Charles Arm.

9. To synthesize the results of the mark and release studies with wild bivalve larvae distributions to examine the possible fate of artificially produced scallop larvae released in Charles Arm and evaluate this method as a strategy for enhancement and recruitment for scallop aquaculture.

CHAPTER 1

Development and testing of calcein for practical use with *Placopecten magellanicus* larvae

1.1 Introduction

Several marking methods involve chelation or replacement of calcium during calcium carbonate deposition. The antibiotic tetracycline produces a fluorescent band, visible under UV light, and has been used by several investigators to mark a variety of different species of fish, e.g., eggs and otoliths of larval ayu (*Plecoglossus altivelis*) (Tsukamoto, 1985), and otoliths of larval spot (*Leiostomus xanthurus*) and pinfish (*Lagodon rhomboides*) (Hettler, 1984). At least one successful mark-recapture experiment has been conducted with tetracycline-labeled ayu whereby eggs and larvae were released into a river and recovered 1 to 6 d later, in order to validate the presence of daily growth increments in larval otoliths (Tsukamoto and Kajihara, 1987). Calcein was proposed by Wilson *et al.* (1987) as a safer replacement for tetracycline in marking fish otoliths. Tetracycline is sometimes detrimental to the health of fish, causing reduced activity and cessation of feeding. A later study by Monaghan (1993) supported the superiority of calcein to tetracycline as a chemical marker in summer flounder (*Paralichthys dentatus*): unlike tetracycline, calcein bands were more intense and the calcein staining procedure was less toxic to the fish. In any study using chemical markers it is important that neither the marking process nor the chemical itself affects the animal in any way. The chemical, therefore, must be non-toxic and remain detectable on the animal for the duration of the study.

Calcein (3,6-Dihydroxy-2,4-bis-{N,N'-di(carbomethyl)-amino-methyl} fluoran)

binds with alkaline earth metals, and although the process has not been studied it appears that calcein is permanently incorporated into calcareous tissue when present during calcification (Rowley and Mackinnon, 1995). Calcein is readily absorbed by tissues, binding permanently to newly deposited calcium, showing little toxicity and fluorescing bright green when irradiated with blue light, thus making it very attractive for use in marking, identification and growth studies. These features have allowed calcein to be employed in marking bones and scales of fish (Wilson *et al.*,1987) and in other calcified structures of invertebrate taxa such as brachiopods, bryozoans, gastropods, cephalopods, echinoids, ophiuroids, asteroids, holothuroids, polychaetes, crustaceans and bivalves (Rowley and Mackinnon, 1995). Fluorescent dyes also offer a number of advantages including: high affinity to very low concentrations of the substance to be stained; absence of distributional error characteristics in absorbance measurements; and the possibility for *in situ* studies (Castell and Mann, 1994). Calcein, therefore, has potential for marking a variety of calcified larval structures, including mollusc shells, echinoderm plates and skeletal elements, and, in this study, the newly-forming shell of the larvae of the giant scallop.

Giant scallops reach the D-veliger stage 3 to 4 d after fertilization (Couturier *et al.*, 1995). At age 0 to 3 d, giant scallop larvae are lecithotrophic. Secretions by the shell gland and mantle epithelium begin the assembly of calcium carbonate crystals and an organic matrix to form the prodissoconch I larval shell (Crenshaw, 1980; Jablonski and Lutz, 1980). It is possible to induce mature giant scallops to spawn, fertilize the eggs and

maintain the developing larvae for up to 4 d under hatchery conditions. At the onset of the formation of the larval shell, D-veligers can be immersed in a bath of calcein which then becomes incorporated in the new shell growth. Once the veligers are marked they are ready to be released into the natural environment.

The fundamental assumption of chemical marking is that neither the marking process nor the introduced chemical changes the behaviour, physiology, or biochemistry of the animal (Nielsen, 1992). High concentrations of, or long exposure to, any chemical can be harmful (Tsukamoto, 1985), so every proposed chemical marker and marking process should be carefully evaluated. It is also required that chemical marks remain identifiable on the marked animals throughout the desired interval. An experiment was performed to test the efficacy of the staining process and the toxicity of the stain.

1.2 Objective

To describe a staining protocol for the fluorescent stain, calcein, for use with giant scallop larvae, and to test the efficacy of this staining procedure with newly developed D-veligers.

1.3 Methods

Study site

Adult giant scallops were obtained from a commercial shellfish farm (Thimble Bay Farms) located at Charles Arm, Notre Dame Bay, Newfoundland on July 20, 1994 (trial 1) and on July 24, 1994 (trial 2). These scallops served as naturally conditioned broodstock for the purposes of this study. Field laboratories were established in a processing plant and cabin located at the site.

Experimental design

Two trials were undertaken to investigate the efficacy of the stain. Triplicate samples of larvae (250 per mL - trial 1 and 40 per mL - trial 2) were exposed to 5 concentrations of the stain plus a control (no stain, 10 µm filtered seawater), for 5 exposure times of 10, 100 min, 8, 16 and 24 h (trial 1) and 2, 8, and 24 h (trial 2), followed by recovery periods in 10 µm filtered seawater for 24 and 48 h (trial 1) and 24 h (trial 2) at 15°C. The calcein concentrations were 500, 200, 100, 10, 1 mg/L and 0 mg/L for the control. The calcein, in powdered form, was obtained from Sigma Chemical (Lot 123H0594).

Spawning and D-veliger production

Larvae were obtained by inducing adult giant scallops to spawn. The adults were taken from pearl nets hanging at about 3 m depth on the farm site and brought back to the processing plant, where they were placed in a 1 m^3 Xactics PVC tub containing 10 µm filtered seawater. Scallops were induced to spawn by the method of Desrosiers and Dubé (1993), but on a smaller scale. At the onset of spawning, individual male and female scallops were placed in 5-L plastic buckets filled with 10 µm filtered seawater and allowed to complete spawning. Fertilization was accomplished by adding sperm to the egg suspension with a pipette until a ratio of approximately 5 to 10 sperm per egg was obtained. This was determined by dark field microscopy. Eggs were left for a few hours and monitored using an inverted microscope to confirm that cell division was taking place. About 15 million fertilized eggs were then transferred to each of 6 pre-cleaned, covered, 1m^3 Xactics PVC tub filled with filtered seawater and left for 36 h. Larvae were then monitored to confirm development into straight hinge D-veliger larvae. When needed, D-veligers from the tubs were collected on 50 µm screens and washed into 5-L plastic buckets filled with filtered water.

Calcein preparation

The calcein stock solution was prepared using the method of Rowley and Mackinnon (1995) by dissolving 0.5 g of calcein in one litre of 10 µm filtered seawater in a glass jar. Sodium hydroxide (2.3 mL from a stock solution of 40 g/L), and concentrated HCl (if necessary), was added to bring the pH close to that of ambient seawater (pH~8.10).

Aliquots (2 mL) of a D-veliger suspension were placed in 30 mL tissue culture flasks with calcein stock solution and/or filtered sea-water (final volume of 30 mL) to give final concentrations of 0, 1, 10, 100, 200 and 500 mg calcein per litre

Data analysis

A 2 mL sample was taken from each of the flasks at appropriate time intervals and larval survival evaluated with an inverted microscope. Samples were preserved in 4% formalin for estimating abundance, survival and mark intensity. Fluorescence was determined on an arbitrary 5 point scale ranging from 0 (no fluorescence) to 4 (bright fluorescence). Preserved stained larvae were examined on a monthly basis to check for loss of fluorescence.

After 24 h immersion, larvae in the staining solutions were removed using a 50 µm screen. The larvae were rinsed with clean 10 µm filtered seawater to remove any

residual calcein solution, then returned to the tissue culture flasks with 30 mL of filtered seawater (trial 1, high density) or to buckets filled with 8 L of filtered seawater (trial 2, low density).

Single Factor Analysis of Variance (ANOVA) was performed to compare mortalities in all concentration groups, for the different time periods sampled, within each trial.

1.4 Results

Mortality

In the first trial mortality remained below 10% for all concentrations (Figure 1) during the staining period with the exception of the 500 mg calcein/L concentration, which had 15% and 16% mortality at immersion times of 16 h and 24 h, respectively. There was a significant difference between the 500 mg calcein/L and all other concentrations, including the control at the 16 h sample (ANOVA, $F_{(5,17)} = 4.841$, $p < 0.05$). No difference in mortality was observed among groups at any of the other immersion times during the staining period (ANOVA, $p>0.05$). An increase in the mortality of larvae was observed after the larvae were removed from the stain (recovery

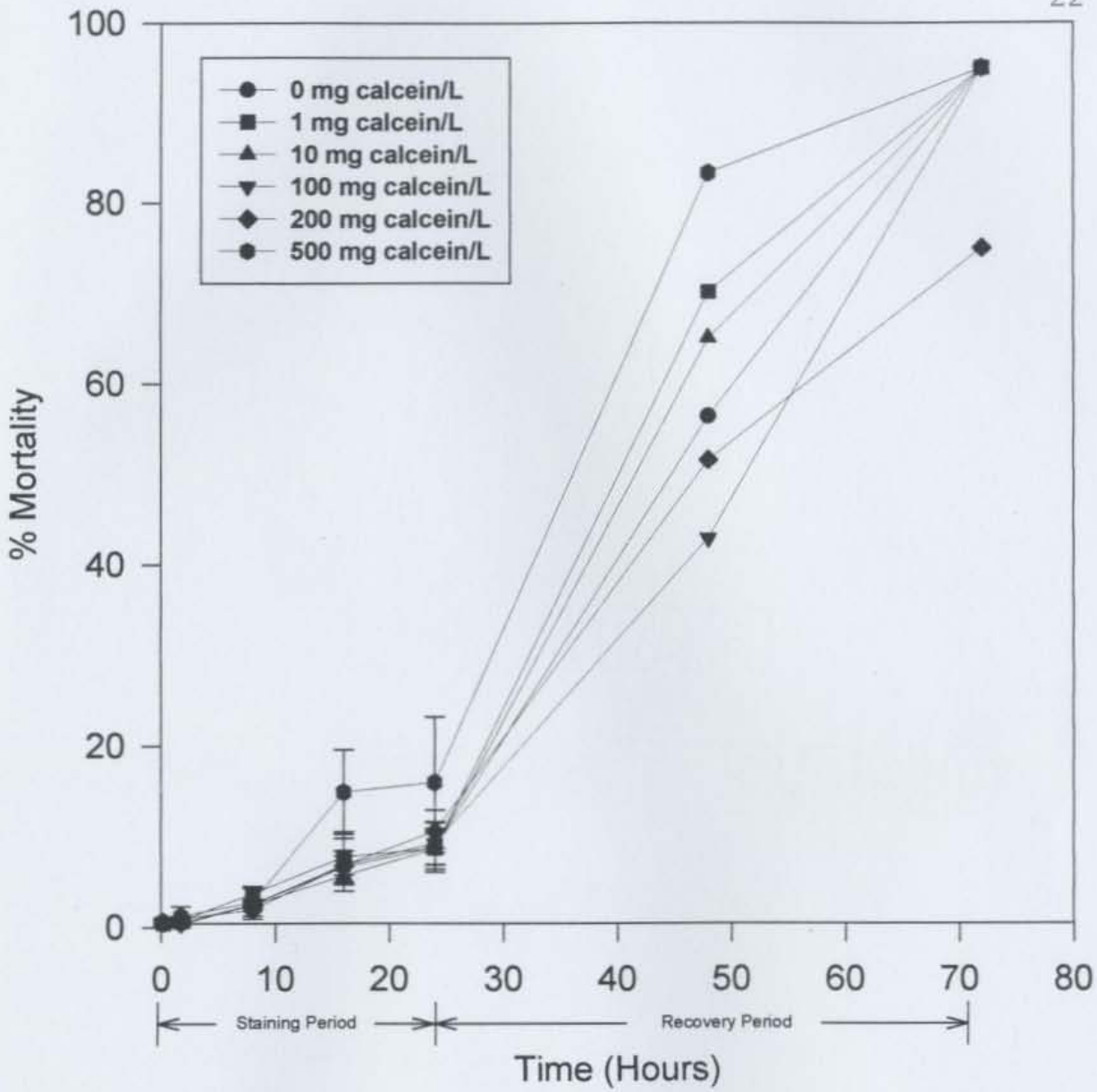

Figure 1: Mortality of 2-day-old giant scallop veligers stained with calcein at various concentrations for 24 h at ~250 veligers/mL (Trial 1). Values represent mean +/- standard error of 3 replicates.

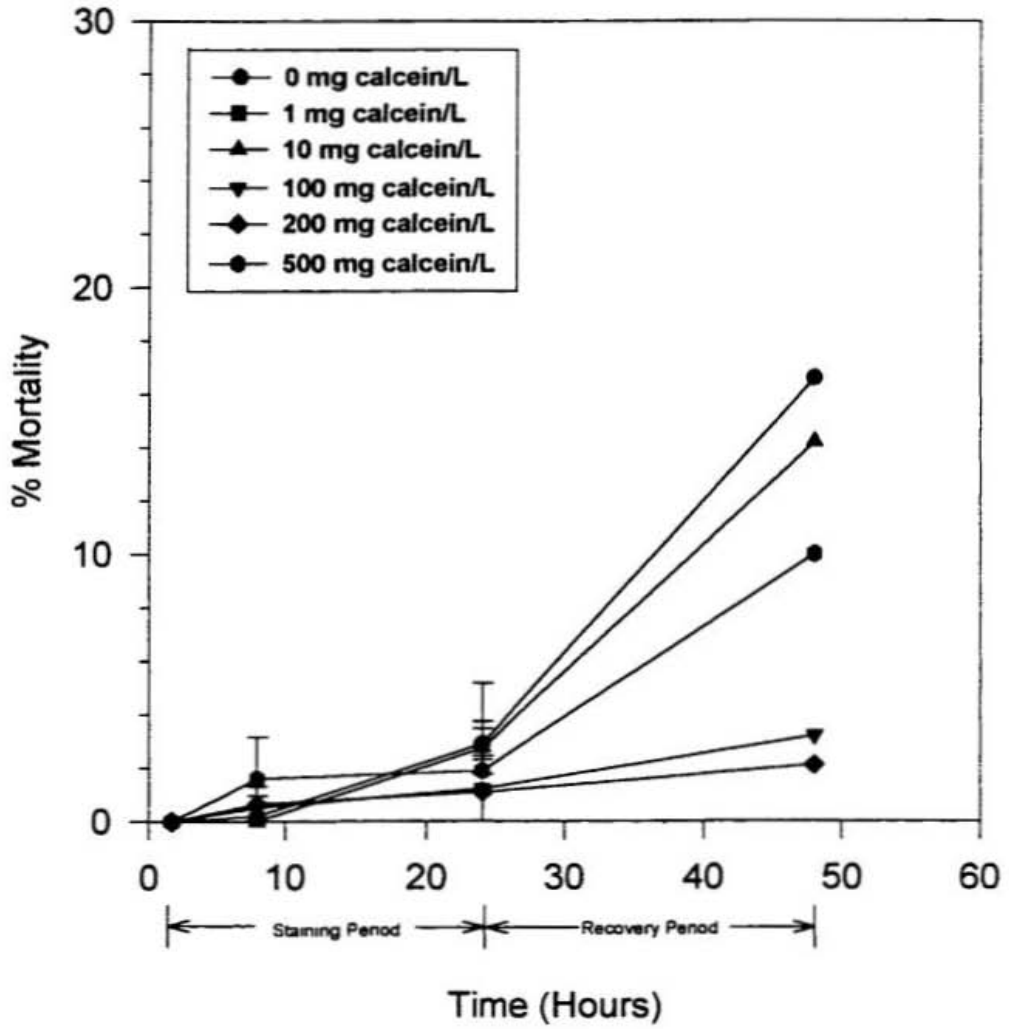

Figure 2: Mortality of 2-day-old giant scallop veligers stained at various with calcein at various concentrations for 24 h at ~ 40 veligers/mL (Trial 2). Values represent mean +/- standard error of 3 replicates.

period). After 24 h recovery, all treatments including the control groups exhibited greater than 45% mortality. After 48 h recovery, mortalities were ~95% at all concentrations with the exception of the 200 mg/L (mortality ~75%).

In the second trial, no mortalities over 20% were observed for any of the staining concentrations or the control during the staining or recovery periods (Figure 2). No mortalities greater than 4% were observed in the 100 and 200 mg calcein/L concentrations even during the recovery period. There was no significant difference in percent mortality for each concentration group and the control (ANOVA, $p>0.05$) during the staining period. Mortalities increased over the study period for the control group (0 mg calcein/L) (ANOVA, $F_{(2,8)} = 91.06$, $p<0.05$), and 10 mg calcein/L (ANOVA, $F_{(2,8)} = 23.65$, $p<0.05$) but no significant increase in percent mortality was evident for the 100, 200 and 500 mg calcein/L groups (ANOVA, $p>0.05$). Microscopic observation during the recovery period revealed that protozoans were prevalent in the control group while larval samples previously bathed in calcein were protozoan free.

Fluorescence

Fluorescence was observed in larvae after as little as 8 h immersion in the stain (Figure 3), but was faint and visible only in the highest concentration of stain (500 mg calcein/L). After 16 h immersion, larvae in both the 500 and 200 mg calcein/L

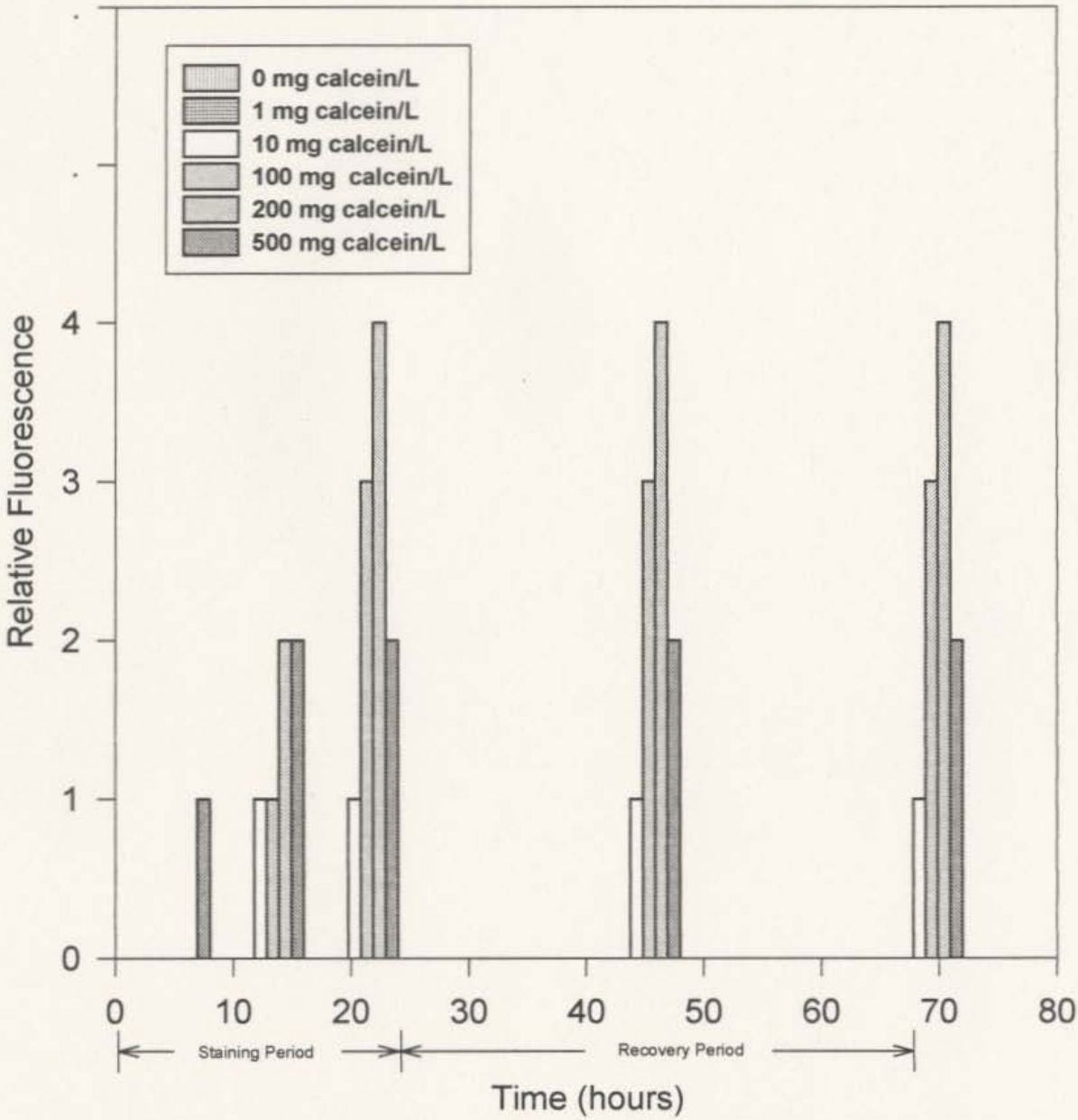

Figure 3: Relative fluorescence of 3-day-old giant scallop veligers stained with calcein for up to 24 h, and after 24 and 48 h recovery in filtered seawater. Fluorescence of 0 = undetectable, 1 = fair, 2 = satisfactory, 3 = good and 4 = excellent.

concentrations were stained satisfactorily and a faint fluorescence was observed in the 100 mg calcein/L concentration. After 24 h, the 200 mg calcein/L samples showed excellent fluorescence. Fluorescence was undetectable in the control or the 1 mg calcein/L concentration after 24 h immersion. No depletion of fluorescence was observed during the recovery period in any of the stained veligers. Preserved larvae have retained their fluorescence with the same intensity after two years.

1.5 Discussion

Mortality

Although high measurements of percent mortality were observed in trial 1 in all calcein treatments they did not differ from the control treatment. In contrast, during trial 2 the control group had the highest mortality observed (Figure 2). Some factor, other than the calcein bath, must be responsible for the increase in mortality of the controls. The difference in percent mortality observed between the two trials (Figures 1 and 2), especially during the recovery period, suggests two possibilities.

Firstly, the difference may be attributable to the larval density in the tissue culture flasks. Trial 1 used a very high density of ~250 veligers/mL while during trial 2 density was reduced by almost 80% to 40 veligers/mL. The higher densities mean that less time

is taken for metabolic waste to reach critical levels and for oxygen to be depleted. The extremely high mortalities (>40%) observed during the recovery period of trial 1 (Figure 1) were unsatisfactory, and would render the staining procedure impractical for marking the large numbers of giant scallop larvae needed for a field experiment. Lowering the density of the veligers in the flask, for trial 2, would reduce stress and decreased percent mortality would be expected. Not only was the density lower during the staining period, but once removed from the stain these veligers were placed in 8 L of water, thereby greatly reducing the density (6 veligers/mL) during the recovery period. Large numbers of larvae could possibly be supported by using larger incubation vessels (buckets or tanks), i.e., a lower density, and possibly with aeration.

Secondly, different batches of scallop veligers were used in the trials. These batches of veligers may be of a different genetic makeup, related possibly to the genetics of the broodstock or to the ripeness of the gametes (Couturier, 1986) and may account for the differences observed in percent mortality between the two experimental trials.

Large numbers of protozoans were observed in the control group (trial 2) after 24 h post-stain, whereas none were observed in the samples bathed in calcein. This finding is of some interest and may be noteworthy for further study. It may be possible that calcein has some effect on protozoans or bacteria such as reducing their abundance.

Fluorescence

Fluorescence was not evident until at least 8 h after immersion, and was faint at this time (Figure 3). Stronger fluorescence was observed at 16 h for the 200 and 500 mg calcein/L samples, but the greatest fluorescence was seen in the 200 mg calcein/L sample after 24 h.

Giant scallop veligers could be satisfactorily marked through immersion in this stain for a minimum of 16 h at a concentration between 100-200 mg calcein/L. Although better fluorescence was observed after a longer immersion time (Figure 3), it is more convenient to use 16 h because scallops can be placed in the stain in the evening and used the following morning. Furthermore, the shorter the length of time that the veligers are handled, the less likely that mortalities will occur. A fluorescent marker need only be detectable thus a minimum amount is required. Using ~150 mg calcein/L would be sufficient for fluorescence in giant scallop veligers and would make more efficient use of the calcein. A concentration of 125 mg calcein/L was previously used by Wilson *et al.* (1987) to mark otoliths of larval and juvenile fish although, Rowley and Mackinnon (1995) used a calcein solution of 500 mg/L to stain bivalves as well as other taxa. The results of this experiment indicate that only 1/3 that concentration need be used for giant scallop larvae but higher concentrations may be needed for other taxa.

Calcein is useful in its ability to mark giant scallop larvae. Not only is it non-toxic to the larvae, it also provides a bright, permanent fluorescent mark, that has a long life in preserved samples.

CHAPTER 2

Release and recapture of calcein stained giant scallop veligers at Charles Arm, Notre Dame Bay, Newfoundland.

2.1 Introduction

Earlier enhancement studies at Charles Arm, Notre Dame Bay, during which millions of 3-day-old giant scallop larvae were released, did not result in increased spat collection (Dabinett and Couturier, 1994). There are several possible explanations for the lack of observed increase in spat yield. Firstly, the veligers produced by hatchery techniques may have been moribund and therefore did not survive to set. Secondly, the released larvae may have been transported out of the site to set elsewhere. Thirdly, the predation level may have been sufficiently high that the number of released larvae that survived to set was too low to make a significant difference. Fourthly, too few larvae may have been released. Charles Arm holds approximately 10 million m^3 of water, so releasing only a few million larvae would probably not result in a high percentage increase of spat found on the collectors as compared to previous years. Finally, the veligers may have been retained in the site but the collection method or placement of collectors within the site may have been inappropriate to provide any substrate for the released larvae.

Before staining techniques were developed, studies on the movements and distribution of larvae had to deal with unknown populations. Since a staining method for oyster larvae with neutral red was developed by Loosanoff and Davis (1947), many stains have been used successfully for a variety of invertebrate larvae. For a comprehensive review see Levin (1990). Despite the availability of appropriate stains there are very few studies in which invertebrate larvae have been tracked directly in the field. Most tracking studies have combined information on larval distributions with larval sources, behaviour,

recruitment patterns and relevant hydrodynamic properties (Levin, 1990).

Mark, release and recapture methods are among the most direct means of studying larval dispersal. They are rarely attempted and Millar (1961) is the only reported successful attempt.

2.2 Objectives

The staining protocols described in Chapter 1 were used to stain batches of scallop larvae prior to release at a single point within a sheltered site for recapture studies. The purpose of this mark, release and recapture experiment was: 1) to determine the spatial distribution of the veligers which may be moved by swimming, wind transport or current/water movement; 2) to establish if the veligers are being entrained within Charles Arm; and 3) to employ stained veligers, upon recaptured, to measure their growth rates under *in situ* conditions. Temperature and chlorophyll-*a* data collected by a CTD meter will be used as measures of *in situ* conditions.

2.3 Methods

Study site

The study area is located at Charles Arm, Notre Dame Bay (Figure 4a and b). This is a commercial mussel (*Mytilus edulis*) and giant scallop farm. The site is located in a sheltered narrow inlet with a shallow sill (5 m) at the mouth. The inlet extends for nearly 2 kilometers and has buoyed head ropes anchored shore to shore across the site. The farm occupies nearly 100 hectares and holds about 10 million m^3 of water with a maximum depth of 20 m (Figure 4c and d)

Staining

Batches of *Placopecten magellanicus* veligers were produced using standard hatchery techniques and stained using the protocols developed in Chapter 1, i.e, immersion in 150 mg calcein/L for 16h.

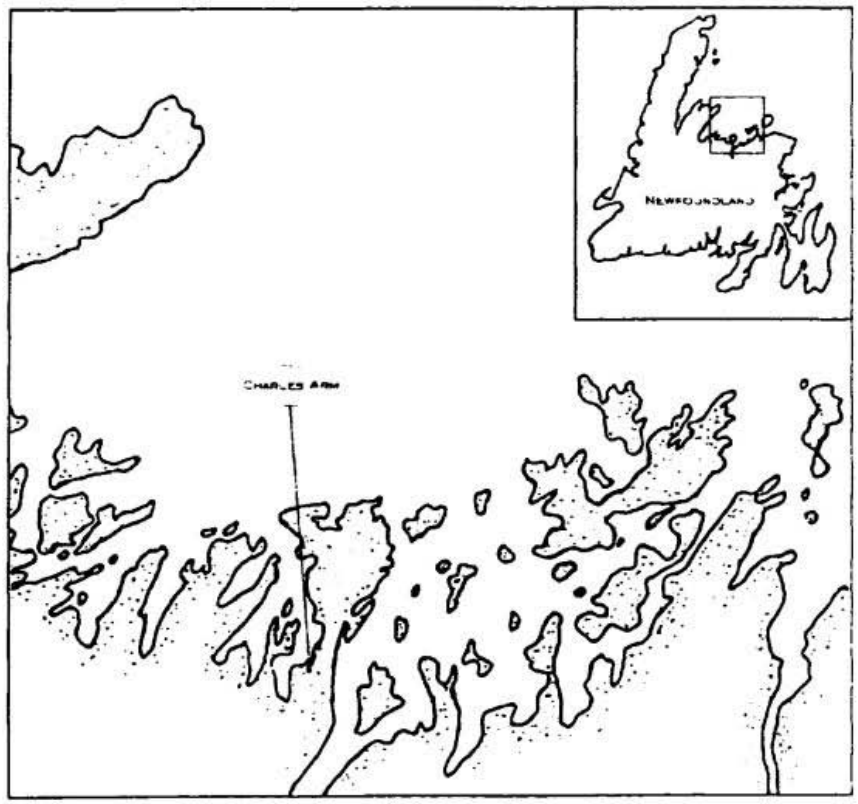

Figure 4a: Location of study site at Charles Arm, Notre Dame Bay, Newfoundland.

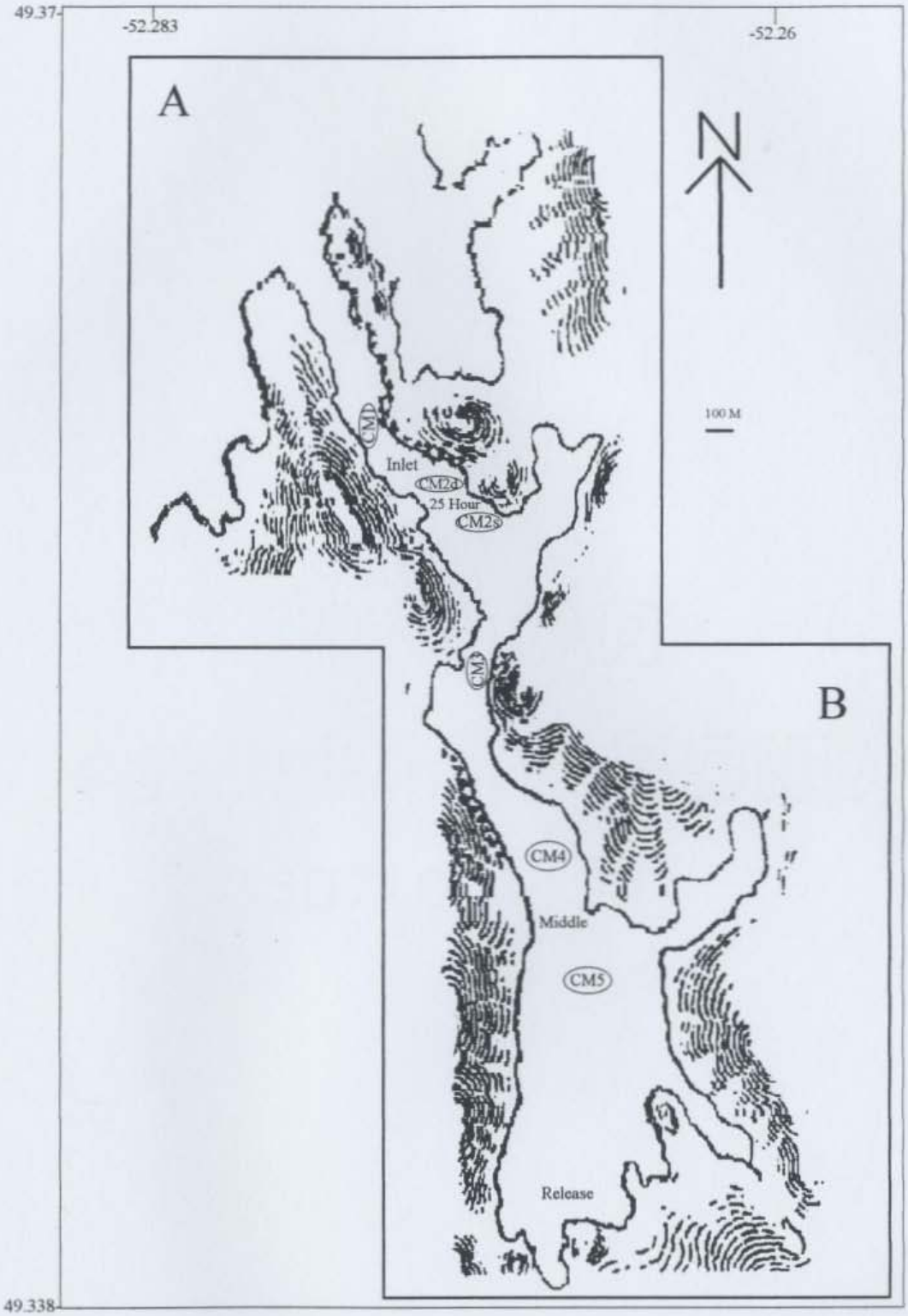

Figure 4b: Overview of Charles Arm showing "release", "middle" and "inlet" stations, current meter locations, and "25 hour" station for tidal influences. Box A = Mouth of Charles Arm and B = End of arm.

Figure 4c: Overview of the "Mouth" of Charles Arm showing location of headropes, collector bags (C) for scallops, and maximum depths. The depths have been corrected to show water heights during lowest low tide. Stippled areas indicate areas which are less than 3m deep or are shoals which break the surface during low tide.

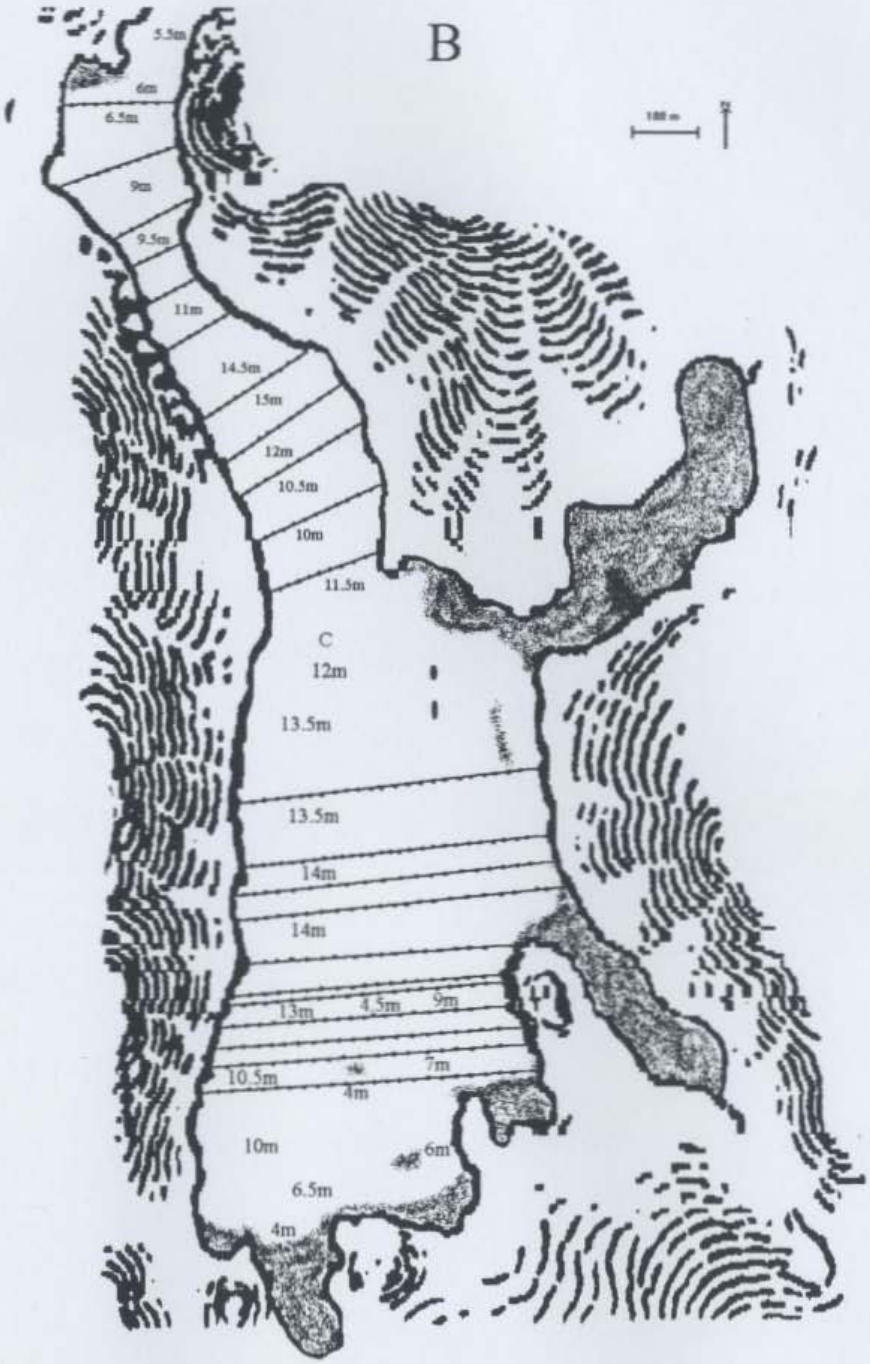

Figure 4d: Overview of the "End" of Charles Arm showing location of headropes, collector bags (C) for scallops, and maximum depths. The depths have been corrected to show water heights during lowest low tide. Stippled areas indicate areas which are less than 3m deep or are shoals which break the surface during low tide.

Release trial 1994

Stained larvae were filtered from the calcein bath with a 50 μm screen. Larvae were washed with 10 μm filtered sea-water and placed in 16-L buckets filled with 11.5 L filtered water (~175 veligers/mL) until the time of release (within 2 h). Larvae were released only at high tide. The buckets of stained larvae were transported to the release point in the farm's harvest boat and emptied gently over the side of the boat by tilting the partly submerged bucket in the water. Eighteen million (1.8/m^3) stained veligers were released in the first trial on July 27 at 17:47, with an observed 17.25 million larvae viable. On August 19 at 08:00, a second batch of 21 million larvae (2.1/m^3) was released. Mortality was observed to be 30% and approximately 15 million larvae were viable.

Release trial 1995

Stained larvae (15 million and 27 million) were released on two occasions (August 12 and 25, respectively) at the same release point as in 1994.

Recapture trial 1994

Recapture of marked veligers was attempted with horizontal plankton tows at two depths (1 m and 5 m) at three different stations (release, middle and inlet). Tows were conducted 1,14, 21, and 72 h after the first release on July 27 with a 30 cm diameter 64-µm-mesh net. Subsequent tows were completed on August 6, 17, 19, 23, 24 and 26th, September 8 and October 4 (10, 21, 23, 27, 28, 30, 43 and 69 d after release). The second release of stained larvae occurred 3 wk later on August 19. Tows completed after July 29 were performed with a 50 cm diameter 64-µm-mesh net. Plankton samples were preserved in 5% buffered formalin until microscopic examination for stained larvae. Towing times varied from 1 to 3 min, depending on the observed concentration of plankton in the water. Towing times were reduced on occasion to prevent the plankton net from clogging. A General Oceanics, Inc. 2030R Standard Flowmeter was used to quantify the volume of water filtered during each tow.

Recapture trial 1995

Recapture of stained veligers was accomplished using oblique tows, done in triplicate, at the same three stations designated in 1994. Tows were completed with the same 50 cm diameter 64 µm plankton net and performed 2, 4, 8, 16, and 24 h after release and then weekly. The first release date was August 12 and subsequent tows were

performed on August 13, 19, 25, 26, 28, September 7, 21 and 30th (1, 7, 13, 14, 16, 26, 40 and 49 d later) with the second release of larvae on August 25. The oblique tows at the release and middle stations were 70 m long, dropping 1 m in depth for every 10 m of horizontal movement. Distance and depth consideration allowed a 50 m oblique tow at the inlet station only. To aid in the replication of tows and to ensure that the boat remained on the sampling station, fluorescently painted Javex bottles were attached to a fixed headrope 10 m apart. Sampling began at one end of this headrope. Samples were pre-filtered through a 500 µm screen to remove any larger planktonic animals and then preserved in 5% formalin until examined for stained larvae.

CTD (Seabird) casts

Water column structure was examined with a conductivity, temperature, depth meter (CTD, manufactured by Seabird Electronics Inc., Washington USA), equipped with an fluorometer to measure chlorophyll-*a* concentration. Temperature and chlorophyll-*a* contour plots were prepared with Surfer (Win 32) software, Version 6.01 (Golden Software Inc. Colorado, USA, 1995).

Microscopic examination

Preserved samples were filtered onto a 50 µm screen and re-suspended in 25 mL of 2 µm filtered seawater. Twenty five 1 mL subsamples were examined with a Zeiss Axiovert Inverted Microscope with a standard blue filter set (Zeiss 487709), Chromatic Beam Splitter 510, Barrier Filter BP 515-565 and Exciter filter range 450-490 nm. When stained scallop veligers were found, shell height and length were measured with a calibrated eyepiece. To shorten search time, samples collected within a week of release were size graded to less than and greater than 186 µm (nitex mesh screen measured on the diagonal). Only those samples with larvae smaller than 186 µm were examined. Samples collected after the first week but not later than 2 wk after release were separated into less than and greater than 295 µm with only those samples with larvae smaller than 295 µm examined.

2.4 Results

Larvae were ~60-80 µm in shell height upon release in 1994. Larvae remained within the site for over 40 d in 1994 with maximum shell heights of 240 and 270 µm observed for the July 27 and August 19, 1994 releases, respectively. Growth rates of 3.53 and 3.85 µm/d were observed for the 1st and 2nd releases, respectively (Figure 5a).

Larvae were 70 µm in shell height upon release in 1995. A maximum shell height

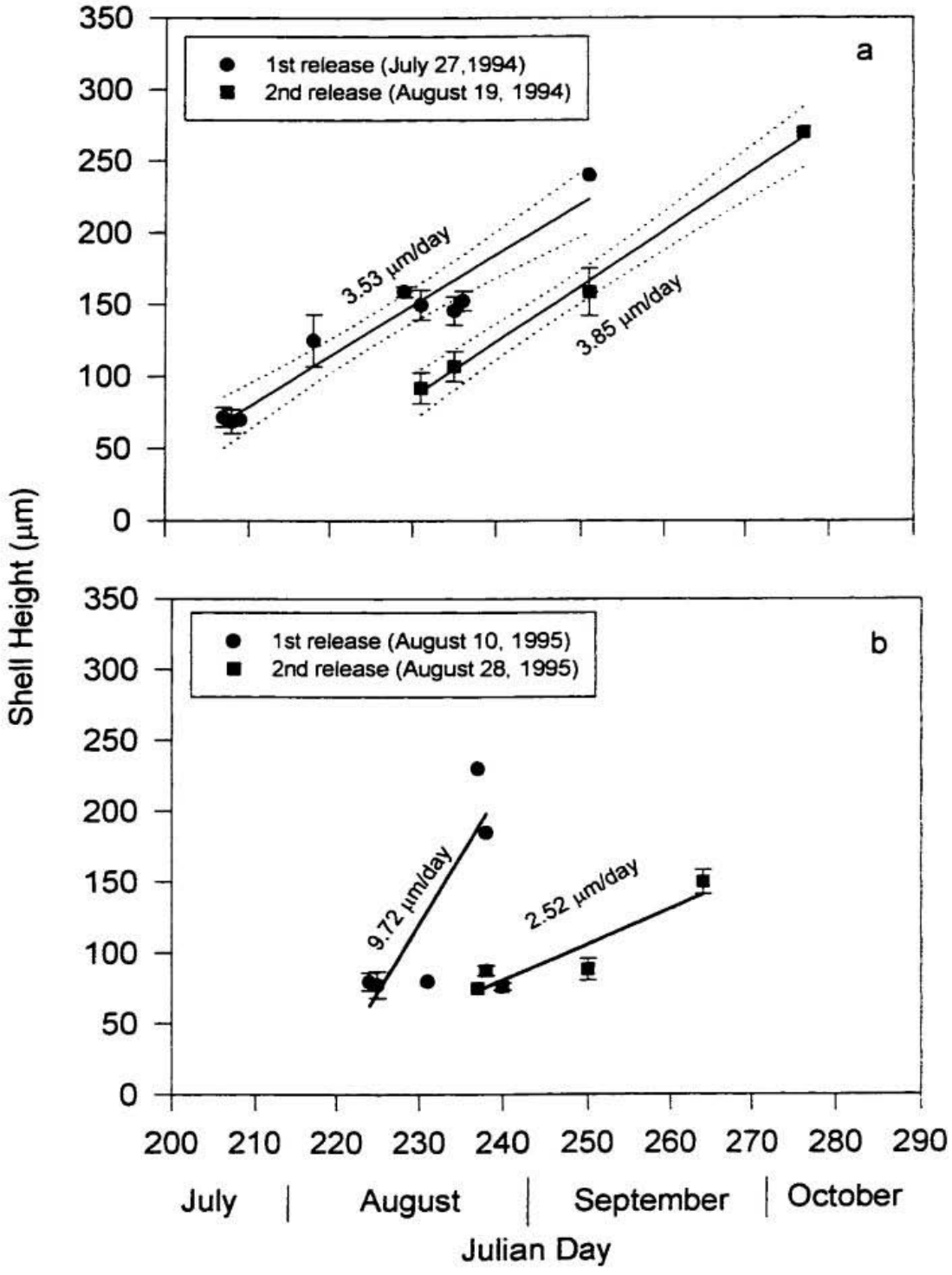

Figure 5: Growth rates of released calcein stained 3-day-old scallop veligers during 1994 (a) and 1995 (b). Values are mean sizes (+/- standard deviation) for each sample date. Dotted lines represent the 95% confidence intervals.

of ~240 µm and a growth rate of 9.72 µm/d were observed within 3 wk for the first release on August 10, 1995. Larvae of the second release remained within Charles Arm for over 30 d and had an observed growth rate of 2.52 µm/d (Figure 5b).

In 1994, surface temperature peaked during late July with 18°C observed at all three sampling stations. Temperatures remained greater than 15°C until early September at the release and inlet stations but remained above 16°C at the middle station until late September. Temperatures greater than 14-15°C were observed to a depth of 7 m at all sampling stations from late July to late September in 1994 (Figure 6).

In 1995, surface temperature reached its maximum during early to mid-August with 14, 18 and 17°C observed at the release, middle and inlet stations, respectively. The temperature declined rapidly to 12°C (September and October). At a depth of 7 m, temperatures above 12°C were not observed until early October at the release station, late September at the middle station and early August at the inlet station.

Temperatures greater than 15°C were observed near the surface from early July to late September during 1994, whereas temperatures above 15°C were recorded only from late July to late August in 1995.

In 1994, the first indication of a phytoplankton bloom appeared in late July at the release station, peaking at 10 µg chlorophyll *a*/L (Figure 7). At the middle station, the chlorophyll-*a* maximum remained around 8 µg/L but was present at depths of greater than 10 m. Another bloom occurred in early September with an observed concentration of 11 µg/L chlorophyll-*a* at 14 m.

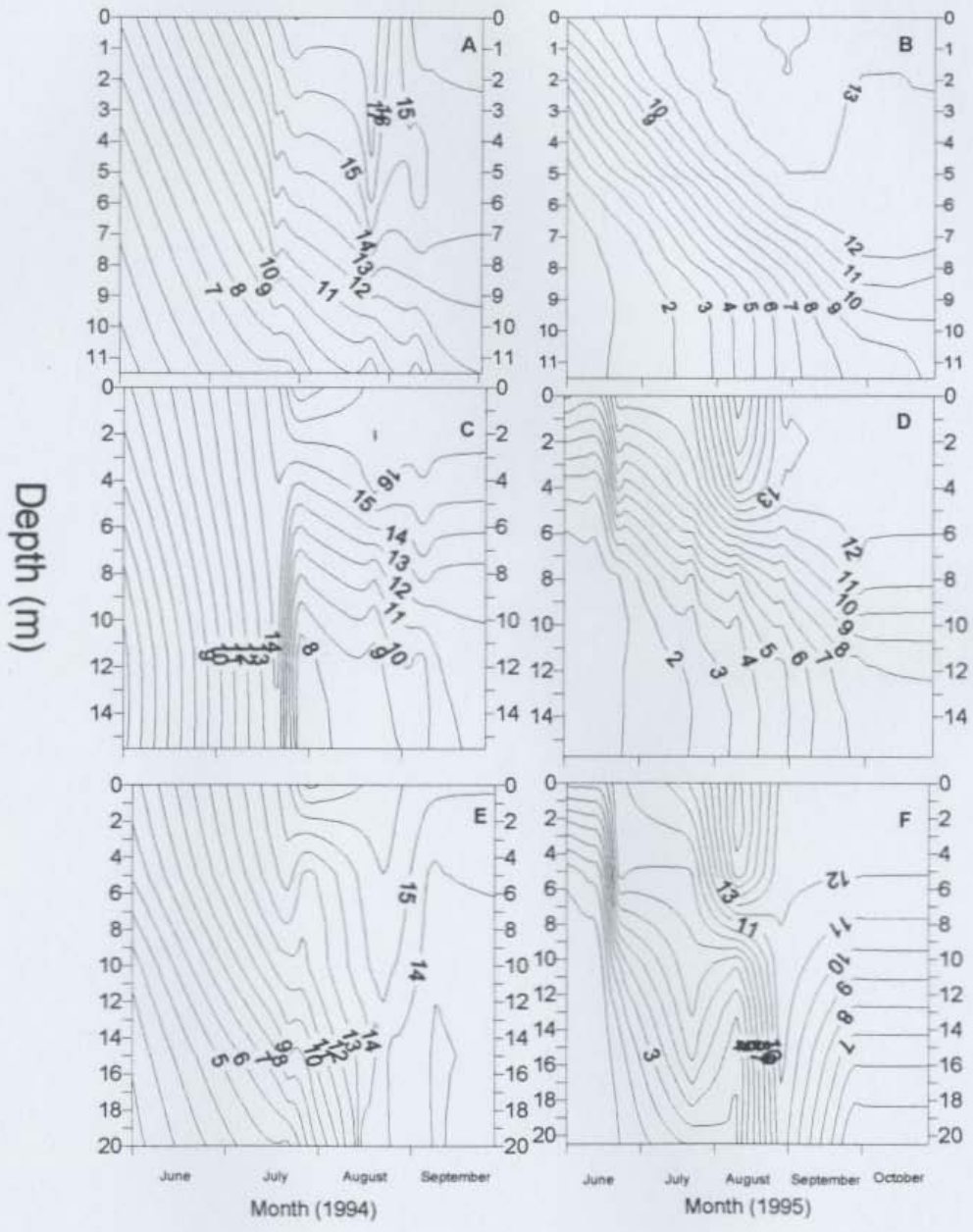

Figure 6: Contours of temperature (C) during 1994 and 1995 in Charles Arm at the sampling stations (release = A & B, middle = C & D, and inlet = E & F).

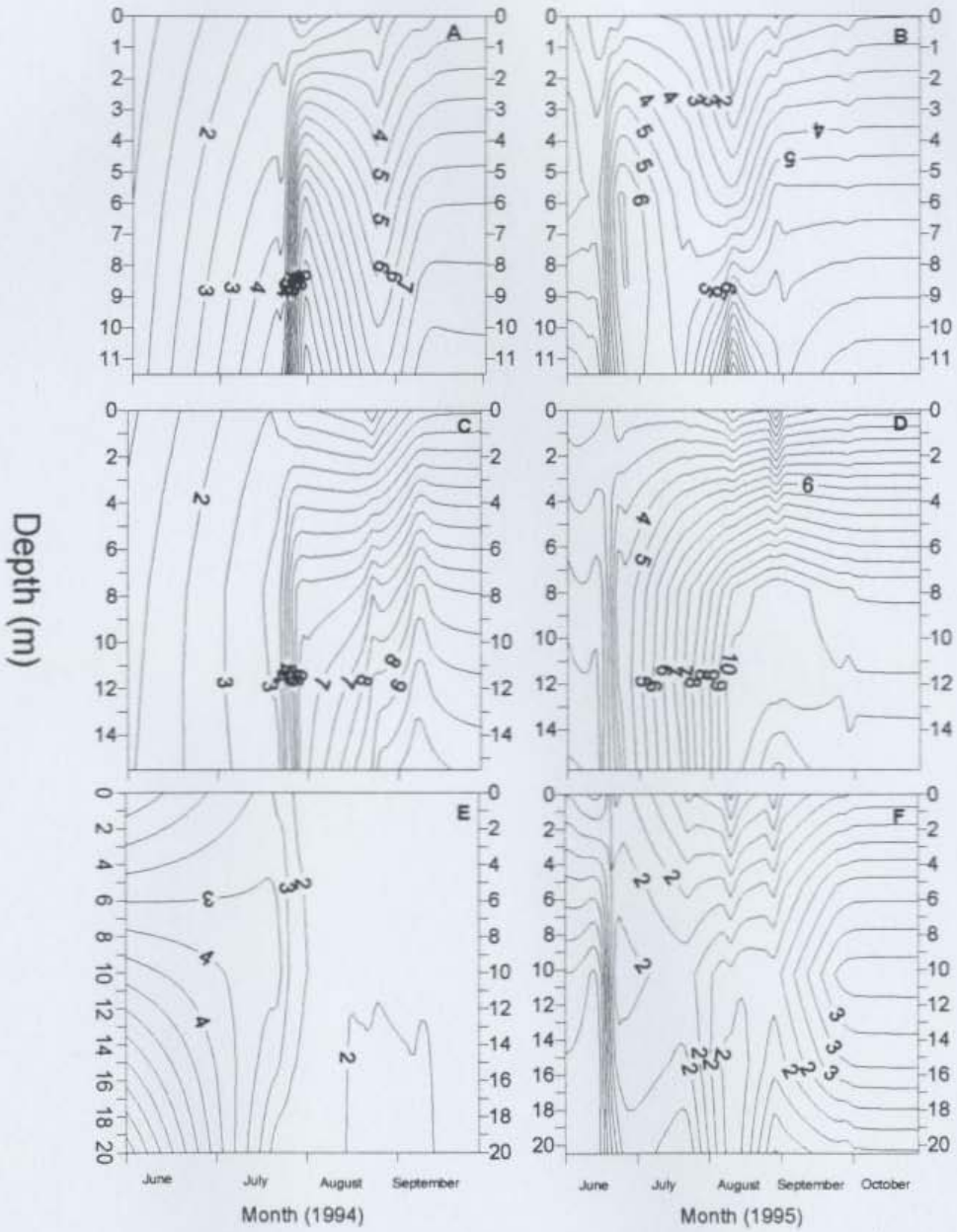

Figure 7: Contours of chlorophyll-a (ug/l) during 1994 and 1995 in Charles Arm at the sampling stations (release = A & B, middle = C & D, and inlet = E & F.

In 1995, a phytoplankton bloom occurred at the release station in the middle of June (Figure 7). Chlorophyll-*a* concentration reached a maximum of 7 µg/L with the peak between 6 and 8 m depth. A second bloom occurred during early August with chlorophyll-*a* concentrations reaching 13 µg/L (11 m depth) at the release station and 10 µg/L at the middle station. The chlorophyll-*a* concentration at the middle station remained high over the study period with values greater than 10 µg/L observed at depths greater than 8 m.

Stained scallops were recaptured at all three sampling stations during 1994 (Tables 1a and 1b) and 1995 (Tables 2a and 2b) and ranged from 70 to 270 µm in shell height.

Table 1a: Summary of stained giant scallop veligers found over the sampling season of 1994 (from release trial 1). Total in sample is an estimate, where indicated by an asterisk (*), based on stained larvae found in a subsample of a tow, otherwise, the entire sample was searched. Average size is for veligers found at all three (Release, Middle and Inlet) stations for each sample date. NA = not applicable.

Date Retrieved	Site Found	Number Found	Total in Sample	Range (µm)	Average Size (µm)	Standard Deviation
July 27	Release 1 m Middle 5 m	5 8	5 8	60-80	68.8	8.2
July 28	Release 1 m	2	70*	70	70	0
August 6	Middle 1 m Inlet 5 m	5 2	85* 34*	105-160	125	18
August 17	Release 5 m Inlet 1 m Inlet 5 m	1 4 3	1 68* 50*	155-165	158.8	3.5
August 19	Release 1 m Middle 5 m Inlet 1 m Inlet 5 m	1 15 6 9	1 15 102* 153*	145-160	149.8	10.4
August 23	Release 1 m Release 5 m Middle 5 m Inlet 1 m Inlet 5 m	4 9 5 3 6	4 9 5 51* 102*	130-170	145.6	9.7
August 24	Release 1 m Middle 5 m Inlet 1 m Inlet 5 m	1 2 6 1	17* 34* 102* 34*	140-160	152.5	6.8
September 8	Release 1 m	1	17*	240	240	NA

Table 1b: Summary of stained giant scallop veligers found over the sampling season of 1994 (from release trial 2). Total in sample is an estimate, where indicated by an asterisk (*), based on stained larvae found in a subsample of a tow, otherwise, the entire sample was searched. Average size is for veligers found at all three (Release, Middle and Inlet) stations for each sample date. NA = not applicable.

Date Retrieved	Site Found	Number Found	Total in Sample	Range (μm)	Average Size (μm)	Standard Deviation
August 19	Release 1 m	2	2	85-100	92	10.6
August 23	Release 5 m Release 1 m	2 2	2 2	95-120	107	10.4
September 8	Middle 5 m Inlet 5 m	1 3	17* 3	140-175	158.7	16.5
October 4	Inlet 1 m	1	17*	270	270	NA

Table 2a: Summary of stained giant scallop veligers found over the sampling season of 1995 (from release trial 1). Total in sample is an estimate, where indicated by an asterisk (*), based on stained larvae found in a subsample of a tow, otherwise, the entire sample was searched. Average size is for veligers found at all three (Release, Middle and Inlet) stations for each sample date. NA = not applicable.

Date Retrieved	Site Found	Number Found	Total in Sample	Range (μm)	Average Size (μm)	Standard Deviation
August 12	Release Middle Inlet	10 6 2	10 6 2	70-90	79.7	6.3
August 13	Middle Inlet	2 4	2 4	70-95	77.5	9.4
August 19	Inlet	1	1	80	80	NA
August 25	Release	1	1	230	230	NA
August 26	Inlet	1	1	185	185	NA

Table 2b: Summary of stained giant scallop veligers found over the sampling season of 1995 (from release trial 2). Total in sample is an estimate, where indicated by an asterisk (*), based on stained larvae found in a subsample of a tow, otherwise, the entire sample was searched. Average size is for veligers found at all three (Release, Middle and Inlet) stations for each sample date. NA = not applicable.

Date Retrieved	Site Found	Number Found	Total in Sample	Range (μm)	Average Size (μm)	Standard Deviation
August 25	Release	1	1	75	75	NA
August 26	Release	2	2	85-90	87.5	3.5
August 28	Middle Inlet	3 1	3 1	75-80	76.3	2.5
September 7	Release Inlet	2 1	2 1	80-95	88.3	7.6
September 21	Middle Inlet	2 1	2 1	140-155	150	8.7

2.5 Discussion

Plankton towing was chosen for the recapture of released stained larvae for several reasons. Firstly, bivalve veligers are generally less than 300 µm in maximum dimension, negatively buoyant, swim weakly, and their ability to avoid towed nets is probably negligible (Mann, 1986). Secondly, a plankton tow allows for a greater volume to be filtered in a shorter amount of time than does a small pumping system. Finally, a plankton net was readily available and practical to use on the access boat. Modern gauze unencased nets have an initial filtration efficiency of greater than 85% (Tranter and Smith, 1968), but for convenience 100% efficiency was assumed in this study.

Readings from the flow meter used in the early tows of 1994 were very inconsistent and at times, provided no readings or even negative readings were observed. For this reason no attempt was made to quantify the number of bivalve veligers per given volume in 1994.

In 1995, because tows were completed over a known distance, it was possible to calculate the number of bivalve larvae and stained larvae found per unit volume, but no attempt was made to estimate the total number of stained larvae present in the study area. The total number of stained larvae found in a given subsample and an estimate of the total number in the entire tow are reported because the scope of this project was to recapture these stained larvae to obtain growth measurements. The large volume involved and the minute fraction of water filtered by plankton tows made it impractical to estimate losses of

larvae out of the system.

The depth stratified tows may not give representative sampling of the species present and abundance of scallop veligers may be influenced by any tidally (Levin, 1986) or diurnally influenced changes in depth (Tremblay and Sinclair, 1990a and 1990b), and any vertical migration as a function of food and temperature stratification (Gallager *et al.*,1996; Manuel *et al.*, 1996). The use of depth integrated sampling by oblique or vertical net tows, such as was used to investigate scallop larvae on a large horizontal spatial scale (Tremblay and Sinclair, 1988), is an option to eliminate any vertical influences . Any vertical migrations which do occur are then assumed to have no effect on the ultimate horizontal position of the larvae. While these tows will capture representatives from all depths, they provide no data on depth distribution, a particular problem in stratified systems (Tremblay and Sinclair, 1990a; Raby *et al.*, 1994) where information on horizontal dispersal is required. Again the retrieval of stained larvae was not done to study vertical movements in the water, although this technique is well suited for further research in this area. The sampling protocol was altered after it was realized that the towing protocol in 1994 possibly missed aggregations of bivalve larvae due to the stratified plankton tows. Therefore oblique towing regimes were adopted for 1995.

It is important to note that regardless of when the stained veligers were found they were recaptured at all the stations sampled (Tables 1 and 2). Therefore, current patterns and wind effects were dispersing the larvae around the entire lease. The detection of the stained veligers at the inlet site also suggests that a percentage of the released larvae were

being lost from the Charles Arm system, but the proportion is not known. Stained veligers were also recaptured within Charles Arm up to 43 d after release (Table 1a). This provides some evidence for the retention of released veligers. What is not clear was the percentage of released veligers that was retained within Charles Arm. Therefore, enhancement may be achieved and the negative results at Charles Arm pertaining to Dabinett and Couturier (1994) may have been due to poor placement of collector bags, or that too few larvae were released in those trials. If the percent survival of larvae is small, it would not be until hundreds of millions of larvae are released and retained, that one would find a significant increase in giant scallop settlement.

Growth rates under *in situ* conditions were determined for each batch of stained released larvae. Rates of larval development for giant scallops have been derived from laboratory studies conducted at 12-15°C (Culliney, 1974; Tremblay,1988). During larval growth, the shell increases and changes shape, paired eyespots and a foot develop and at about 240 to 300 µm settlement and metamorphosis occur. Settlement usually occurs 30 to 40 d post-fertilization, which implies a growth rate of 5-6 µm/d. The 1994 results indicated (Figure 5) that growth rates were 3.57 and 3.85 µm/d. However, in 1995, the growth rate for the first release (9.72 µm/d) was much faster than that for the second release (2.52 µm/d) and that in 1994. This not only indicates a seasonal difference in the growth rate of larval giant scallops but also differences from year to year. These differences may be attributed to changes in temperature or food conditions. It may also be due to differences in gamete quality from batch to batch.

Feeding rates of adult giant scallops have been correlated with temperature and also with food abundance in some habitats (MacDonald and Thompson, 1986). The effect of temperature in regulating the rate of larval growth and development is known through laboratory experiments on a wide variety of invertebrate larvae (Scheltema, 1986). The quality and quantity of food was demonstrated long ago to affect larval growth (Davis and Guillard, 1958). Data collected from the Seabird (CTD) during 1994 and 1995 can help to explain these differences.

The growth rates of scallop larvae from each release did not differ greatly in 1994. Temperature plots for the release and middle stations (Figure 6 a and c) indicated that by the time the larvae were released from the first batch on July 27, surface temperatures had reached a maximum of around 18°C but remained there for only a short period. The temperature dropped to 16°C in early August and remained there throughout the sampling season, including the time the larvae from the second release remained in the water column. Water temperatures did increase over the summer and remained greater than 14°C (Figure 6 a and c), even at the depths of the observed chlorophyll-*a* maximum (Figure 7 a and c). Chlorophyll-*a* concentrations remained high after the initial bloom during the last week in July. Overall, the larvae in the water column during the 1994 study period were subjected to similar physiological conditions and subsequently, growth rates remained similiar.

Observed growth rates for larval release trials in 1995 were different from each other and also from those of 1994 (Figure 5 a and b). Maximum temperatures observed at

the release and middle stations (Figure 6 b and d) were 14 and 18°C, respectively, when the first release of larvae was attempted on August 10, and remained high throughout the period in which these larvae remained in the water column. Chlorophyll-*a* concentrations had also peaked during this time (14 µg/L and 10 µg/L) at the release and middle stations, respectively. These concentrations were also higher than those observed during 1994 (Figure 7 a and c). The observed temperatures and high chlorophyll-*a* concentrations may explain the higher growth rate of 9.72 µm/d. These two parameters also explain the slower growth rate of 2.52 µm/d observed for the second release trial on August 28, 1995. Although chlorophyll-*a* concentrations of August 25, 1995 (second release trial) remained similar to those observed on August 10, 1995 and to those of 1994, temperatures had dropped and remained below 13°C. This was much lower than the temperatures observed during 1994 and during the first release trial of 1995. Therefore, it seems that temperature plays a significant role in the determination of growth of scallop larvae.

The estimated average growth rate of field-caught larvae from straight-hinge to late pediveliger was estimated to be 2.77 to 3.37 µm/d by Parsons (1994) but did range from 0 to 7.14 µm/d between successive spawning periods. Laboratory studies have reported growth rates ranging from 1.3 to 7.2 µm/d (Culliney, 1974; Couturier, 1986; Hurley *et al.*, 1987; Gallager *et al.*,1996; Manuel *et al.*, 1996)

Since food abundance and temperatures are so important for growth and survival, it is possible that the growth rate differences reported here are a direct result of the conditions present at time of release and over the pre-settlement period. This raises

several points in any monitoring or enhancement study. When monitoring the stages of larval growth in the natural environment in order to predict spatfall, it would be prudent not only to measure the size of the bivalve larvae in question but also to measure characteristics of the water from which they are retrieved in order to determine if optimal conditions are present. With any enhancement study the release of the larvae should be timed with favorable conditions in the environment. Evidence of loss of larvae from the site, assuming that stained larvae found at the Inlet station are being transported out of the area during ebb tides, indicates that very large numbers of D-veligers may have to be released in order to cause significant increase in spat collection.

CHAPTER 3

Abundance and distribution of bivalve larvae in Charles Arm, Newfoundland

3.1 Introduction

Bivalve larval dispersal can be addressed from a number of different perspectives and on a variety of spatial and temporal scales. Depending on the species, the time in which dispersal occurs can be several minutes, over a tidal cycle, or months (Scheltema, 1986).

Literature debating whether bivalve larvae, because of their relatively high density, will generally act as inert particles or whether they can actually demonstrate some control over their vertical distribution at very low swimming speeds (probably <1.0 cm/s) has been reviewed by Mann (1986). More recent studies by Gallager *et al.* (1996) and Manuel *et al.* (1996) indicated that giant scallop veligers do control their vertical distribution in the water column and that this movement is a function of light, food, and temperature stratification. The distribution of giant scallop larvae has been assumed to be a simple function of surface current, direction and speed (Posgay, 1979). Boicourt (1982) described how horizontal currents clearly dominated larval transport because daily transport distances of the order of kilometers per day are commonplace in coastal or estuarine systems. Tremblay and Sinclair (1988) reported that no local aggregation can be self-reproducing, with the possible exception of Georges Bank where a semi-persistent gyre may retain the spawning products long enough for them to complete development and settle. A later study by Robinson *et al.* (1992), postulated that the northern part of Passamaquoddy Bay, in the Bay of Fundy, acts as a larval nursey area for the scallop

population in the area, whereby, a link between the oceanography of the area and the early life history patterns of scallops was demonstrated.

Eddy-diffusion, the random dispersion of suspended particles that results from turbulent flow, is also involved in larval dispersal. This horizontal movement can be measured on the sea surface by the change in mean distance among freely drifting objects. The rate of eddy-diffusion and the consequent dispersion of larvae from one another, as well as the variance in duration of planktonic development, will determine how many larvae of the same cohort (i.e., originating from the same spawning episode) will remain together in the plankton and subsequently settle together in one place (Strathmann, 1974). Hudon and Fradette (1993) also demonstrated the importance of wind-induced advection with their field study of larval decapod dispersal.

Active aggregation by giant scallop larvae in the area of the thermocline is a distinct possibility (Tremblay and Sinclair, 1988; Gallager *et al.*, 1996) although, on Georges Bank, larvae appear to be distributed as passive particles in areas of low stratification and highly aggregated above or within the pycnocline in stratified areas (Tremblay and Sinclair, 1990b). Mesocosm simulations conducted by Silva and O'Dor (1988), Gallager *et al.* (1996) and Manuel *et al.* (1996) reported diel vertical migration in all cases. Scrope-Howe and Jones (1986) observed that bivalve larvae move into the chlorophyll maximum during the night while the mesocosm studies indicated that giant scallop larvae move close to the surface during this time. With many parts of the ocean and coastal regions exhibiting marked differences in rate and direction of currents related

to depth, the vertical position of larvae in the water column may have important consequences for the rate and direction of their horizontal dispersal.

Active vertical migration and local current patterns may determine the locations of giant scallop larvae within Charles Arm. Spatial patterns have closely reflected known oceanographic properties (Robinson *et al.*, 1992) and may aid in determining if artificially produced giant scallop larvae, released in Charles Arm, may be entrained there.

3.2 Objectives

To determine: 1) growth rates of bivalve larvae under natural conditions; 2) if different aggregations of bivalve larvae (mussel, scallop, etc.) exist within Charles Arm; 3) to see if the greatest concentration of bivalve larvae is located at the end of Charles Arm, i.e., near the release station of the larval sampling in the mark and recapture study; 4) to identify possible circulation patterns within Charles Arm; and 5) to use wild aggregations of larvae to help predict the fate of artificially enhanced aggregations of larvae produced using quasi-hatchery techniques.

3.3 Methods

Study site

See Chapter 2 for details.

Experimental design

In order to examine temporal and spatial variability in bivalve larvae, triplicate oblique tows were performed at the three sampling stations (Figure 4b, Chapter 2) over the study period by the same methods as outlined in Chapter 2. Samples were taken on the ebbing tide shortly after high tide (except on August 19 and 28, 1995, when sampling was on the flood tide shortly before high tide) to reduce the effect of other tidal influences.

Microscopic examination

Preserved samples were filtered onto a 50 μm screen and resuspended in 200 mL of 2 μm filtered seawater. Triplicate 1 mL subsamples were then counted for larval abundances. Larvae in 1 mL subsamples were also sized using a inverted phase microscope fitted with an ocular micrometer.

Data analysis

Unpaired Student's t-tests were performed on the data to identify any differences in the mean size of bivalve larvae found at each sample station for the particular sample date over the sampling season. These tests were performed using Jandel Scientific's Sigmaplot Version 2.0. Size class frequency histograms were also constructed and cohorts separated such that mean size could be calculated. Growth rates were determined by dividing the differences in mean sizes by the days between sample dates. To determine minimum sizes of bivalve larvae from the first natural spawning episode it was assumed that 3 μm/d growth would have occurred (Parsons, 1994). Abundances of bivalve larvae were calculated by

$$\text{Number of larvae} \cdot L^{-1} = \frac{\text{Number of larvae in a tow}}{\{\text{volume of tow } (m^3) \div 1000 \text{ L}\}}$$

where, Volume = Distance of tow (m) × area of mouth of net (m^2).

The results were also plotted for each sample station over the sampling period and single factor ANOVA and student t-test performed on the data to see if any difference in numbers of bivalve larvae existed within Charles Arm at the three sampling sites. All statistical analyses were performed using Microsoft Excel Version 5.0c.

Deployment of drift bottle drogues (surface currents)

Javex bottles were filled with sand so that the bottle would float with only 1 to 2 cm of the bottom visible when placed in seawater. Sets of 20 bottles were colored with a fluorescent paint so that different releases would be distinguishable. Three releases were completed at the end of Charles Arm, two during the flood tide (July 6 and September 30, 1995) and one during ebbing tide on July 20, 1995. Two other drift bottle surveys were completed near the mouth of Charles Arm on July 18 and August 10, 1995. These were on the ebb and flood tides, respectively. Base maps were constructed by photocopying a nautical chart of the study area and converting the images to bitmap files using a computer scanner. Paint Shop Pro version 3.12-32 was used to create maps for the surveys.

Current meters

Six Interocean Systems, Inc. Model S4 current meters were placed at different positions within Charles Arm (Figure 4b, Chapter 2) on June 13, 1995 and retrieved on October 10, 1995. All meters were supported by weighted aluminum frames which were lowered to the bottom, allowing the meter to be suspended ~1 m from the bottom. These positions held the current meter at a depth of 5 m for current meters (CM) 1 and 2d, 4 m for CM 3, 10 m for CM 4 and 12 m for CM 5. Current meter 2s was attached to a mussel

headrope and suspended 1m below the surface. This meter rose and fell with the tide, allowing it to remain 1m below the surface. Data on temperature and current speeds were obtained for the study period.

3.4 Results

Bivalves had spawned on or before Aug 12, 1995 (Figure 8) with another, smaller, spawn occurring early September (Figure 9). There were significant differences in larval abundance among the sample stations on all sampling dates (ANOVA, $F_{(2,26)} = 384.027$, 9.324, 28.646, 15.012, 8.645, and 61.940 for August 12, 19, 28 and September 7, 21 and 30, respectively, $p<0.05$). Numbers of bivalve larvae were highest during early August with up to 23 larvae/L found at the release site, which was significantly greater than numbers found at the middle and inlet sites (t-test, d.f. = 12, t-value = 21.377, $p<0.05$ and d.f. = 13, t-value = 22.048, $p<0.05$, respectively). Within one week abundance had dropped at the release site (23.2 to 2.8 larvae/L). At this time (August 19) the middle station had a significantly greater abundance of larvae/L (3.6 larvae/L) than at the release staion or the inlet station (3.0 larvae/L) (t-test, d.f. = 16, t-value = -4.896, $p<0.05$ and d.f. = 15, t-value = 2.861, $p<0.05$, respectivley). When abundance increased slightly on September 7, 1995 a significant difference was observed between the

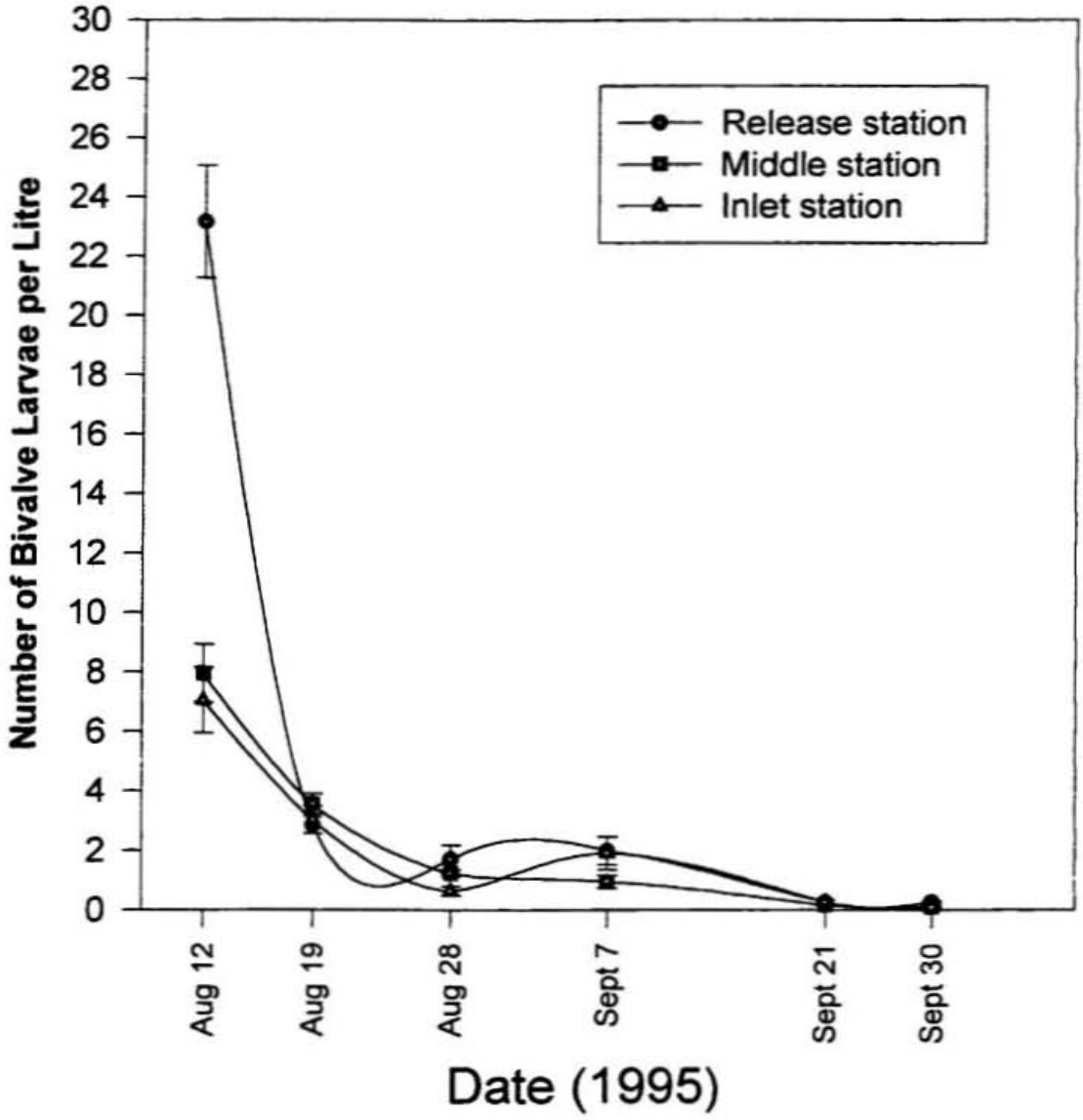

Figure 8: Average number of bivalve larvae per litre collected from three sampling stations (release, middle and inlet) in Charles Arm during the two month sampling period in 1995. Values are means of triplicate samples with vertical bars representing standard deviation.

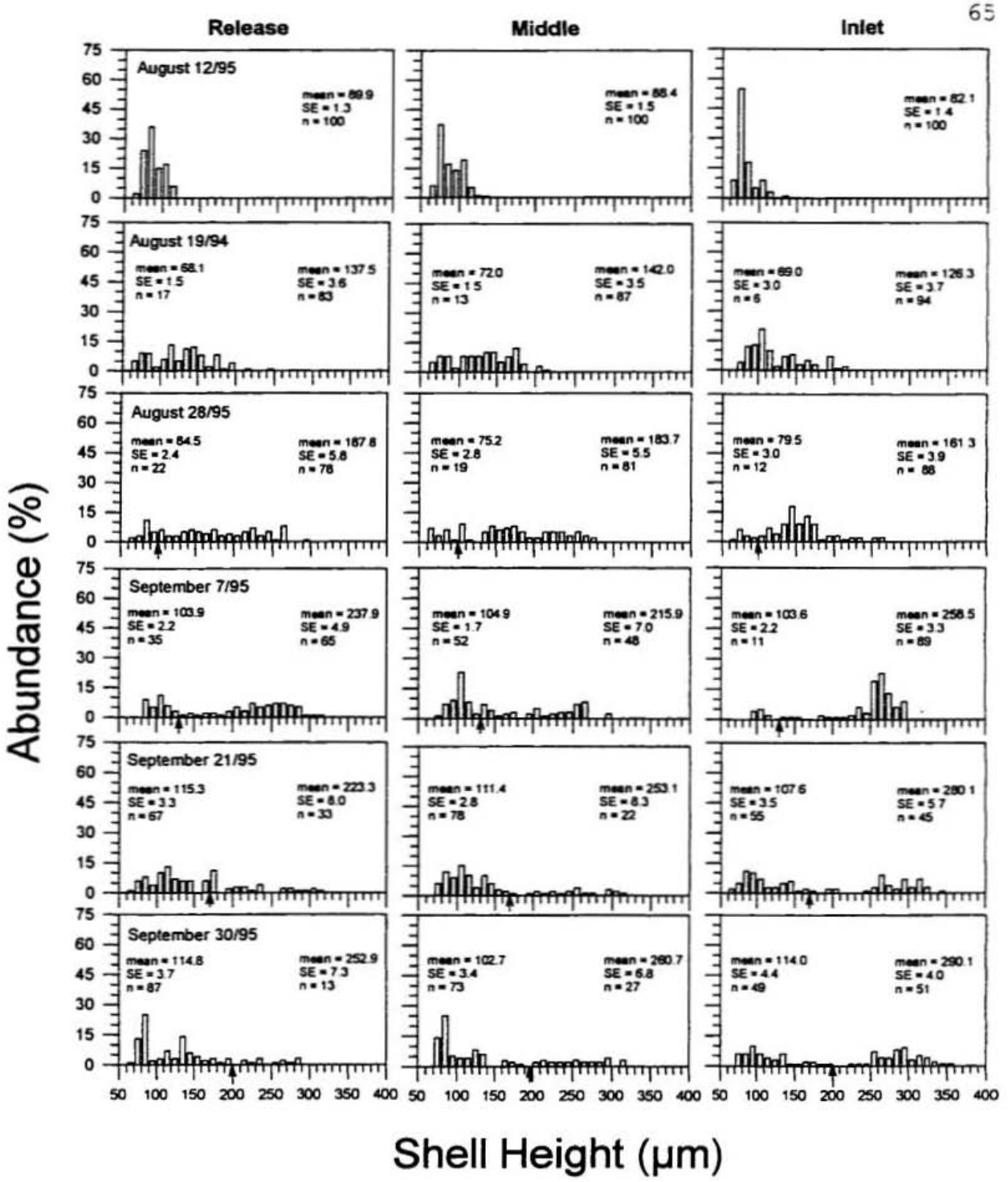

Figure 9 : Shell height frequency distributions of bivalve veligers found at the three sample stations in Charles Arm throughout the sampling period in 1995. (SE = standard error, n = number of larvae measured). Arrows indicate where cohorts are assumed to be separate.

abundance at the release station (2.0 larvae/L) and the middle station (1.0 larvae/L) (t-test, d.f. = 11, t-value = 5.922, $p<0.05$), and also between the inlet station (1.9 larvae/L) and the middle station (t-test, d.f. = 10, t-value = -4.710, $p<0.05$), while no significant difference was observed between the release and inlet stations ($p>0.05$).

Shell heights of bivalve veligers ranged from 60 µm to 360 µm and the mean shell height ranged from 68.1 to 290.1 µm (Figure 9). The temporal pattern of bivalve larval abundance consisted of two cohorts. The first was observed on August 12, and the second, on August 28, with both identifiable by the increase of smaller (less than 120 µm) bivalve larvae. During the sampling periods separation of the cohorts was not distinct. A 3 µm/d growth rate was assumed based on Parsons (1994) observed growth rates of 2.77-3.37 µm/d. Based on this assumption, separation of the cohorts was estimated by calculating the minimum expected size of the smallest larva of the previous sampling period. It is also acknowledged that these growth rates were observed for giant scallop larvae and that all bivalve larvae are sampled here.

Larval abundance was initially high and shell height low. Over the sampling period the abundance decreased while average shell height increased.

Mean shell heights at the release and middle stations were significantly different from the mean shell height of the inlet site on August 12, 1995 (t-test, d.f. = 198, t-value = 4.170 and 3.073 respectively, $p<0.05$). No significant difference was found between mean larval shell heights on August 19 and 28 ($p>0.05$), but all stations were different from each other on September 7 ($p<0.05$) with the inlet site having the highest mean shell

height. On September 21, the mean shell heights were similar between the release and middle stations (t-test, $p>0.05$), although they were significantly higher than the mean shell height observed at the inlet station (t-test, d.f. = 198, t-value = -3.097 and -3.776, respectively, $p<0.05$). During the last sampling period on September 30, there was no difference between mean shell heights at the release and inlet stations (t-test, $p>0.05$), but recaptured larvae from these sites had significantly greater mean shell heights than that observed at the middle station (t-test, d.f. = 198, t-value = -6.487 and -4.840, respectively, $p<0.05$).

Growth rates were higher for cohorts of the August 12, 1995 event then for cohorts of the second event on August 28 (Tables 3 and 4). Overall growth rates for the August 12 cohort ranged from 5.7 µm to 6.2 µm/d (Table 3). Growth rates also decreased over the sampling period for the August 12 cohort, with the exception of the inlet station. Average growth rates for the August 28 cohort ranged from 1.3 µm to 1.6 µm/d (Table 4).

Table 3: Summary of growth rates (µm/d) of bivalve larvae in Charles Arm during the 1995 sampling season for the first spawning episode around August 12, 1995. Values are estimates based on a change in mean cohort size.

RELEASE

Date	Aug 12	Aug 19	Aug 28	Sept 7	Sept 21	Sept 30	
Aug 12	-	6.8	6.1	5.7	-	-	
Aug 19	-	-	5.6	5.3	-	-	
Aug 28	-	-	-	5.0	-	-	
Sept 7	-	-	-	-	setting	-	
Sept 21	-	-	-	-	-	setting	
Sept 30	-	-	-	-	-	-	Overall Average
Average		6.8	5.9	5.3			6.0

MIDDLE

DATE	Aug 12	Aug 19	Aug 28	Sept 7	Sept 21	Sept 30	
Aug 12	-	7.7	6.0	4.9	-	-	
Aug 19	-	-	4.6	3.9	-	-	
Aug 28	-	-	-	3.2	-	-	
Sept 7	-	-	-	-	setting	-	
Sept 21	-	-	-	-	-	setting	
Sept 30	-	-	-	-	-	-	Overall Average
Average		7.7	5.3	4.0			5.7

INLET

DATE	Aug 12	Aug 19	Aug 28	Sept 7	Sept 21	Sept 30	
Aug 12	-	6.3	5.0	6.8	-	-	
Aug 19	-	-	3.9	7.0	-	-	
Aug 28	-	-	-	9.7	-	-	
Sept 7	-	-	-	-	setting	-	
Sept 21	-	-	-	-	-	setting	
Sept 30	-	-	-	-	-	-	Overall Average
Average		6.3	4.5	7.8			6.2

Table 4: Summary of growth rates (μm/d) of bivalve larvae in Charles Arm during the 1995 sampling season for the second spawning episode around August 28, 1995. Values are estimates based on a change in mean cohort size.

RELEASE

Date	Aug 12	Aug 19	Aug 28	Sept 7	Sept 21	Sept 30	
Aug 12	-	-	-	-	-	-	
Aug 19	-	-	1.8	1.9	1.4	-	
Aug 28	-	-	-	1.9	1.3	-	
Sept 7	-	-	-	-	0.81	-	
Sept 21	-	-	-	-	-	-	
Sept 30	-	-	-	-	-	-	Overall Average
Average			1.8	1.9	1.2		1.6

MIDDLE

DATE	Aug 12	Aug 19	Aug 28	Sept 7	Sept 21	Sept 30	
Aug 12	-	-	-	-	-	-	
Aug 19	-	-	0.4	1.7	1.2	-	
Aug 28	-	-	-	3.0	1.5	-	
Sept 7	-	-	-	-	0.5	-	
Sept 21	-	-	-	-	-	-	
Sept 30	-	-	-	-	-	-	Overall Average
Average			0.4	2.4	1.1		1.3

INLET

DATE	Aug 12	Aug 19	Aug 28	Sept 7	Sept 21	Sept 30	
Aug 12	-	-	-	-	-	-	
Aug 19	-	-	1.2	1.8	1.2	-	
Aug 28	-	-	-	2.4	1.2	-	
Sept 7	-	-	-	-	0.3	-	
Sept 21	-	-	-	-	-	-	
Sept 30	-	-	-	-	-	-	Overall Average
Average			1.2	2.1	0.9		1.4

A counterclockwise circulation pattern was evident in the end (release station area) of Charles Arm (Figure 10a, b and c) during both flood and ebb tides. Wind effects were also evident even though winds were very light during the release and further observation of the drift bottles. Drift bottles tended to travel in the direction of the wind (Figure 10a and c), although a circular current prevailed and some bottles did leave the open area in the end of the Arm. Bottles leaving the open area began travelling back to the end of the arm once the tide turned (Figure 10a). Many small eddies were also evident in the small coves in the end of the Arm (Figure 10b). Drifters in the mouth of Charles Arm (inlet area) during ebb tide travelled out of Charles Arm and then westward into the larger Notre Dame Bay (Figure 10d). Drifters released during the flood tide indicated surface currents moving toward the end (release station area) of Charles Arm, with eddies occurring around points along the shore (Figure 10e). Estimated average surface current speeds were 5.0, 5.2 cm/s (Figure 10a and c) and 3.6 cm/s in the end of Charles Arm and 7.7 cm/s in the narrows (Figure 10b). At the mouth of Charles Arm averages of 8.1 cm/s (Figure 10d) and 4.8 and 6.5 cm/s were observed depending on the drifter's route (Figure 10e).

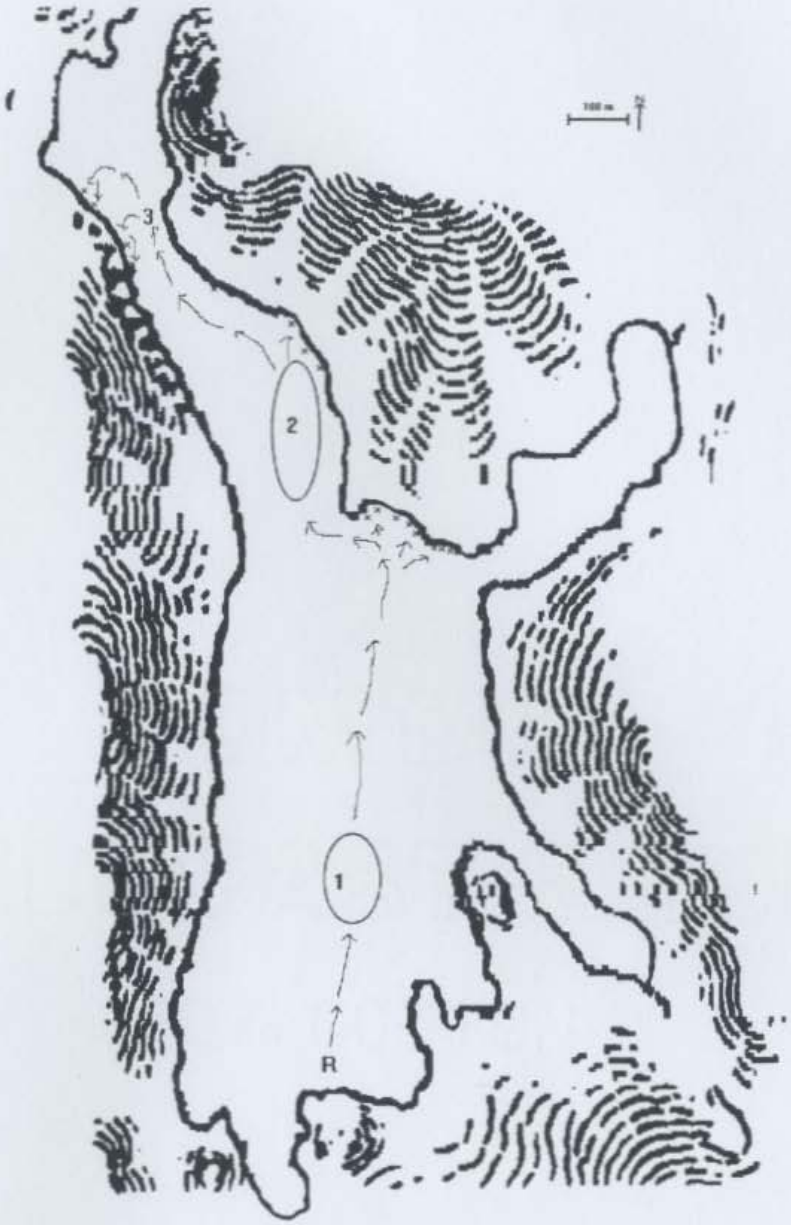

Figure 10a: Surface current map of the end of Charles Arm. Arrows indicate the direction of the current drogues. R = release area of the drogues at 06:45 on July 6, 1995 (low tide). Times indicated were: 1 = 10:15, 2 = 13:00, 3 = 15:00 and all drogues ashore at 15:50. Circles encompass the area of the drogues at the times surveyed. Winds = light to moderate southwest. High tide @ 13:52 and Low tide @ 20:12.

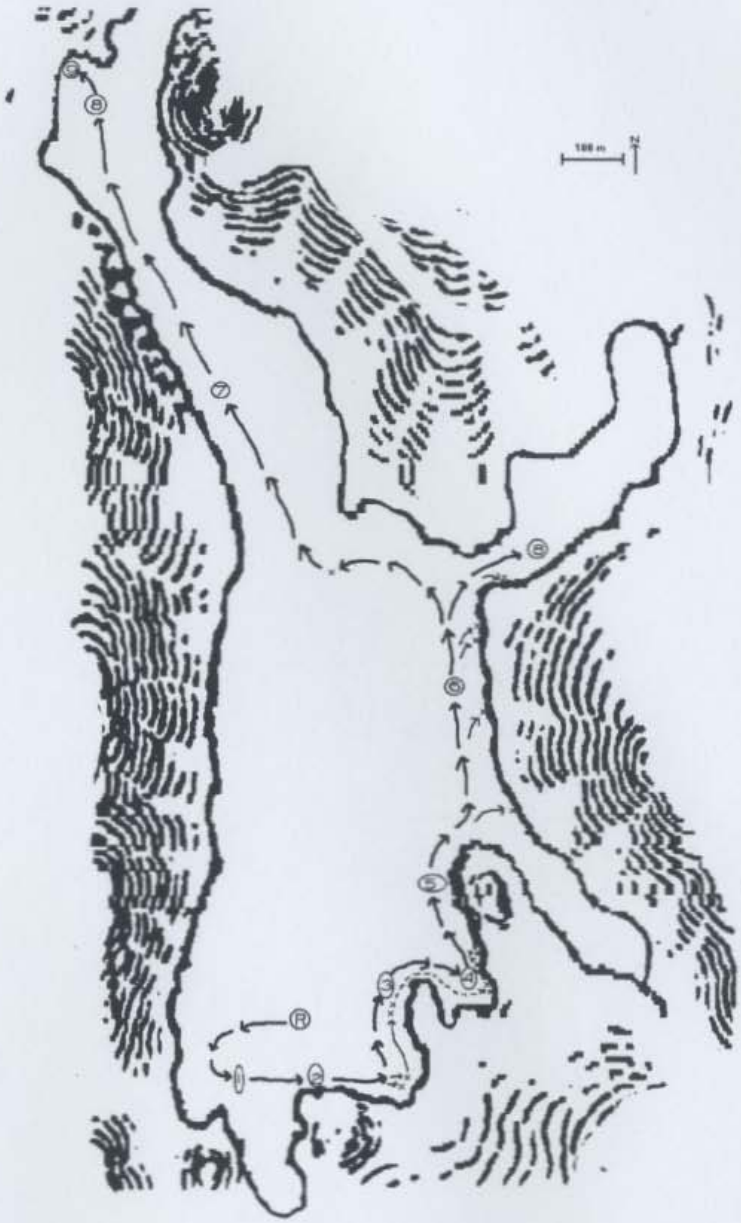

Figure 10b: Surface current map of the end of Charles Arm. Arrows indicate the direction of the current drogues. R = release area of the drogues at 14: 05 on July 20, 1995 (High tide). Times indicated were: 1 = 15:10, 2 = 16:24, 3 = 17:35, 4 = 18:15, 5 = 21:40, 6 = 12:45 (July 21), 7 = 13:45, 8 = 14:50 and 9 = 17:35. Circles encompass the area of the drogues at the times surveyed. Winds = no wind. Low tide @ 20:27, High @ 02:32, Low @ 08:37 and High @ 15:02.

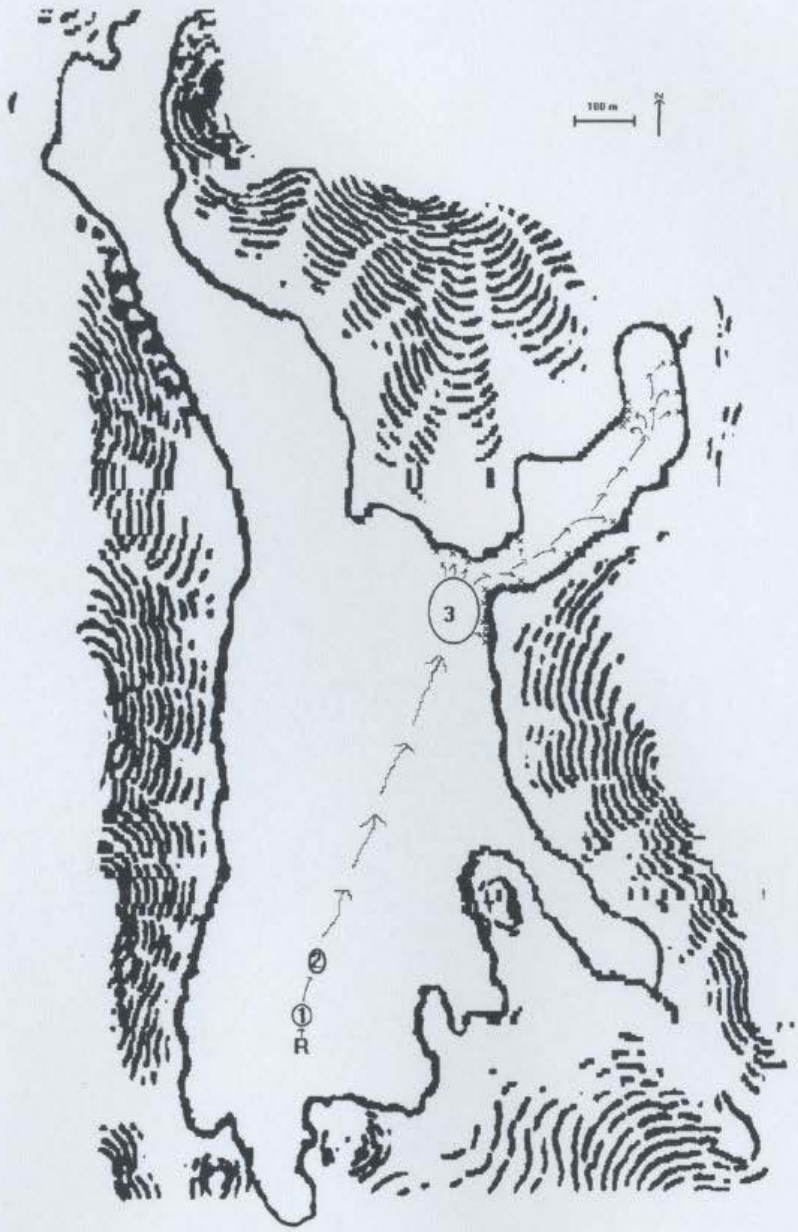

Figure 10c: Surface current map of the end of Charles Arm. Arrows indicate the direction of the current drogues. R = release area of the drogues at 07:00 on September 30, 1995 (low tide). Times indicated were: 1 = 07:18, 2 = 08:00, 3 = 10:00 and 11:30 the drogues were ashore. Circles encompass the area of the drogues at the times surveyed. Winds = very light southwest switching to mild-moderate southeast after 08:00 survey. High tide @ 11:57.

Figure 10d: Surface current map of the Inlet of Charles Arm. Arrows indicate the direction of the current drogues. R = release area of the drogues at 12:50 on July 18, 1995 (High tide). Times indicated were: 1 = 13:25, 2 = 14:15, 3 = 14:50, 4 = 15:20, 5 = 15:35 and 6 = 16:00. Circles encompass the area of the drogues at the times surveyed. Winds = none to very light southerly. Low tide @ 18:32.

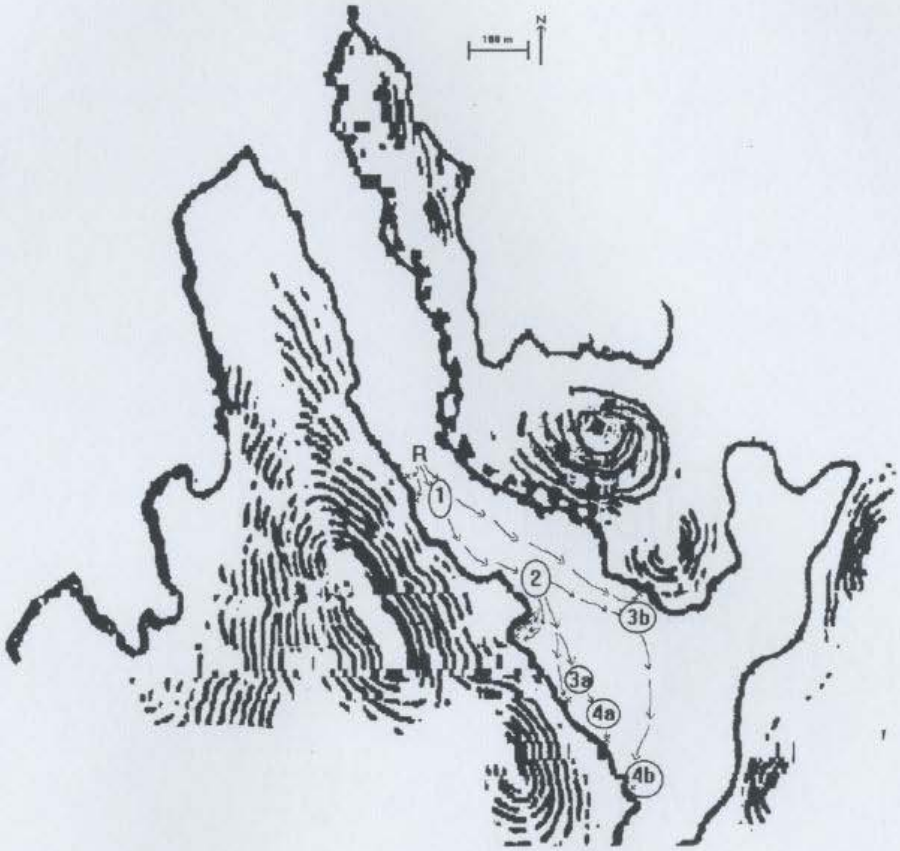

Figure 10e: Surface current map of the Inlet of Charles Arm. Arrows indicate the direction of the current drogues. R = release area of the drogues at 14:30 on August 10, 1995 (Low tide). Times indicated were: 1 = 14:40, 2 = 15:10, 3 = 16:55, and 4 = 17:40. Circles encompass the area of the drogues at the times surveyed. Winds = none to very light northwest wind. High tide @ 20:17.

Temperatures at the current meter stations increased over the study period with maxima of 18-19°C occurring at site 1 to 3 (mouth and middle of Arm), during early to mid August (Figure 11). Temperatures reached an early peak of around 10°C in late June but then dropped back to 4°C. Temperatures at site 2s (Surface current meter) were 6 to 7°C higher than all other sites until mid-July. Sites 4 and 5 (end of Arm) illustrated a gradual increase in temperature over the study period to a maximum of around 11°C.

Current speeds near the mouth of Charles Arm (CM1) reached a maximum of 48.4 cm/s with a mean of 6.21 cm/s. The surface current meter showed similar results to CM1, but illustrated that current speeds were faster than those recorded from the current meter near bottom (CM 2d), with maximum speeds of 28.9 cm/s and a mean value of 4.21 cm/s. Current speeds in the end of Charles Arm were reduced to mean values of 2.37 and 0.89 cm/s (CM4 and CM5, respectively). Maximum speeds never exceeded 12.6 cm/s (Table 5).

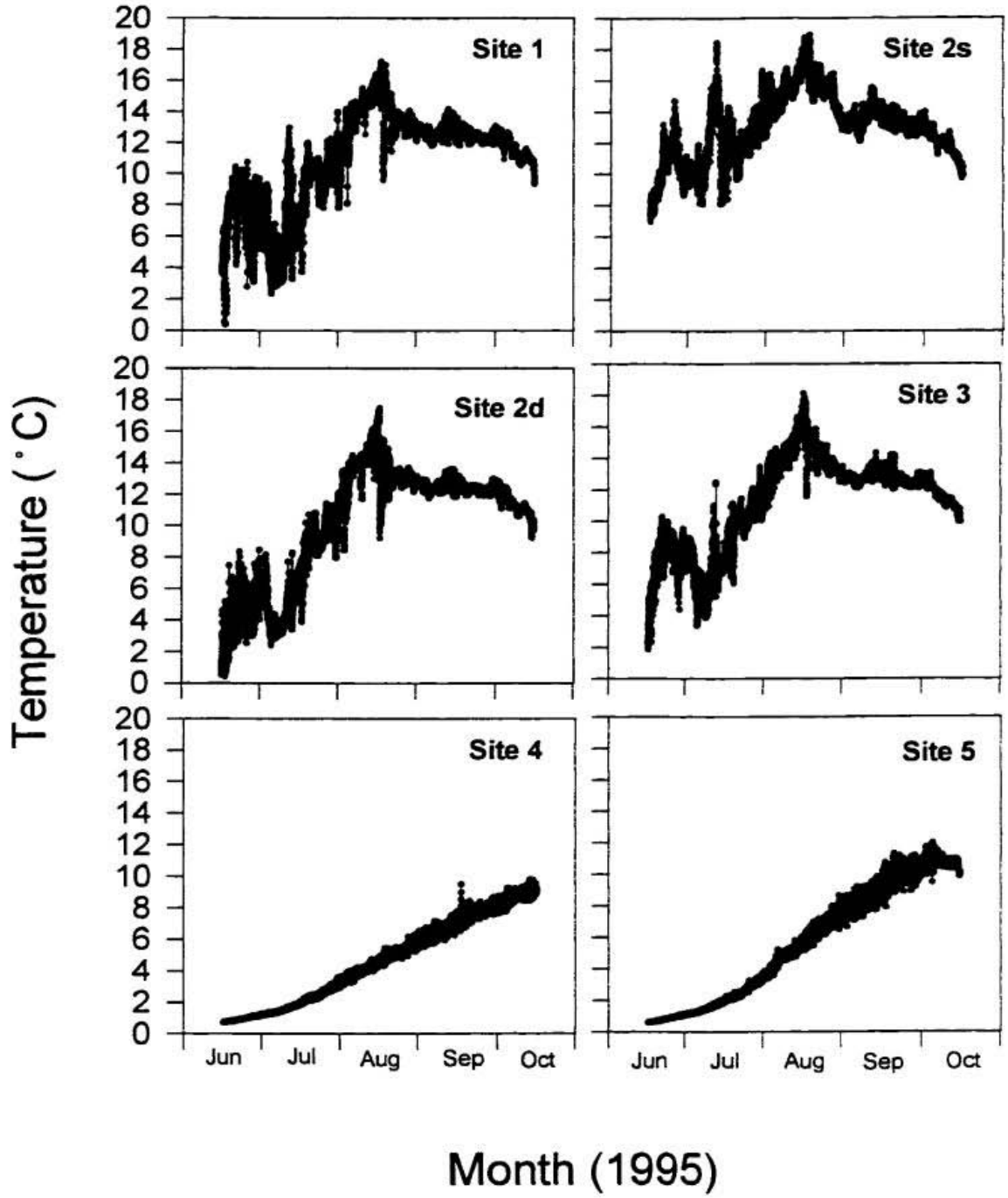

Figure 11: Temperature profiles in Charles Arm. Data collected from the current meters over the sampling period. Site 1 = inlet station, site 2s and 2d = 25 hour station, site 3 and 4 = middle station and site 5 = end of Arm (release station).

Table 5: Summary of current speed data obtained from the current meters placed within Charles Arm from June 13 - October 10, 1995. Values are in cm/s.

Statistic	Current Meter Site					
	1	2s	2d	3	4	5
Mean	6.21	6.20	4.21	7.57	2.37	0.89
Standard Error	0.06	0.05	0.04	0.05	0.01	0.01
Minimum	0.0	0.0	0.0	0.0	0	0
Maximum	48.4	44.4	28.9	39.5	12.6	9.2
95% Confidence Level	0.12	0.10	0.08	0.10	0.02	0.02

3.5 Discussion

Monitoring of spawning events can be achieved by measuring larval occurrences (Barber and Blake, 1991) and is especially effective if size frequency distributions are also estimated (Parsons, 1994). In Charles Arm, larval abundance was greatest (Figure 8) on August 12 when shell height was least. This infers that a recent spawning event occurred around August 12 because shell heights were in the 60-120 μm range (Figure 9). Abundance was in the order of 23 larvae/L at the release station (end of arm), whereas only a third of that number was found at the middle and inlet stations (8 and 7 larvae/L, respectively). The following week (August 19) all three stations showed equal abundance. This may indicate that the first spawning event (possibly mussels) occurred at the end of Charles Arm and the majority of larvae were staying within the area while the remainder were finding their way out of the system. It may also be argued that the spawning bed of giant scallops and/or mussels is outside the Charles Arm system and larvae are being transported into the area and being aggregated within the end of Charles Arm.

Previous work on monitoring of mussel larvae has been carried out in Charles Arm (Penney, 1993). Data from this study (1995) are similar to those recorded by Penney (1993) in 1991, but very different to those for 1990. Penney (1993) noted that in 1990 plankton tows at 1 m depth revealed that mussel veligers were already present in high numbers (greater than 40/L) by the late June. Two other peaks were recorded in mid-July and early August with abundances less than 20 larvae/L. These peaks were composed of

setting size larvae (greater than 250 μm) and it was argued that these larvae were from the early spawn and were aggregating in the surface water to set. During this study regular plankton tows were not started until the release of stained larvae on August 12, and there were still low numbers of larvae in the water. A series of tows on July 25 indicated low numbers of larvae (less than 1/L) and qualitative observations on gonads of both mussels and scallops suggested that no spawning event had occurred by this date in 1995 (T. Mills, pers. comm.).

In 1995, another influx of larvae or spawning event occurred around August 28 because of the increase in abundance of large numbers of D-stage bivalve larvae less than 100 μm in shell height (Figure 9). Data from 1995 were also similar to that observed by Penney (1993) in 1991. Penney (1993) reported that the first plankton tows were conducted in mid-June but veligers were not observed until August with a peak of 200 veligers/L recorded on August 12. In the present study, there was a 3-fold decrease in abundance from August 12 to August 19 (Figure 8) therefore abundances may have been much higher if tows had been completed the week previous to August 12. The decreases in abundance may be a result of several factors. Firstly, since predators are present in the water, predation and natural mortality could account for decrease in numbers. Secondly, larvae also may be transported to inhospitable environments, i.e., high turbulence, washed ashore, etc. Thirdly, larvae may be transported out of the system due to water currents and tides although the fact that more larvae were usually observed within the release area seems to indicate an aggregation of larvae in this area. Finally, larvae may be exhibiting

depth regulation and aggregating below the maximum depth of 7 m which the plankton tows did not sample.

Debate continues on whether bivalve veligers are passively drifting particles in the water or capable of active aggregation in water currents. Penney (1993) confirmed extreme variability in depth distributions of planktonic mussel veligers in Charles Arm, and he demonstrated a significant depth relationship to proportion of mussel veligers greater than 250 μm. Those mussel veligers greater than 250 μm tended to increase from bottom to top of the water column, which may indicate an affinity for near surface depths by settlement size larvae. Tremblay and Sinclair (1990a) found that in mixed areas of the water column on Georges Bank scallop larvae were distributed evenly but in stratified waters larvae were concentrated above the pycnocline. In areas where the pycnocline was well developed the differences in the centre of mass of the larvae were associated with the differences in the position of the pycnocline. Scrope-Howe and Jones (1986) observed bivalve larvae moving into the chlorophyll maximum during the night, thereby exhibiting vertical migration. Therefore, veligers may not have been sampled in the present study because of this active vertical movement.

The shell height frequency graphs (Figure 9) of August 12, 19 and 28 indicate that small veligers were found throughout the sampling period, which may indicate some trickle spawning after the main episode or invection of larvae from outside the Charles Arm system. An alternate explanation is that there was large variation in the growth rate of veligers from a cohort. A further peak in larval abundance around August 28-

September 7 (Figure 8) and the presence of a bimodal distribution in shell height (Figure 9) indicated another spawning episode. This second episode was likely that of scallops and the first episode of mussels which is typical of this site (T. Mills, pers. comm.). Thimble Bay Farms is a giant scallop and blue mussel farm, so there are spawning populations of both species within the Charles Arm site. Because the bivalve larvae were not identified it is also possible that the peaks in larval abundance were representative of spawning episodes from bivalve populations outside of Charles Arm.

A third spawning event seems to have occurred around September 30 with larvae smaller than 100 μm making up almost 40% of the total number. Larval abundance was less than 1 larva/L and larvae were confined to the release and middle stations. This suggests that these new larvae are not from a source outside the Charles Arm system.

The majority of bivalve larvae represented in the September 21 and 30 larval samples were from the smaller size class and were less abundant than those sampled on August 12 indicating that larvae from the first spawning episode must be reaching and/or must have reached settling size and were settling out. The presence of larger larvae at the inlet site during September, which were sampled on the ebb tide, is evidence for the loss of pre-settlement larvae from the site. Larvae sampled on September 7, and estimated to be from the first cohort, have mean shell heights that were significantly different from each other. Larvae greater than 250 μm were observed at the release and middle stations on September 7 but mean shell heights were 237.9 and 215.9 μm, respectively. These areas have been suspected to be more suited to the settlement of mussel larvae and were

the areas of concentration for both mussel and scallop collectors within the lease. The inlet station was an area of high current from which larvae were presumably flushed out on ebbing tides (discussed later). The observed value of 258.5 µm mean shell height observed on September 7, 1995 at the inlet station may be further support for Penney's (1993) suggestion that settlement-size mussel larvae may have an increased affinity for the near surface layers, although the greatest densities occur in the subsurface depths. Settlement size mussel larvae in Charles Arm on September 7, 1995 may have aggregated near the surface and set in favorable areas, whereas others may have been transported out of the area by surface currents. In 1995, larvae were not of settlement size (greater than 250 µm) until early September. This is atypical of Charles Arm, where settlement size larvae usually occur in mid-July (T. Mills, pers. comm.; Penney, 1993), but it was very similar to reports from 1990 and 1991, when settlement was delayed until early August and early September, respectively (Penny, 1993).

Significant differences in the mean shell heights of larvae on August 12 and September 7 also indicate that the larvae were not randomly distributed but were being aggregated in certain areas, both horizontally and vertically. With the limited sampling stations and the lack of depth stratified tows in this study, comments on this would only be speculation. More research is needed on the active components of the forces controlling larval aggregation/dispersal within this area. In doing so, better predictions of spatfall and more efficient placement of collectors for both scallops and mussels would be achieved.

Estimated growth rates from the mean shell heights of the first cohort indicated

that they were similar for all three stations from August 12 to August 28 (Table 3). This was consistent with the growth rate determined by the release and recapture of stained larvae mentioned in Chapter 2. Differences in growth rates on sampling dates from August 12 to September 7 were observed. Average growth rates decreased from 6.8 to 5.3 µm/d for the release station, and from 7.7 to 4.0 µm/d for the middle station (Table 3). Growth rates of 6.3 to 7.8 µm/d for the inlet station showed an increase in growth but this is believed to be misleading due to the presence of settlement size larvae (discussed earlier). A more noticeable decrease in growth rate was observed in the larvae from the second spawn. Rates of 1.6, 1.3 and 1.4 µm/d were observed at the release, middle and inlet stations, respectively (Table 4). These slower growth rates were likely related to the water column characteristics that were determining the growth of the veligers found within them. Even though Parsons (1994) did not find any correlation between average growth rate and temperature, citing the fact that the temperature range was only 13.3 to 15.9°C over the larval planktonic duration, other laboratory studies show that temperature does have a significant effect on growth of scallop larvae (Hodgson and Bourne, 1988; MacDonald, 1988). Temperature profiles from the current meters (Figure 11) illustrated that temperatures were at the annual maximum (18-19°C) during mid August and then began to decline. By August 28, when the next cohort of veligers was present, temperatures had dropped almost 5°C to ~13°C at current meter sites 1 to 3. Current meters at site 4 and 5 were in deeper water (10-12 m) and the temperature profiles from current meters 4 and 5 reflected how the bottom water temperature gradually increased

over the summer from less than 1°C and was at a maximum of 12°C in mid-October when the meters were retrieved. It is not believed that larvae were subjected to these lower temperatures found at depth because plankton sampling at different depths in Charles Arm during 1990 and 1991 (Penney, 1993), revealed that larvae were generally most abundant in the top 7 m of the water column with few veligers (less than 10%) below this level. CTD temperature profiles (Figure 6, Chapter 2) of the release and middle stations showed that temperatures reached a maximum of 17-18°C in early August and by early September had decreased to 12-15°C. Essentially, slower growth of the second larval cohort appears to be related to cooler water temperatures.

Scrope-Howe and Jones (1986) observed that bivalve larvae moved into the chlorophyll-*a* maximum during the night. Raby *et al.* (1994) found that the vertical distribution of veligers was not related to chlorophyll-*a* except at night in stratified conditions. In these stratified systems, gut chlorophyll-*a* was associated with water chlorophyll-*a* concentration but in mixed waters larval gut content was similar at all depths. Chlorophyll-*a* maxima of 13 µg/L occurred in the release station in early August (Figure 7). It was during mid-August that chlorophyll-*a* maxima of 12 µg/L was observed at the middle station. The combination of high temperatures and chlorophyll-*a* concentrations would account for the high growth rates observed for both the stained larvae (Chapter 2) and for the larvae captured during the plankton tows. The decreased growth rates of the second cohort may again be explained by the decreased chlorophyll-*a* concentrations combined with lower temperatures at the release station, where values had

declined to less than 6 µg/L and remained low during the time these larvae were in the water. Interestingly, the chlorophyll-*a* concentration at the middle station peaked during late August and remained high for the rest of the study period. Although concentrations were high, temperature was low, thus it may be more critical for larvae to have good growing temperatures in the presence of high food levels. These higher concentrations of chlorophyll-*a* (greater than 6 µg/L) were also in much greater depths (greater than 9 m) during the time the second cohort was in the water column. If mussel larvae (Penney,1993) and other bivalve larvae remain in the upper water column it is likely that they do not avail of the higher food concentrations located at depth. Giant scallop larvae were observed by Gallager *et al.* (1996) and Manuel *et al.*, (1996) to remain above the thermocline regardless of the distribution of food. If a thermocline existed at depths less than 9m during the time giant scallop larvae were in the water column in Charles Arm then they were restricted from this food source and may explain the decrease in growth rates observed in larvae sampled during September 21 (Table 4).

Swimming speed is slow during the planktonic stage of scallop larvae (2mm/s) (Couturier *et al.*, 1995), although it is possible for the larvae to swim significant distances vertically in the water column (172.8m/d). Larvae are believed to be horizontally distributed by currents. Tremblay and Sinclair (1992) reported that the retention of scallop larvae on Georges Bank appears to be due to physical processes alone but . In this context, larval dispersal depends not only on the length of time the larvae are in the water column but also on the direction and speed of the currents to which they are subjected as well as

their vertical position in the water column (Manuel *et al.*, 1996). Figures 10a through 10e represent an attempt to map the current patterns of Charles Arm. At the end of Charles Arm it was observed that a counterclockwise circulation pattern (Figure 10b) with eddy currents that may trap some of the larvae present in the water column for a period of time. Although the presence of surface currents (Figure 10a) indicates that larvae do leave the end of Charles Arm and travel through the middle narrower area, larvae in the water column may be returned if they are within this area when the tide changes or by eddy currents if they are caught in the incoming current and returned to the end (release station area). Recaptured stained larvae were retained in this area for more than 30+ days and were of setting size when they were recaptured. Furthermore, stained larvae were also found in the inlet station area but not until a week after release (Table 2, Chapter 2). It was illustrated (Figure 10d) that any larvae in the water column of this area during ebbing tide would be transported out of the Charles Arm system and taken further down the bay. It is very unlikely that any of these exported larvae would find their way back to this lease. Subsequently, Figure 10e showed that larvae in the water column at the inlet station during flood tide would be transported into the Charles Arm system. Furthermore, the larvae would be transported along the western shore and up into the middle and possibly the end of Charles Arm.

Estimated surface current speeds from the drifters were similar to those observed from the current meters (Table 5). Current speeds above 6 cm/s were observed at CM 1, 2s and 3, 3-4 cm/s faster than greater depths. Most noticeable were the mean current

speeds (2.37 and 0.89 cm/s) from the current meters (CM 4 and 5, respectively) located in the end of Charles Arm. The vertical position of larvae in the water column therefore has an important consequence for the rate and possible direction of the horizontal dispersal with a slower dispersal expected for those larvae situated in the slower currents at depth. Depth related current speeds and direction are found in many parts of the ocean and coastal regions (Scheltema, 1986). Tremblay *et al.* (1993) suggest that the depth of larval drift is important because the strength of the around and cross-bank flows on Georges Bank varies with depth. Given that bivalve veligers do exhibit vertical control, it is reasonable to suggest that larvae deeper in the water column in the end of Charles Arm were subject to much slower current speeds than those at surface, thereby minimizing the likelihood of being flushed out of the system. Penney (1993) suggested that settlement size mussel larvae, which are normally concentrated at depth, come to the surface layers to set. Such behaviour may explain the results found during this study (Figure 9) if the majority of these larvae were mussels. The large numbers of settlement size larvae found at the inlet station on September 7 may represent those larvae that were entrained in the end of Charles Arm because they were deeper in the water column and were not subjected to currents that would possibly remove them from the system until they moved to the surface layers to find suitable substrates. Although a majority of larvae are found in subsurface waters (Penney, 1993), some larvae can be found in the top 1 m of water. Therefore, it is very likely that larvae in these surface currents would make their way out of the system.

The possibility does exist for enhancement of artificially produced aggregations of scallop larvae. Evidences exists for the entrainment of scallop larvae but results also indicate a loss of scallop larvae by direct current out of the Charles Arm system. A very intensive production of hundreds of millions of viable D-veligers would be required to produce the numbers necessary to augment spat collection significantly. Only a small percentage of released, artificially produced, scallop veligers would likely set on collectors for the following reasons: losses of larvae out of the system, natural losses by means discussed earlier (predation), and the probability of placing collectors in the areas where settlement will take place. The likelihood of retaining a greater number of released scallop veligers may be significantly increased by raising larvae to settlement size and then releasing them on site. This would decrease losses out of the system by reducing the amount of time the veligers are in the water column and thereby reduce the time that they are subjected to predation and the prevailing surface currents within Charles Arm.

CHAPTER 4

Tidal influence on quantatative sampling of bivalve veligers within Charles Arm

4.1 Introduction

The influence of diel and tidal rhythms can be a significant problem when interpreting zooplankton data from a time series of samples made at regular intervals over a day or longer (Omori and Ikeda,1984). Furthermore, if lunar or tidal effects are important then regular interval sampling of one week or month may miss important events over the tidal/lunar cycle. Tenfold variations in larval abundance can also be observed at one site within a single tidal cycle, and differences in abundance which are possibly diel have been observed previously (Levin, 1986; Newell *et al.*, 1991). Therefore, attempts to estimate larval availability should incorporate short-term tidal and diel variability, as well as time of day.

Nelson (1953,1955), cited in Mann (1986), observed that the distribution of oyster veliger larvae was affected by tidal currents, where in weak tides, larvae were found on both the ebb and flood tides in equal numbers, whereas in stronger tidal regimes larvae predominated on the flood tide. Studies on the distribution and transport of mesoplankton during tidal cycles (George, 1995) revealed that mollusc larvae were found in higher abundance during flood tide in the northern German Wadden Sea.

Reviewed literature by Mann and Wolf (1983) indicates that swimming speeds of bivalve larvae range from 0.17 to 10.0 mm/s. These speeds permit vertical positioning if larvae are able to respond to tidally induced cues such as changes in salinity, temperature, pressure or current velocities. Laboratory studies reported in Newell *et al.* (1991) indicate

tidally related responses of bivalve larvae to: salinity (Haskin, 1964), temperature (Mann and Wolf, 1983), and hydrostatic pressure (Bayne, 1963; Mann *et al.*, 1983).

In 1994, the Newfoundland Aquaculture Industry Association initiated a larval monitoring program to predict spatfall to assist mussel farmers in circumventing the low mussel seed supply problem by optimizing spat collection. Plankton tows were done at several farms around the province and from larval numbers and size-frequency distributions the best time was determined to place collectors in the water. To date there is no means of standardization with respect to tide levels.

If tidal state affects distribution of larvae then predictions at specific sites may be in error depending on sampling times. The variation in abundance of bivalve larvae over a tidal cycle adds a significant factor to any sampling regime. Monitoring programs that do not sample according to the tides may be inadequate. This variation in abundance which occurs over the tidal cycle will be investigated here.

4.2 Objective

To determine the variation in total bivalve larval density during tidal and diurnal cycles.

4.3 Methods

Sampling

Oblique plankton tows were completed at the following times: on August 19, 1995: 08:30. - August 20/95: 09:30; and on August 27, 1995: 09:40 - August 28, 1995: 10:30, at the sampling station ("25 hour" Figure 4b, Chapter 2) using a 64 μm mesh, 50 cm diameter plankton net. Triplicate tows were taken every 90 min for more than 24-h. On August 19, 1995 (neap tides), sampling started at low tide and ended 25 h later (two tidal cycles) at slack low tide. On August 27 (spring tides), sampling began at slack high tide and ended 25 h later at high tide.

The overall horizontal distance sampled was 70 m with a vertical drop of 7 m (1 m drop for every 10 m horizontal). To aid in the replication of tows and to ensure that the boat remained on the sampling station throughout the night, 8 fluorescently painted Javex bottles were attached to a fixed headrope 10 m apart. Sampling began at one end of this headrope. Upon retrieval of the plankton net the sample was pre-screened through a 500 μm screen to eliminate ctenophores and copepods. The sample was then preserved in 5% buffered formalin until analyzed.

Samples taken 1, 4, 8, 16 and 24 h after release of stained larvae on August 12 and August 25, 1995 at the release, middle and inlet stations were also examined. Sampling protocols were detailed in Chapter 2.

Physical data (current meters)

Measurements of temperature, salinity and depth were obtained by Model S4 current meters (Interoceans Systems, Inc.) deployed on June 12, 1995 and retrieved on October 13, 1995. Data were recorded every 20 min. Data from current meters 2d and 2s were examined because of their proximity to the sampling station.

Abundance and net flux

Preserved samples were resuspended in 200 mL of 10 µm filtered sea-water. Three aliquots of 1 mL were taken from the resuspended sample and examined under 40x magnification on an inverted microscope. Calculations were then performed, as per methods used in Chapter 3, to determine the number of bivalve larvae per litre of seawater at the sampling station.

One tidal regime consists of two flood and two ebb tides. Possible transport into or out of Charles Arm, was studied by summing the abundance of larvae from each sample of the two flood phases and ebb phases and then summing the values separately for flood and ebb conditions. The resulting two sums consisted of an equal sample number (9 samples, total number = 18).

Sizing

Selected preserved samples from the August 19, 1995 sampling were resuspended in 200 mL of 10 µm filtered sea-water. Aliquots of 1 mL were taken from the homogenized sample. Each subsample was then scanned using an inverted or a Olympus Ch-2 microscope and measurements (shell height and length) taken on 100 randomly selected larvae. Ocular micrometers were calibrated before every use using a stage micrometer. Total magnification was 100x.

Data analysis

Descriptive statistics (mean, standard deviation, sample variance, and minimum and maximum size) and ANOVA ($\alpha = 0.05$) were performed on the data using the data analysis of Microsoft Excel Version 5.0c software.

4.4 Results

The mean number of veligers at the "25 hour" sampling station was 3.67 larvae/L on August 19 and only 1.21 larvae/L on August 27, 1995. Sample variances were 1.80 and 0.20, respectively.

There was a significant difference between the means for these two dates (ANOVA, $F_{(1,320)} = 480.470$, $p<0.05$). On August 19, 1995 there was a large variation in the number of larvae present as the tidal cycle progressed (Figure 12). Within 3 h (from 16:00 to 19:00) there was over a 6-fold increase in the number of veligers present (1.28 larvae/L to 6.51 larvae/L). A similar situation was observed on August 27, 1995 with significant differences in the number of larvae/L over the sampling period (ANOVA, $F_{(17,158)} = 48.520$, $p< 0.05$). The difference between the highest and lowest numbers observed was not as great as that for August 19. Only a 4-fold difference was observed between the 14:00 sample (mean = 2.13 larvae/L) and the 09:30 sample (mean = 0.57 larvae/L) (Figure 12).

Peaks of larvae were observed during mid-flood to high tide and mid-ebb to low tides on both sampling dates.

Bivalve veligers obtained from the selected sampling periods on August 19, 1995 showed a size range of 114.2 to 130.7 μm (mean values) which were significantly different (ANOVA, $F_{(4,499)} = 3.080$, $p<0.05$) (Figure 13).

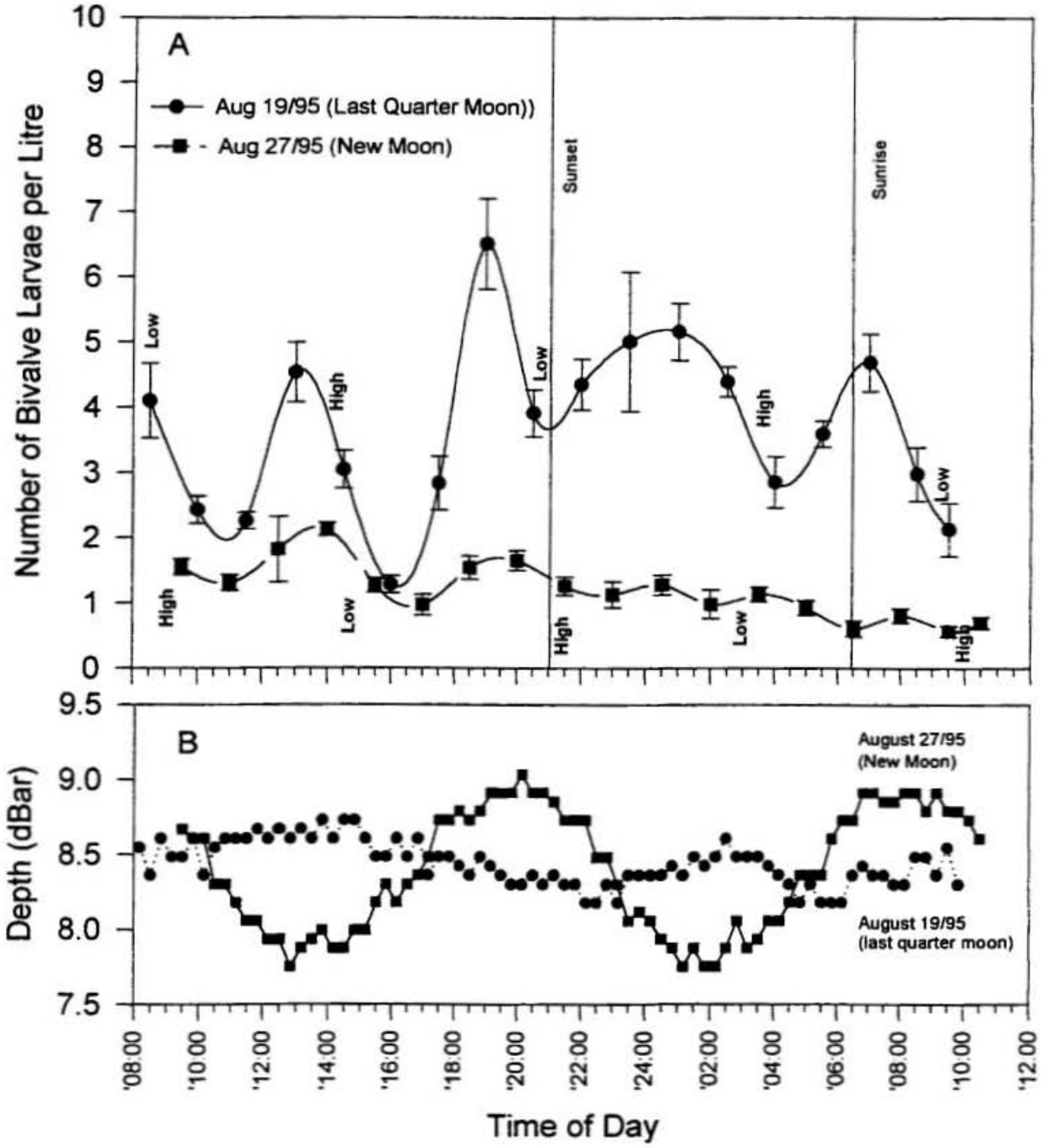

Figure 12 : (A) Average number of bivalve larvae per litre from the 25 hour station at Charles Arm on August 19 and 27, 1995. Vertical bars = standard deviation. High and Low represents time of tidal high and low.
(B) Depth related pressure (dBar) from CM 2d over the sampling period.

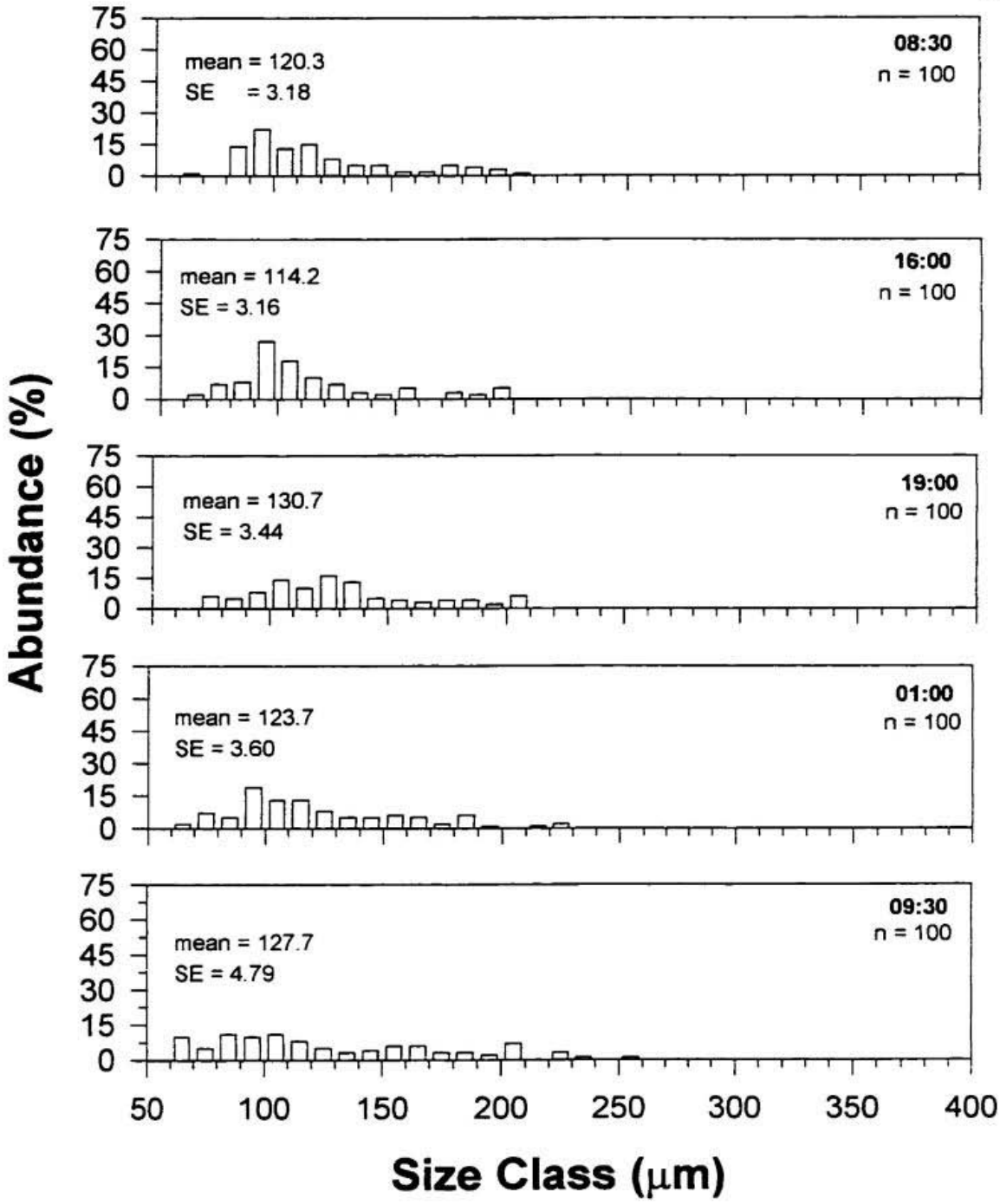

Figure 13 : Shell height frequency distributions of bivalve larvae from Charles Arm on August 19, 1995, collected from the 25 hour station. (SE = standard error, n = number of larvae measured). Time of day is indicated in the right corner of each panel.

Percent abundance (for the selected samples) of bivalve larvae observed on August 12, 1995 and August 25, 1995 at the three sampling station is illustrated by figure 14 (a&b). On August 12, the release station had significantly more bivalve larvae (range 10-23 larvae/L) than either the middle or inlet stations (t-test, d.f. = 47, t-value = 12.401, and d.f. = 55, t-value = 12.506, respectively, $p<0.05$), whereas the middle and inlet stations contained only 4-10 veligers/L and were not found to be significantly different (t-test, $p>0.05$). On August 25, bivalve larval abundance declined below 5 veligers/L (Figure 14b) to 20% of the numbers observed on August 12. There was a significant difference in abundance among the mean values for larval numbers at the three stations (ANOVA, $F_{(2,134)} = 4.060$, $p<0.05$).

Variation in abundance within a station was observed to be almost 2-fold on August 12 and August 25. All stations on August 12 and on August 25 revealed a significant difference in larval abundance over the tidal cycle (ANOVA, $p<0.05$).

Mean size range was 89.9-97.5, 83.4-90.1, and 74.2-82.5 µm shell height on August 12, 1995 for the release, middle, and inlet stations, respectively (Figure 15). Only the release and inlet stations showed a significant difference in the shell heights of the larvae (ANOVA, $F_{(4,499)} = 3.860$ and 5.940, respectively, $p<0.05$). On August 25, 1995, mean shell heights were about 60 µm greater than those collected on August 12, with ranges of 153.2-163.4, 139.9-155.3, and 133.3-144.1 µm for the release, middle and inlet stations, respectively (Figure 16). No significant difference was found within the sites (ANOVA, $p>0.05$) in contrast to the situation on August 12.

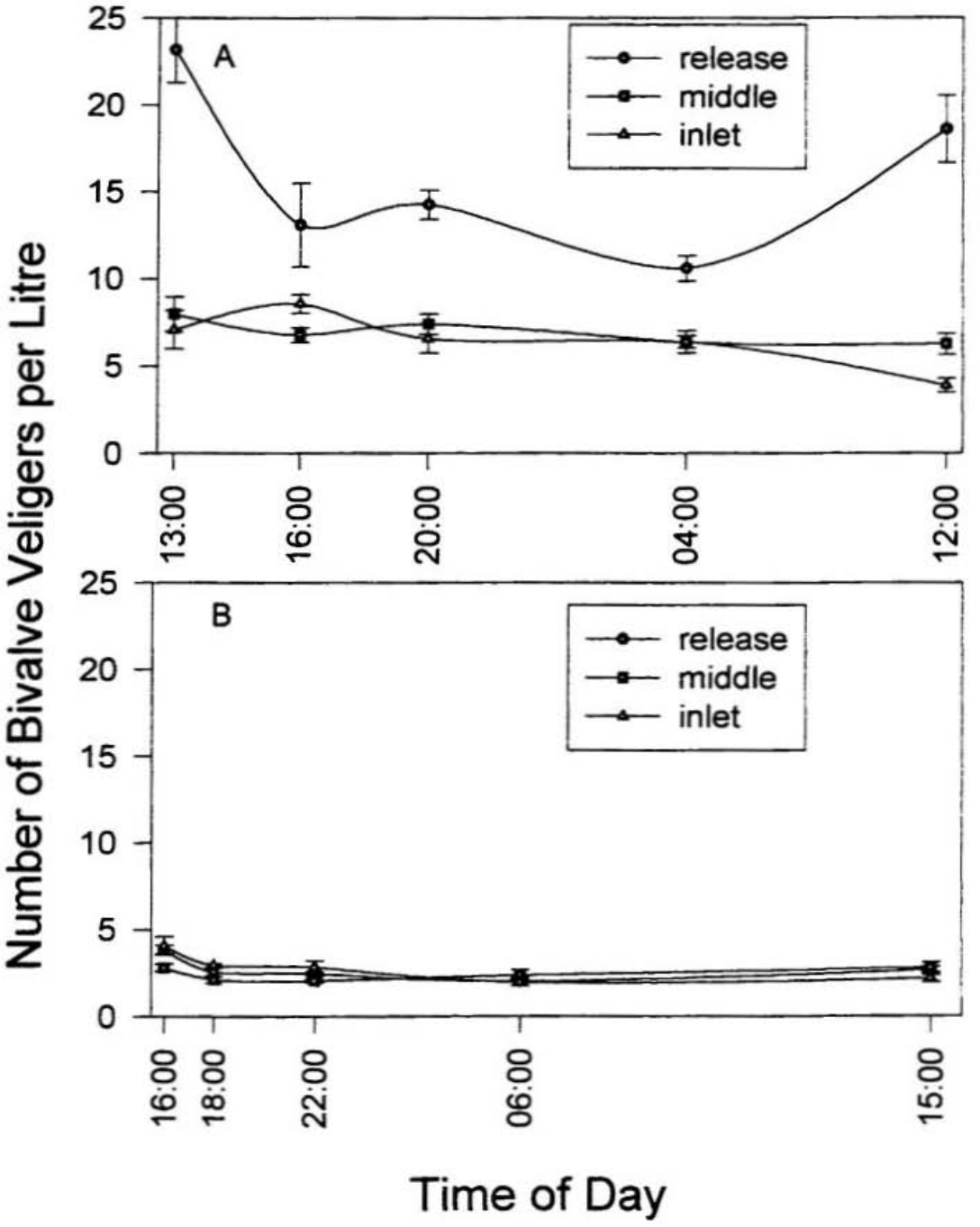

Figure 14 : Summary of bivalve larvae abundance over a 25 h period in Charles Arm on A) August 12 and B) August 25, 1995 at the release, middle and inlet stations.

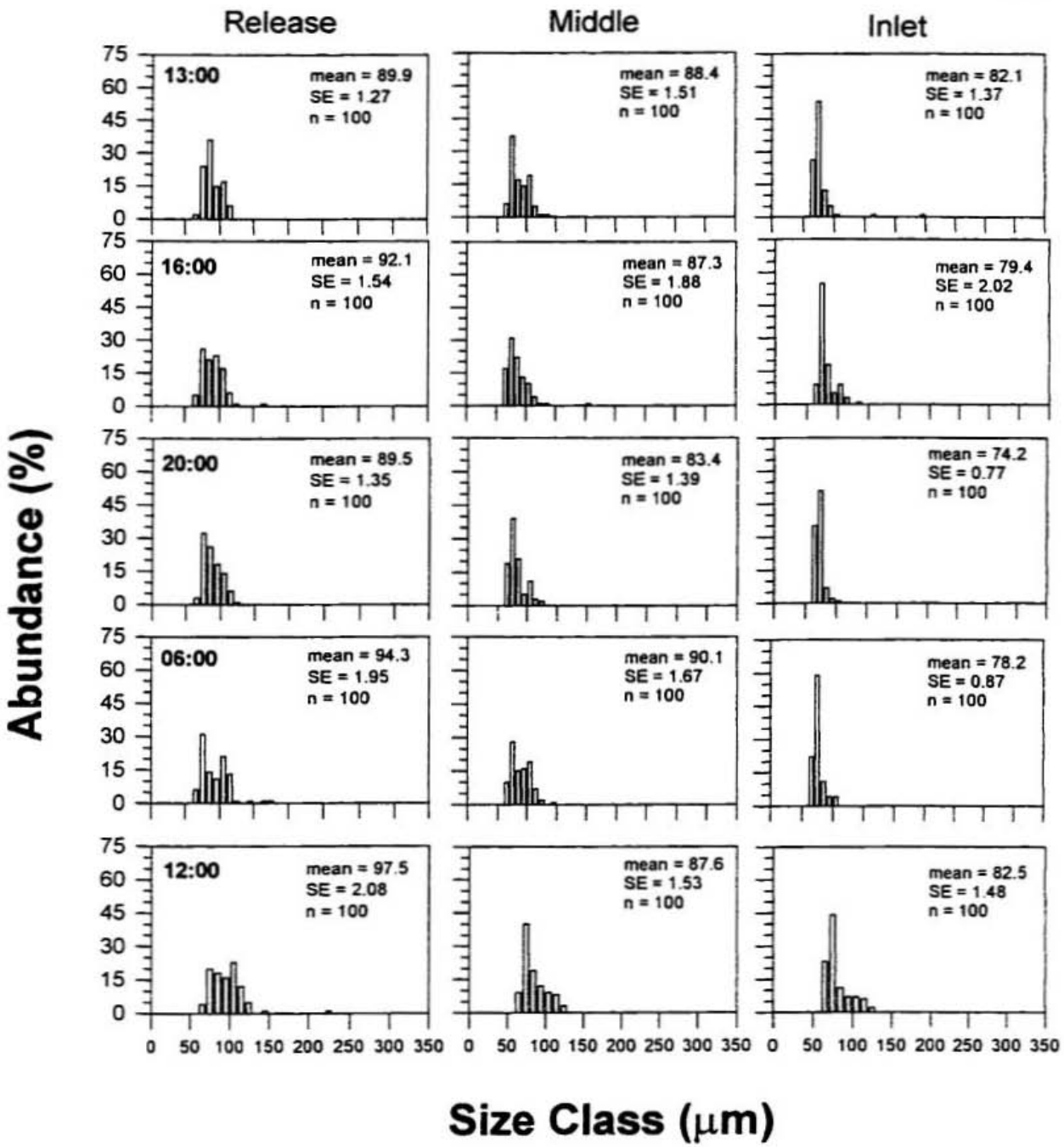

Figure 15 : Shell height frequency distributions of bivalve larvae from Charles Arm on August 12, 1995 at the release, middle and inlet stations. (SE = standard error, n = number of larvae measured)

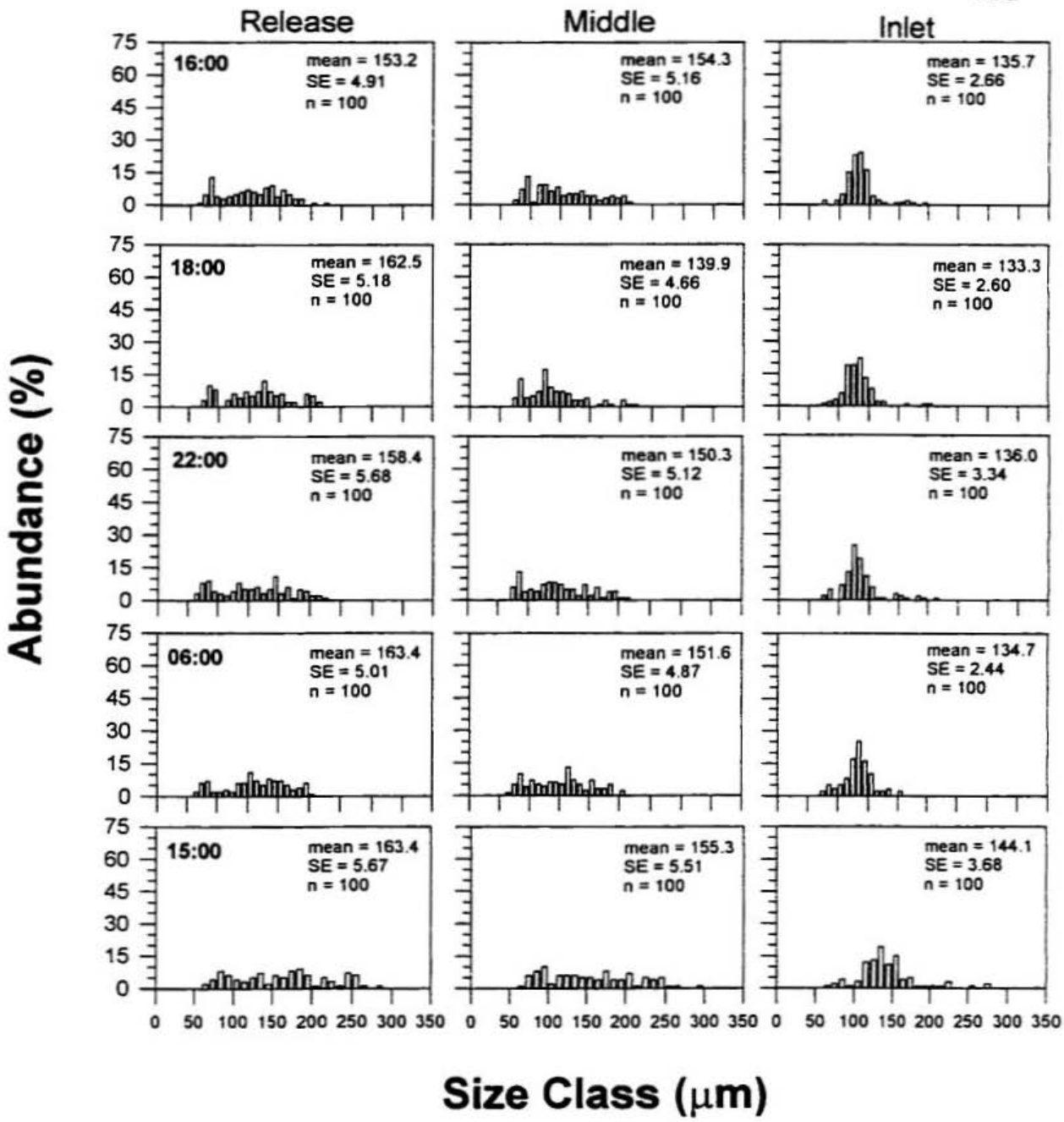

Figure 16 : Shell height frequency distributions of bivalve larvae from Charles Arm on August 25, 1995 at the release, middle and inlet stations. (SE = standard error, n = number of larvae measured)

Estimates of net flux of bivalve veligers indicated a net transport into Charles Arm on August 19, 1995 with 7.79% of those veligers transported into Charles Arm remaining there (Table 6). On August 27 there was a net flux of veligers out of Charles Arm, with 28.27% more leaving Charles Arm, than the total entering Charles Arm.

Table 6: Summation of flood and ebb phases by numbers of bivalve larvae/L during a 25 hour tidal regime experiment in Charles Arm on August 19-20, 1995 and August 27-28, 1995. (+) means transport into Charles Arm, (-) means transport out of Charles Arm, (%) percentage of bivalve larvae from flood, which lack in the ebb tide and remain in Charles Arm.

Date	Σ individuals of 2 outflow (ebb) phases	Σ individuals of 2 inflow (flood) phases	absolute difference	in %
August 19-20	31.68	34.36	+2.68	+7.79
August 27-28	12.16	9.48	-2.68	-28.27

4.5 Discussion

Transport of planktonic organisms from or into Charles Arm may not yet be completely interpreted quantitatively. As described in George (1995), a continuous plankton recording over a complete tidal cycle is necessary, as well as biomass estimates. Water transport and its velocity also need to be investigated because of possible lateral transport. Nevertheless, in this study, some differences were observed over the tidal and diel cycles.

Differences in the mean numbers of bivalve larvae present on Aug 19, 1995 (3.7 larvae/L) as compared to Aug 27, 1995 (1.2 larvae/L) were most likely explained by several factors, the first being a loss of larvae out of the system. Evidence presented in Chapter 2 indicated that some stained larvae had travelled from the release station to the inlet station. Larvae present in the inlet station during ebb tide would be washed out of the system. The hydrodynamics of this area (Chapter 3) suggest that larvae going out of the Charles Arm system would be transported further out in the bay and would not re-enter Charles Arm. Estimates of net flux (Table 6) revealed that 7.79% of larvae transported into Charles Arm remained inside on August 19, but on August 27 there was a loss of bivalve veligers out of Charles Arm (28.27% of total coming in). This loss would be shown directly in abundance estimates.

The second reason for the decrease in numbers of bivalve larvae is settlement. Illustrating size classes of the larvae present on August 28 (Figure 9, Chapter 3), indicates

that there were larvae of a size >200 μm and possibly nearing the setting stage. Any setting veligers would, in effect, be removed from the plankton.

Finally, Nelson (1953,1955) noted that older scallop larvae tended to be more abundant deeper in the water column. The older larvae in the present study, therefore, may have been missed by the sampling because the plankton tows only sampled to a 7 m depth.

If the setting size larvae were mussels, we can expect to observe setting size larvae in the upper water column (Penney,1993). Considering that 1) a percentage of larvae in the surface waters were likely to be transported to the inlet, 2) larvae present in the inlet station during ebb tide would be lost, and 3) there was a net flux of veligers out of the system, it can be assumed that the majority of veligers lost out of the system were mussels of setting size.

Abiotic factors possibly influence the distribution and/or behaviour of larvae. Tides can play a major role in circulation and are known to influence larval availability (Levin,1986). The tides may act to passively transport larvae (Stancyk and Feller, 1986) or to stimulate active behavioural responses (Christy and Stancyk, 1982; Cronin and Forward 1986). Levin (1986) studied plankton overlying a mudflat over four separate 12 h tidal cycles in order to identify short-term, tidally induced variations. In the daytime, bivalve veligers did exhibit a distinct bimodal abundance pattern, suggesting an oscillation of a large patch of larvae. Results of this study (Figure 12a, Figure 14a and b) also showed a bimodal pattern during the daylight hours on both the August 19 and 27 sampling dates, as well as on August 12 and 25. During the nocturnal period this pattern still existed, although the difference in numbers of larvae present was not as great. Nocturnal samples in Levin (1986)

also yielded less variable abundance patterns. Such information suggests that patches of larvae exist within Charles Arm and that their distribution was aggregated. Greene (1990) points out that plankton nets are suited for sampling zooplankton on intermediate and larger spatial scales and that variability amongst samples (as observed in Figure 12a, Figure 14 a and b), is typically the result of zooplankton patchiness and not necessarily an artifact of sampling method. Significant mean shell height differences observed at the 25 hour station on August 19 (Figure 13) and at the release and inlet stations on August 12 (Figure 15) indicated the transport of a patch of bivalve veligers, back and forth, over a point source during the tide cycle.

Differences in the variation of larval abundance among the sampling dates was best explained by the magnitude of the tides. Newell *et al.* (1991) referred to several reports that described examples of vertical moves of bivalve larvae with tidal stage in well stratified coastal estuaries, but in systems with greater tidal ranges, reports of no observed changes in larval abundance with tidal stage was noted because of consequent increases in turbulence. Therefore, possible responses by the larvae to environmental cues are overridden and the larvae behave as inanimate particles in their distribution. Neap tides of August 19, 1995 showed the greatest variation in abundance with time of day. The spring tides on August 27, 1995 allowed for better mixing of the water and thereby eliminated any effect on larval abundance which may be due to active vertical migration of the larvae.

The difference in larval abundance between daylight and nocturnal observations and the magnitude of the variation in abundance may be due to stimuli which activate behavioral responses such as swimming or sinking. Bivalve larvae swim upwards in vertically oriented straight lines with periods of upward movement alternating with periods of passive sinking (Mann and Wolf, 1983). Depth is therefore regulated by these alternating periods of sinking and swimming. Many factors have been reported to stimulate active aggregation in larval bivalves. Tremblay and Sinclair (1988) suggested that active aggregation by giant scallop larvae at the thermocline is possible given that bivalve larvae can occupy different depths during the day compared to the night. An exception to this was observed at one of the stations, but it was suggested that larvae were responding to a density difference rather than a temperature difference because larval distribution corresponded better with the pycnocline caused by salinity stratification. Further work showed this to be the case (Tremblay and Sinclair, 1990a and b). Mesocosm studies by Gallager *et al.* (1996) which showed strong diurnal migration which resulted in aggregations of giant scallop larvae at the thermocline during the day and at the water/air interface during the night is consistent with the trends observed by Tremblay and Sinclair (1988). There also are reports of active migration of late stage oyster larvae in relation to tidal circulation within some estuaries (Kunkle, 1958), but without simultaneous measurements of the parameters mentioned, the reasons for the difference are not clear. Scrope-Howe and Jones (1986) observed bivalve larval movement during the night into the chlorophyll maximum, which was observed to be below 7 m in Charles Arm in 1995 (Figure 7, Chapter 2). In the present study, larvae were not sampled at

these depths. All of these reasons suggest active depth regulation by bivalve veligers, and migration below the sampling zone (0-7 m) would result in a decrease in larval abundance as well as in the magnitude of variation in abundance caused by tidal flows. The absence of greater than 200µm shell height larvae during two sampling times (22:00 and 06:00) for the 25 hour experiment on August 25 (Figure 16) indicates that these larger larvae may well be migrating out of the sampling zone. Gallager *et al.* (1996) observed that only scallop larvae greater than 230µm descended below a thermocline. During this study, the larger larvae may be descending to areas of high food concentration (9m) and would be able to do so even if a thermocline existed. If larvae are aggregating at the surface during the night, and these numbers are spread over a wider horizontal area, then fewer numbers of larvae would be sampled during the night, as compared to the day, with the oblique plankton tows used in this experiment. To see if aggregations near surface are occurring during the night, one would have to complete horizontal plankton tows at the surface over a 24 hour cycle.

Herein lies a serious problem with bivalve larval monitoring programs. Data collected at one point in time at weekly intervals may not accurately represent abundances of larvae present, and may therefore lead to incorrect predictions of spatfall numbers. The findings of the present study does not agree with the findings of Newell *et al.* (1991) in that larvae did not predominate on the flood tide nor did they occur in equal numbers on flood or ebb tides (Figure 12). During this study, it is important to note that larvae were less predominant on the high and low tides with the highest numbers occurring around mid-tide to late ebb or late flood levels. When differences of tidal heights were greater the variation

in abundance was lower. At times when the tidal range was at its lowest, the variation in abundance of larvae increased dramatically (Figure 12). A monitoring program should incorporate more frequent sampling for larvae, sampling at the same time of tide, or even sampling at different times over the tidal cycle on any particular day to get a better understanding of the possible variation. Data on distribution patterns (i.e., currents and wind direction) should also be a important part of the monitoring program, providing information on net flux into or out of a system. It would be also be advantageous to sample the full water column, if at all possible, to eliminate possible active vertical migration effects.

SUMMARY

Calcein has been shown to be non-toxic to many bivalve veligers including the giant scallop (Rowley and Mackinnon, 1995). Immersion of millions of 3-day-old scallop larvae (less than 100/mL) into a calcein solution containing 150 mg/L for 16 h was sufficient to stain large numbers of giant scallop veligers simultaneously. The stain (mark) was visible under blue or UV light and was retained by the animal for over two years in preserved specimens. Because the giant scallop shell grows throughout the life cycle, it is possible to use calcein to stain older veligers, post-set and even juveniles. Calcein, therefore, can be useful for studies on giant scallop veligers and post larvae in growth experiments both in the laboratory and in field experiments such as this one. Not only will calcein supply an identification mark but also a benchmark for growth studies and also for release-recapture studies in the environment.

Mark, release and recapture of stained giant scallop larvae was successfully completed in Charles Arm. Stained larvae were recaptured from 1 h to over 30 d post release at the three sampling stations. Growth estimates and larval distribution patterns were obtained from this data.

Estimated growth rates of giant scallop larvae were 3.53 μm/d and 3.85 μm/d in 1994, and 9.72 μm/d and 2.52 μm/d in 1995. Thus, growth rates can differ between and within years. Timing of the release of hatchery produced larvae is important in order for the new larvae to encounter favorable conditions. Temperature and chlorophyll-*a* concentration

were found to influence growth rates, and should therefore be measured to aid in the prediction of larval spatfall. Data from 1994 and 1995 were similar to that of 1990 and 1991 reported by Penney (1993). It was suggested that delayed increases in the water temperature postponed both the spawning and settlement of bivalve veligers. Higher temperatures (greater than 16°C) and high chlorophyll-*a* levels (greater than 13 µg/L) resulted in more favourable growth rates, exceeding 7 µm/d. Although chlorophyll-*a* concentrations may remain high, if the temperature declines, the growth rate may decline. When both temperature and chlorophyll-*a* levels diminish, it is expected that growth rates would decline below 2 µm/d.

Retrieval of the stained larvae from the release, middle and inlet stations indicated that giant scallop larvae released at the end of Charles Arm will become depersed throughout the site. The presence of stained larvae at the inlet station revealed that some percentage of larvae released would be lost from the system. It also indicated that naturally produced scallops within Charles Arm would likely finding their way to other areas of Notre Dame Bay.

Current patterns must also be ascertained more fully to understand where aggregations of scallop larvae are most likely located. Eddy currents were formed along the shore where points of land project into the arm and also within the smaller coves along the arm. The counterclockwise circulation pattern, higher larval abundances in the release station area (end of Charles Arm) and the presence of stained larvae within this area indicated the likelihood of entrainment of veligers. Therefore these areas should be

examined more closely for abundances of larvae and thus better locations for deployment of scallop collectors may be obtained.

Current meter and CTD data indicated that two different environments existed within Charles Arm. The bottom water tended to be colder, by almost 6°C, than surface water but also contained a higher concentration of chlorophyll-*a*. Current speeds were also slower in the bottom of the water column compared to the surface water. The higher surface currents, combined with wind drift, were capable of transporting bivalve larvae out of the system. If larvae remain below a critical depth, there was a higher probability that they will remain within the system. Depth stratified sampling which samples the whole water column could corroborate this.

Bivalve larval abundance data indicated that two spawning episodes of local populations occurred during this study, the first in early August and the second in early September. The most likely sources were farmed mussel and/or scallop stocks, but the larvae could not be differentiated. Maximum abundance observed was only 23 larvae/L, much lower than larval abundance values obtained in 1991 (Penney, 1993). Higher numbers of bivalve larvae observed at the release station on August 12, 1995 indicated that either: 1) the spawning took place in the end of Charles Arm on or about August 12, 1995 and these larvae had not yet been distributed to other areas of the site; or 2) circulation patterns within Charles Arm were aggregating bivalve larvae within the end area. The release station always had a higher abundance of bivalve larvae therefore it is assumed that entrainment and aggregation were occurring.

Size frequency of bivalve larvae observed over the study period indicated that two cohorts of larvae remained within Charles Arm during 1995. A possible third cohort may have been present due to the number of small veligers, less than 75 µm in shell height, observed in the water column on September 30, 1995. Larger settlement size veligers (greater than 250µm) were observed at all three stations with greater numbers at the inlet station. Decreased numbers of large veligers at the release and middle sites may be a result of settlement, when the larvae leave the water column. The increased numbers at the inlet site may be evidence for settlement size mussel larvae in the surface water (Penney, 1993) being trapped in the prevailing water currents and transported out of the system. Net outward flux of larvae around this time (August 27, Table 6) indicated that these larger bivalve larvae were being lost from the system.

Tidal variation in abundance and size of larvae was evident, which poses serious implications for any monitoring program which predicts either the timing of spatfall, the numbers of spat settling, or both. Larval abundance, was three times greater over the tidal cycle and significant mean size changes were observed. In many monitoring programs, farmers are recommended to put out their collectors when over 50% of veligers observed are over 250 µm. If samples are taken weekly and at a phase of the tidal cycle when mean shell height is low, the placement of collectors may be postponed with the result that settlement may be missed. Highest abundances of bivalve larvae and greatest mean shell heights were observed at high and low tides with reduced numbers and mean shell heights observed during mid tides. Therefore, it is recommended that plankton tows be completed

consistently on the high or low slack tide times.

The recapture of stained scallop larvae released within Charles Arm has partly determined if scallop larvae released within the system or from natural spawning events will remain within the area. But many other questions have arisen from this study. The fact that stained larvae retrieved after thirty or more days from time of release indicated that some larvae do remain within the Charles Arm system and grew to settlement size. However, stained larvae were recaptured at the inlet station and this, together with current meter data and drift bottle surface current diagrams makes it highly probable that larvae were being transported out of the system on ebbing tides. What is uncertain, and is most critical, is what percentage of released larvae were lost out of the system due to currents. Of the millions of larvae released, much less than 1% were recaptured in plankton tows. A high percentage of these would not have survived to set, or would probably have settled in inhospitable areas. In the present study, giant scallop larvae were only released during high tide. If larvae were released at low tide it may be presumed that subsequent flooding might help retain the released larvae, particularly in the end of the arm. Release of larvae was completed irrespective of tidal season (i.e., neap or spring tide). Presumably, neap tides with less turbulence, would allow for active vertical movement in the water column. This may favor larval retention. Thus any enhancement project for this area releasing scallop larvae may be feasible, but must involve an intensive spawning and incubation regime with the release of hundreds of millions of larvae in order to obtain significant increases in scallop collection.

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IMAGE EVALUATION
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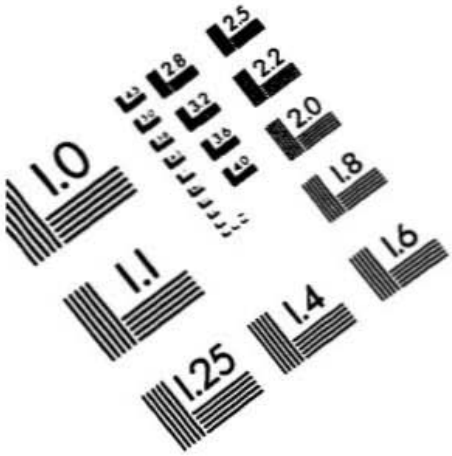

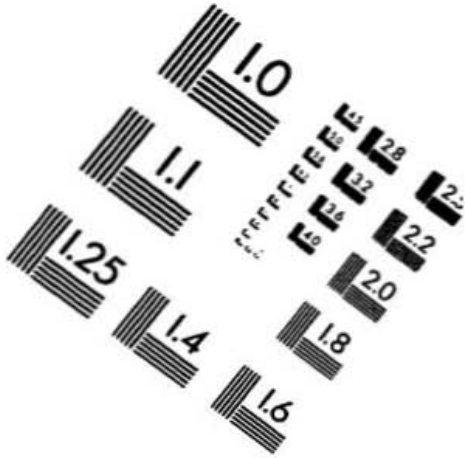

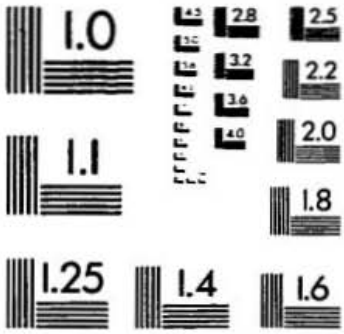

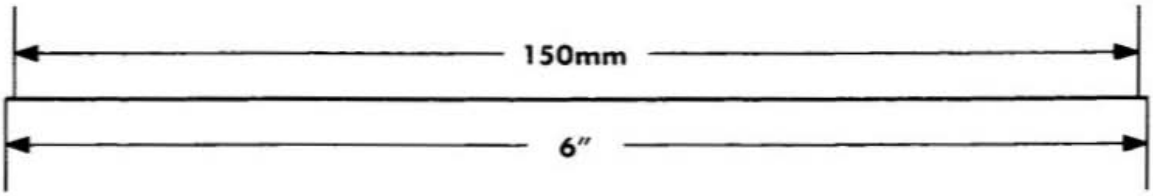

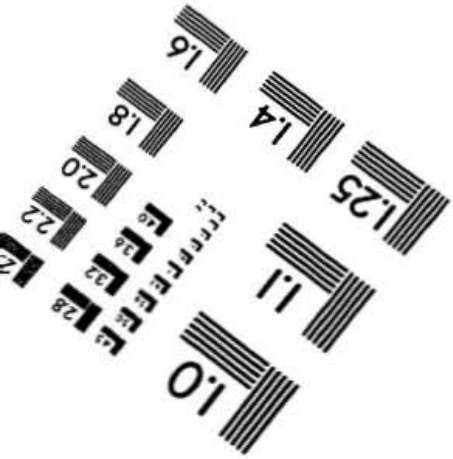

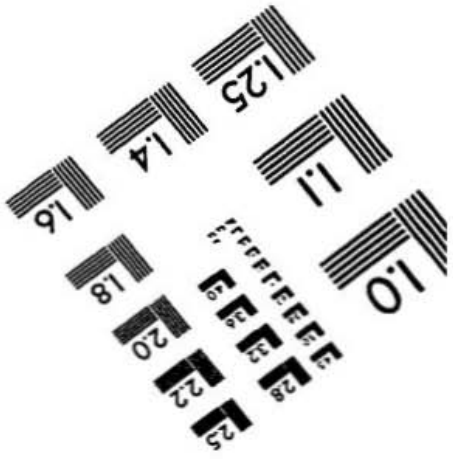

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