# REPRODUCTIVE CYCLE, SPAWNING AND EARLY GROWTH OF SOFT SHELL CLAMS (*MYA ARENARIA*) ON PRINCE EDWARD ISLAND

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**University of Prince Edward Island**

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## ABSTRACT

Reproductive evaluation of male and female soft shell clams (*Mya arenaria*) was investigated using histologic examination of gonads from samples which were collected during spring, summer, and autumn months in the Malpeque Bay and Pinette River areas of Prince Edward Island (PEI) between 1997 and 1999. Clams were classified into 1 of 5 stages, inactive, active, mature, partially spent or completely spent. The data indicated that spawning commences mainly in the month of June and the duration of spawning varies between sites and years.

Triggers of spawning were examined including water temperature and month of year. In 1997, water temperature was significantly associated with the onset of spawning ($P \leq 0.01$). In 1998, month of year was proven to be statistically significant in triggering spawning ($P \leq 0.01$). As there was no consistency between years, it is likely that these parameters do not operate in isolation. Rather, it is likely that a combination of factors contribute to spawning events on PEI.

Several condition indices were tested to determine their accuracy in predicting spawning events. Steamed meat yield, a gravimetric index and a shell condition index were compared to results of histological analysis. Although not statistically significant, it appears that steamed meat yield most closely follows the pattern of mature stage clams ranked using histology.

Larval monitoring was conducted using the plankton pump and plankton net methods to monitor the development of clam larvae in the water and to verify the date of clam spawning. No clam larvae were collected in this process. Seed collection trials were performed in two of the study years using onion sacks filled with monofilament and hung on a longline to capture clam seed. These trials successfully captured clams on the collectors and mean shell lengths in both years reached 8.0 millimeters by the end of September.

# DEDICATION

**To my family, with love.**

## ACKNOWLEDGMENTS

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| | |
|---|---|
| centimeter | cm |
| chi square | $X^2$ |
| degrees Celsius | °C |
| et alii; and others | et al |
| Fisheries and Oceans Canada | DFO |
| gram | g |
| kilometer | km |
| liter | l |
| maximum | Max |
| meter | m |
| micron | µm |
| milliliter | ml |
| millimeter | mm |
| minimum | Min |
| north | N |
| parts per thousand | ppt |
| Prince Edward Island | PEI |
| polyvinyl chloride | PVC |
| p value | P |
| United States of America | USA |
| west | W |
| weight | wt |

# 1. INTRODUCTION

## 1.1 Soft shell clam commercial fishery

The digging of soft shell clams, *Mya arenaria*, is a fishery of commercial and recreational importance in Eastern Canada (Freeman *et al.* 1996) and the New England States (Gulmann *et al.* 2001). The soft shell clam is commonly harvested for its value and use in chowders and as a steamed dish in the restaurant industry. The soft shell clam commercial fishery on Prince Edward Island (PEI) is a long-standing industry with formal catch records dating back to the late 1800s (Robinson 1996).

The harvest of this commercial enterprise on PEI has fluctuated in recent years, with a range between approximately 71 and 421 tonnes per year between 1982 and 1996 (Figure 1) (PEI Department of Fisheries and Tourism 1999). The amount of soft shell clam landings reported in the commercial fishery on PEI increased from 345 tonnes in 1997 and 420 tonnes in 1998, to 450 tonnes in 1999 (Fisheries and Oceans Canada 2002a). The corresponding values for these landings are $761,000, $1,039,000 and $1,237,000, respectively (Fisheries and Oceans Canada 2002a). Although values for soft shell clams are increasing, it is difficult to reliably meet market demand at present without jeopardizing the public resource (Fisheries and Oceans Canada 2002a).

Historical data may not reflect the actual value of this industry to PEI for a variety of reasons. The harvest of soft shell clams is typically a supplemental

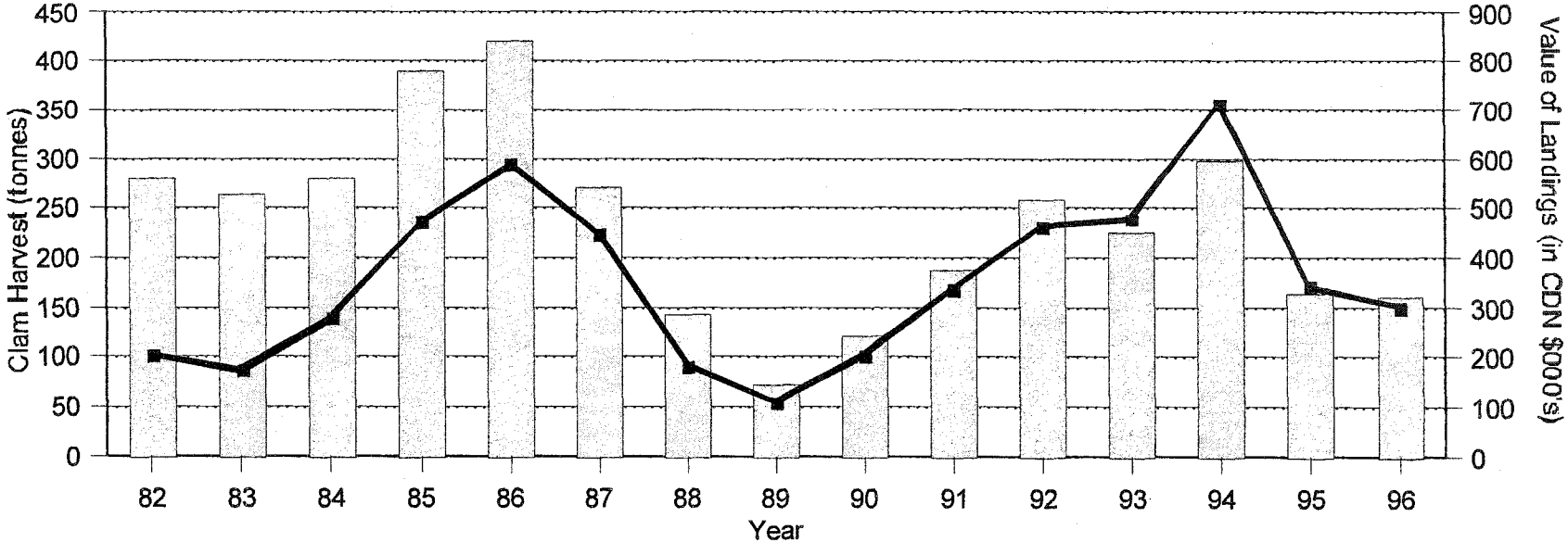


**Figure 1.** Soft shell clam harvest (bars) and value of landings (line) on Prince Edward Island from 1982 - 1996.

fishery that varies in relation to the success or failure of mainstay fishing industries, such as lobsters or crabs (Robert 1981). In a good year for other species, soft shell clam fishing effort, and therefore landings, is typically lower whereas higher effort is placed on fishing clams in a year when other species are scarce.

Additionally, the recruitment of clams, or the settlement of small clams to any given area, is subject to extreme variability both spatially and temporally (Newell 1990, Gulmann *et al.* 2001). Because adult population density is linked to recruitment, variability in the standing stocks results in dramatic population changes from season to season and among locations.

Another source of variation in landing data is caused by the illegal harvest of clams from contaminated beds in closed areas (Robert 1981, Robinson 1996). These closures are commonly linked with high levels of faecal coliform bacteria being present (Fisheries and Oceans Canada 1997a). Not only does this practice affect overall landings, but it poses a human health concern.

A final inaccuracy in the landing data may be attributed to the deficiency of landing statistics from the recreational fishery (Fisheries and Oceans Canada 1996) as many harvest for their own use or arrange for private sale of the clam stocks (Robert 1981, Fisheries and Oceans Canada 2001a). Due to the above practices, it is likely that a large proportion of the landings for soft shell clams are not reported (Fisheries and Oceans Canada 1996 and 2001a) and therefore reported landings are considerably lower than the actual harvest (Fisheries and Oceans Canada 1997a). This results in a need to develop aquaculture techniques for clams.

## 1.2 Soft shell clam biology

The soft shell clam is a benthic infaunal, or substrate dwelling, bivalve mollusc characterized by gills, adductor muscles and equilateral shells with an elongate shape (Maine / New Hampshire Sea Grant College Program 1998). The clam is a filter feeder with mechanisms for sorting and sieving food particles such as phytoplankton and bivalve larvae (Maine / New Hampshire Sea Grant College Program 1998). This marine species is found intertidally, or in the area between the high tide and low tide water marks, and burrows in sand or mud with a compressed muscular foot. Soft shell clams are dioecious, that is, the sexes are separate, and fertilization is external. The resulting zygote develops into free-swimming trochophore and veliger larval stages which metamorphose into the adult form (Manzi 1985).

The soft shell clam is native to the Atlantic Coast and found from Cape Hatteras, USA in the south as far north as Newfoundland and Labrador, Canada (Manzi 1985, Hidu & Newell 1989, MacKenzie and McLaughlin 2000). The species has been successfully introduced to the Pacific coast of North America and Europe (Manzi 1985). The legal harvestable shell length for soft shell clams in Atlantic Canada is 50 mm, or 2 inches, (Fisheries and Oceans Canada 1998) and this size is typically achieved in 5 to 6 years on PEI (Robert 1981). Climate affects the time it takes for clams to reach maturity as it takes only 2 years to reach that size in the east coast of the USA (Brousseau 1987).

### 1.2.1 Reproductive cycle

The reproductive cycle of a bivalve is comprised of numerous interrelated events which can be classified as stages according to a standard protocol. Ropes and Stickney (1965) described the soft shell clam reproductive cycle in five distinct stages, specifically: inactive, active, mature, partially spent, and spent. These stages are explained in further detail in section 2.1.1 and are based on histological analysis. This technique involves viewing a very thin cross section of the specimen under light microscopy.

Researchers in the New England states have conducted in-depth studies on soft shell clam gonad changes and spawning events over time (Coe and Turner 1938, Shaw 1962, Shaw 1965, Pfitzenmeyer 1965, Ropes and Stickney 1965, Brousseau 1978, Brousseau 1987). However, the information from these studies may not be directly applicable to clams in other areas, including PEI, due to differences in temperature, geographic location, or other natural parameters (Brousseau 1987).

Little is known about the developmental potential of aquaculture for soft shell clams on PEI; even less is known about possible factor(s) prompting spawning events or on the spawning pattern in this location. Following and describing reproductive events within clams on PEI may aid in the understanding of clam biology and could provide significant advances in local soft shell clam aquaculture.

### 1.2.2 Larval stages

Adult clams produce gametes and release them into the water. A 50 mm female clam may release between 800,000 and 1 million eggs in a single spawning event, and a 50 mm male is able to produce 25 million sperm (Beal and White 1991). Adult clams synchronously release their gametes into the water, where external fertilization occurs, and within 12 hours the fertilized eggs become trochophore larvae (Beal and White 1991, Doiron 1997). These planktonic larvae are exposed to many perils such as currents, winds and predators.

The trochophores develop shell glands. The larvae then progress to the d-veliger stage in which they acquire a velum for feeding. Over a period of approximately one week, the larvae develop gills for feeding and respiration (Beal and White 1991, Doiron 1997). Ultimately, the pediveliger stage is reached, in which the larvae develop a foot which is used by benthic juvenile clams as a form of transportation. At the pediveliger stage, the clam larvae settle to the substrate and prepare to metamorphose (Beal and White 1991).

At the time of settlement, larvae produce byssus threads to secure themselves to the substrate, or bottom; at this point, the larvae are smaller than a single grain of salt, with shell lengths approximating 235 μm (Sullivan 1948, Doiron 1997). Once the larvae have settled to the bottom substrate, they are referred to as clam seed.

Many clams will not survive the settlement stage for a variety of reasons, including predation by bottom feeders and coming to rest on unsuitable substrate

such as mud which can suffocate young clams. This entire process occurs in a four to six week window in the New England states, depending on water temperatures (Beal and White 1991). The soft shell clam larval period lasts approximately four weeks in the southern Gulf of St. Lawrence (Fisheries and Oceans Canada 1996).

## 1.3 Bivalve larval monitoring

Larval populations can be monitored by means of plankton tows or plankton pump sampling. The collection of bivalve larvae is commonly performed and may be conducted using two distinct methods (Sullivan 1948, Drinnan and Stallworthy 1979, Quayle and Newkirk 1989). The first method is to haul the plankton net a set distance through the water using a boat, while the second approach involves the use of a pump to run a measured volume of water through a mesh screen. These methods are consistent with plankton sampling protocols for oysters, *Crassostrea virginica* (Woo and McIver 1974), and mussels, *Mytilus edulis* (Bernard 1991-1997), used on PEI and in eastern North America (Quayle and Newkirk 1989).

## 1.4 Bivalve seed production and collection

Spreading hatchery-reared and natural seed on intertidal public beds near several towns in northeastern Maine has become a common practice in recent years to enhance stocks for commercial and recreational fisheries (MacKenzie 2000). Hatchery seed is produced at the only clam hatchery in the state, located at Beals Island, where approximately 10 million seed are grown annually (Beal and

White 1991). Natural seed is harvested from areas of higher concentration clam beds after settlement occurs and then the seed is transplanted to lower density areas within the same site (MacKenzie 2000). Currently, soft shell clam aquaculture sites have not been investigated in the New England states.

The success of producing soft shell clam seed in hatcheries is relatively recent. This is partly due to previous difficulties encountered in conditioning and inducing spawning in broodstock clams (Hidu and Newell 1989). One major setback to producing commercially feasible numbers of clams in a hatchery is the cost associated with growing clams to an appropriate planting size (Newell 1990). Depending on juvenile clam growth, the stock may require overwintering in a hatchery, which adds considerably to the cost of production.

To date the hatcheries established in the states of Maine and Massachusetts have been feasible because they are government funded and rely heavily on volunteers to remain in operation. The clam seed produced in these hatcheries is used in the enhancement of public clam beds to support the commercial fishery. The costs associated with the establishment of a hatchery in terms of infrastructure installation, operation, and maintenance, skilled staff and labour can be too costly to supply private industry (Newell 1990).

In the aquaculture of other bivalve species on PEI, hatcheries are not used to produce seed or rear juveniles for grow out. Therefore, it is highly unlikely that a hatchery would be built for the developing soft shell clam aquaculture industry on PEI. Although market values for PEI soft shell clams have increased over time, the

per-unit price of an adult clam is too low to overcome the cost of seed production in a hatchery. In order to successfully cultivate soft shell clams as an aquaculture species, a supply of clam seed is required at a minimal cost and the method of collecting seed must be efficient.

Presently, soft shell clam seed is not being collected from natural, or wild, spawning events on PEI, as aquaculture development is at an early stage and no program exists to enhance commercial or recreational fishing areas. Wild seed collection consists of capturing clam seed from naturally spawning populations. Other types of shellfish aquaculture on PEI rely on wild seed collection, including cultivation of blue mussel (Scarratt 1993), eastern oyster (Medcof 1961), and sea scallop (Pouliot *et al.* 1995).

For all three of these bivalves, a longline system is used to capture wild seed. A longline is a length of rope at the water surface, which is anchored at either end and held afloat with buoys. The main difference between these species is the type of collector hung on the longline system. In mussel aquaculture, a system for seed collection is used which consists of hanging pieces of frayed rope about every 30 centimeters along the longline for seed attachment. Oyster seed is collected using various materials dipped in a thin layer of concrete and fastened to the longline. Lengths of PVC pipe, "Chinese hats", and even oyster shell have been used to capture seed. In sea scallop seed collection, onion sacs stuffed with monofilament are hung on the longline to capture seed.

Timing is important in the placement of seed collectors for all bivalves. In

mussel seed collection, if collectors are hung too early, starfish, which spawn a month earlier than mussels, will set on the collectors first and feed on the mussels that subsequently try to attach. In the case of oysters, if the collectors are deployed too early in the season, large numbers of mussel seed can attach first, thereby out-competing the oyster seed for space and food.

With these occurrences in mind, it is important to know the exact date of spawning for the species whose seed is to be captured. In this way, collectors are placed at the most appropriate time for seed capture. Achieving this goal is highly dependent on the ability to forecast the time of spawning and subsequent seed settlement. This information is currently lacking with respect to soft shell clams on PEI.

## 1.5 Aquaculture

Aquaculture is one of the fastest growing food production activities in the world. Global aquaculture has grown almost 10 percent annually since 1984 (Fisheries and Oceans Canada 2000). Culturing of any organism implies two things: 1) the stock or resource is under private ownership and 2) the natural life cycle of the organism has been altered in some way prior to harvest (Hidu and Newell 1989). In Canada, aquaculture was first used to enhance natural stocks. However, it is now a large-scale commercial industry across the country providing direct and indirect economic benefits to many local and regional economies. All ten

provinces and the Yukon Territory currently have a stake in commercial aquaculture and an increasing interest exists in developing aquaculture within the Northwest Territories (Fisheries and Oceans Canada 2002b). Aquaculture production in 1995 accounted for 7% of total finfish and shellfish production in Canada (Fisheries and Oceans Canada 2002b).

### 1.5.1 Soft shell clam aquaculture

An advanced aquaculture technology such as inducing polyploidy in soft shell clams and other bivalve species on the west coast of the United States has been investigated and utilized by Allen (1987) and others. Triploid clams, or clams with three sets of chromosomes, have been produced by modifying the early larval stages of development to generate a faster growing product (Allen 1987). These soft shell clams do not place effort into reproduction; rather, they focus on growth in terms of shell length.

As mentioned earlier, the state of Maine has been active in culturing soft shell clam seed for the enhancement of public fishing areas since 1987 (Beal and White 1991). The soft shell clam is considered one of the most promising emerging species for aquaculture development on PEI. An emerging species is any new or non-traditional species or fishery which is either in a scientific, experimental or early commercial stage of development (Fisheries and Oceans Canada 2000).

The first experimental soft shell clam lease on PEI was issued by Fisheries

and Oceans Canada in 1993. By the time of this study, 13 of these experimental leases were in operation on PEI (Fisheries and Oceans Canada 2001b). Soft shell clam aquaculture sites issued on PEI had to meet a set of conditions which included that the area could not currently be supporting a natural population of soft shell clams, as the intent was to create additional clam beds and not to privatize a public resource. As the leases were originally without clams, a protocol was established to populate the leased areas. Specified quantities of clams were permitted to be transferred, or relayed, from contaminated and conditionally closed fishing areas to the soft shell clam aquaculture sites.

Before the clams were distributed on aquaculture sites, the bottom substrate was cultivated. This manipulation of the sediment allows for easier self burial for the soft shell clams, as the sediment is less dense and the time it takes to burrow decreases (Fisheries and Oceans Canada 1997c). This method also removes fine sediment, and has been known to capture, or collect, large quantities of newly settled clam seed from spawning events (Woodin 1976).

The transplanted soft shell clams would depurate, or clean themselves of any contaminants, within the aquaculture site as they grow to market size. The transplanted clams could reproduce and aid in the recruitment for future year classes of clams on the lease. The transferring of stock was established on a short-term basis in order to establish a clam bed in the area.

As recruitment was not predictable or consistent, year after year, soft shell clams continued to be transferred from contaminated sites to soft shell clam

aquaculture sites. It is unknown whether or not the adults spawning on the site actually play a role in recruitment to that specific area. As the clams in larval stages are free floating prior to settlement, currents can move the larvae far from their origin. Therefore, another method to ensure recruitment on leases was required. Seed was required for the sites in order to come one step closer to implementing culture practices for soft shell clams.

## 1.6 Objectives

At present, there is considerable interest in the development of soft shell clam aquaculture on PEI. The fostering of aquaculture for this species requires pertinent information with regards to gonadal development, reproductive dynamics and early growth. The objectives of this study include evaluating the reproductive cycle of soft shell clams on PEI, investigating the factor(s) stimulating the onset of spawning, determining when spawning occurs within this species, and assessing the ability to capture seed using collectors.

# 2. GONAD DEVELOPMENT OF SOFT SHELL CLAMS (*MYA ARENARIA*) ON PRINCE EDWARD ISLAND

## 2.1 Introduction

The soft shell clam, *Mya arenaria*, has potential as an aquaculture species on Prince Edward Island (PEI). Reproductive cycle information on this species, in terms of gonad development and spawning, is key to understanding its biology, which is required to culture and manage soft shell clams (Ropes and Stickney 1965). Neither the biology nor aquaculture potential of soft shell clams has been completely studied on PEI.

The gonadal development and time of spawning in clams at selected study sites on PEI were evaluated by histology to determine which gametic stages occurred during summer conditions. Researchers have conducted in-depth studies on soft shell clam gonad changes and spawning events over time, but these studies were particular to local areas within the New England states (Coe and Turner 1938, Shaw 1962, Shaw 1965, Pfitzenmeyer 1965, Ropes and Stickney 1965, Brousseau 1978, Brousseau 1987). Therefore, they may not pertain to clams in other areas, including PEI, due to differences in temperature, geographic location, or other natural parameters (Brousseau 1987). Earlier studies on soft shell clams were conducted on PEI, but their focus was on larval identification (Sullivan 1948) or on determination of the quantities of commercial clam stocks present in public fishery beds (Robert 1981).

Little is known about the developmental potential of aquaculture for soft shell clams on PEI; even less is known of the predictor or predictors of spawning and on the spawning pattern in this location. Questions include: What factors trigger the onset of spawning? When does spawning occur? How long is the duration of spawning? Are soft shell clams on PEI capable of multiple spawning events within a single year? Annual variation may occur in spawning between study sites or within a single site on PEI.

Following and describing reproductive events within clams at different locations may aid in the understanding of clam biology and could provide significant advances in clam aquaculture on PEI. Future aquaculturists might modify the timing and duration of their clam seed collection efforts if provided with results that demonstrate when clam spawning occurs. The study described here was carried out to provide a baseline evaluation of spawning from which other techniques used to estimate spawning could be compared.

### 2.1.1 Clam reproductive cycle

Soft shell clams develop gametes in a well characterized manner that is documented by several studies (Coe and Tuner 1938, Shaw 1962, Ropes and Stickney 1965, Shaw 1965, Porter 1974). The events of reproduction can be described by examining the gonads under a microscope and categorizing the gonads into one of five stages, classified as inactive, active, mature, partially spent, and spent (Ropes and Stickney 1965). In this thesis, this classification was used

to collect data on the clam reproductive cycle and to characterize spawning events. The classification system used in this study was adapted and modified from previous studies (Coe and Tuner 1938, Ropes and Stickney 1965) to identify and understand the reproductive biological activities of soft shell clams. A brief description of each stage follows.

The inactive stage is characterized by a low rate of reproductive activity. Within this stage, it may be difficult to determine the gender of the specimen as the gonad is almost completely void of gametes. Clams in the inactive phase would include mature clams following spawning events, as well as immature juvenile clams prior to sexual maturity and the commencement of their first reproductive cycle. This stage may be found in immature clams at any time of the year.

In the active stage, clams expend energy within their reproductive organs for gamete production. The active phase is the beginning of gametogenesis. It is characterized by the development of immature oocytes during oogenesis in the female clam and the proliferation of primary spermatogonia and subsequent production of spermatozoa or spermatogenesis in the male clam (Ropes and Stickney 1965).

During the mature stage of the reproductive cycle, the alveoli are full of mature gametes as the clam prepares for spawning. Oocyte development continues in this stage with the oocytes filling the lumen. Each oocyte contains a well defined nucleus and nucleolus. As the oocytes increase in size and detach from alveolar walls, they are referred to as ova. In the male clam, spermatozoa are

arranged in radial columns with their tails oriented toward the center of the lumen (Ropes and Stickney 1965). There is no indication of gamete release during this stage.

In the partially spent stage, the clam releases a portion of its gametes into the water column for external fertilization. The alveoli are smaller than in the mature stage and reproductive ducts are partially emptied. However, mature gametes are still present within the alveoli (Ropes and Stickney 1965). Within the New England states, one researcher noted that ova and spermatozoa were released over time, with spawning occurring on more than one occasion (Brousseau 1978).

At the spent stage, most of the gametes have been expelled, spawning has completed and any remaining gametes are located in the periphery of the gonad. Hemocytes or blood cells may be seen grouping together in the connective tissue to reabsorb any unspawned gametes (McGladdery *et al.* 1993). In female clams, primary oocytes appear, histologically, as darkly stained bodies with obscure nuclei. Spherical products of cytolysis are also present (Barber 1996), formerly referred to as inclusions (Ropes and Stickney 1965), which is another sign of the completion of oogenesis. In male clams, spent tissues lack cells in the active phase of spermatogenesis. Follicular cells contain multinucleated non-pyknotic cysts and pyknotic cells, which is a sign of the completion of spermatogenesis (Ropes and Stickney 1965). Since the reproductive process is cyclic, the spent phase merges with and overlaps with the inactive phase.

Each stage is separately classified for ease of identification; however, it

should be noted that within a single clam, more than one stage of the reproductive cycle may be present at any given time as development and spawning are synchronous events. The stage selected for any clam's description is the one most representative of the entire animal. All of the stages represent a continuum of development and regression from which prominent features are selected to classify each clam. A clam may progress through the reproductive cycle in a matter of several weeks or months.

## 2.2 Objectives

The primary objective of this portion of the study was to histologically examine and describe soft shell clam gonad tissues to provide a reference for the evaluation of reproduction in soft shell clams on PEI. A second objective was to determine the reproductive dynamics of soft shell clams, in terms of the sequence and timing of gametogenic development, to explain the frequency and duration of spawning under natural conditions in different regions of PEI. This information is required to optimize the development of this species for aquaculture production. The final objective was to determine the factor influencing spawning events in each year of the study.

## 2.3 Materials and methods

### 2.3.1 Site selections and descriptions

All research took place on PEI (Canada). Three clam sites were chosen for

this study, and they were Oates Point, Barsway, and Gascoigne Cove (Figure 1). Oates Point and Barsway are centrally located on the north shore, within Malpeque Bay, approximately one hour northwest of the laboratory in Charlottetown. Gascoigne Cove is situated on the south shore of eastern PEI, in the Pinette River system, approximately forty-five minutes southeast of Charlottetown.

Sites were chosen in association with and under the advisement of the PEI Department of Fisheries and Tourism, Fisheries and Aquaculture Division, the Federal Department of Fisheries and Oceans (DFO) scientists, and PEI clam producers. Oates Point, Barsway and Gascoigne Cove represented suitable research sites based on a number of qualifying criteria, including biological parameters and logistic concerns.

Biologically, each site currently carried a clam population, and was known to support one in previous years as well. The sites contained areas with shallow and deep water for clam sampling. Logistically, each site was located centrally, each being less than one hour from Charlottetown. As well, all sites were easily accessible for specimen collection via truck or boat. Lastly, each site had been previously monitored by government scientists, thereby expanding the information base on clam population(s) and environmental parameters at the three sites. Scientific permits for the research were granted by DFO for each field season.

#### 2.3.1.1 Oates Point

The Oates Point study site is centrally located along the south side of the

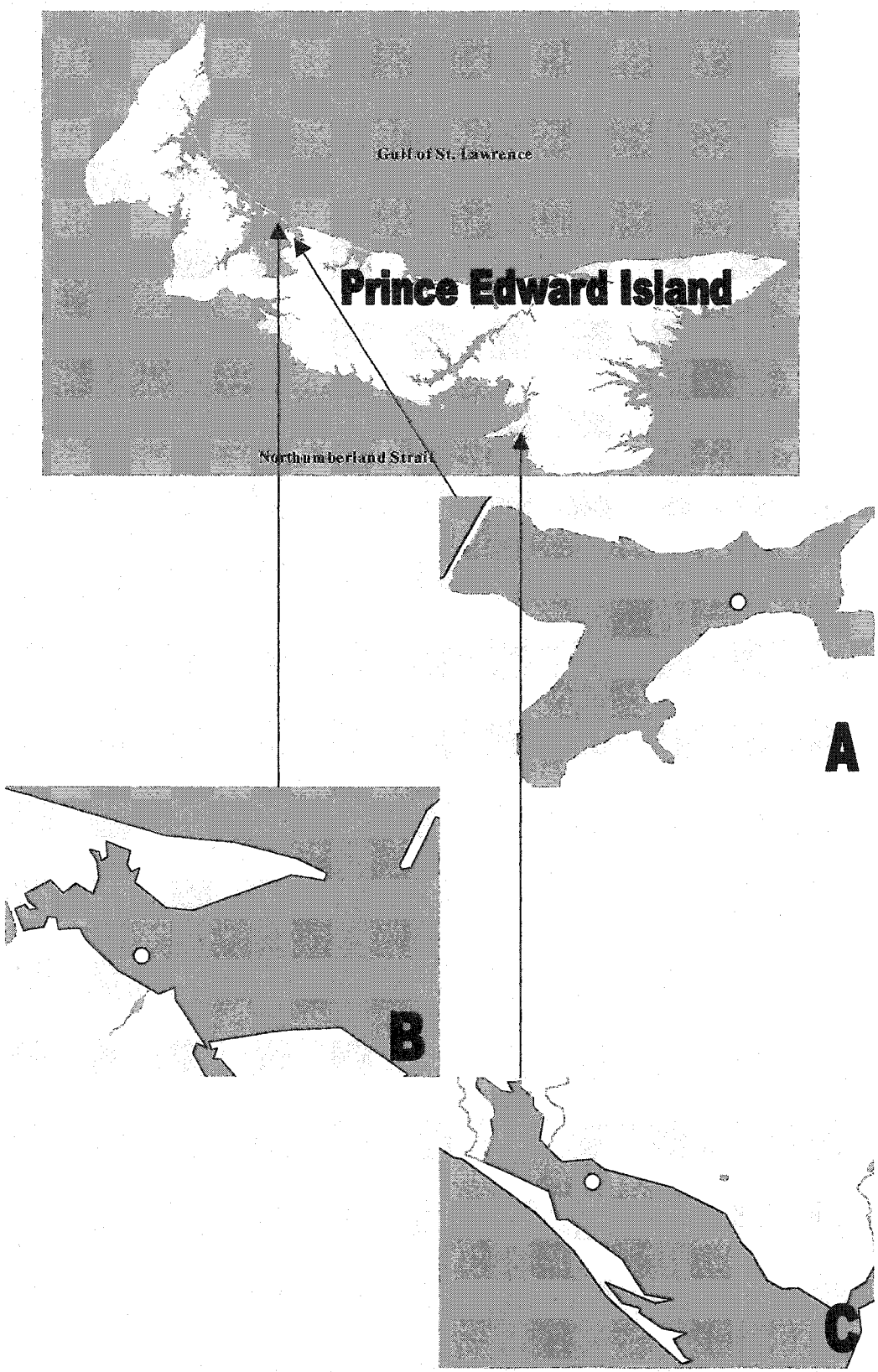


Figure 1. Schematic representation of Prince Edward Island and the three study sites (A) Oates Point, (B) Barsway and (C) Gascoigne Cove. Sampling sites marked with (○ ).

Baltic River at 46 53.1040 N and 63 65.9837 W (PEI Government 2001). This warm water site is the location of a clam lease, which has been in operation since 1993. The area is sheltered from harsh wind and wave conditions as the Baltic River consists of a narrow channel with shallow water and land on both sides. This Y-shaped waterway, located on the north shore, is approximately 4.1 km long and is connected to Darnley Basin, which in turn is fed by the Gulf of St. Lawrence. The Darnley Basin and Baltic River drain into Malpeque Bay.

#### 2.3.1.2 Barsway

The Barsway study site is located in the Darnley Basin at 46 55.3350 N and 63 69.6370 W (PEI Government 2001). This site is a public fishing area. Although Barsway and Oates Point are geographically close, their sites are very different. The Barsway site is an open water area with more wave action and boat traffic than the more sheltered and secluded Oates Point site.

#### 2.3.1.3 Gascoigne Cove

Gascoigne Cove is located along the Pinette River at 46 02.2234 N and 62 90.3223 W (PEI Government 2001). The Gascoigne Cove study site is a public fishing area. Like Oates Point, this area is a warm-water site that is sheltered from extreme weather conditions.

### 2.3.2 Sampling schedule

In 1997, clams were collected at the Oates Point and Barsway sites on a weekly basis from May 23 until July 17, then bi-weekly through to August 26, and then monthly until October 28. At Gascoigne Cove, clams were collected on a weekly basis from May 27 to July 31 and then bi-weekly until November 7, 1997. The sampling at all study sites for 1997 was conducted by staff of the PEI Department of Fisheries and Tourism, Fisheries and Aquaculture Division and sampling was conducted when time allowed within their schedule.

In 1998, clams were collected bi-weekly from April 1 through May 31, and weekly from June 1 through September 16 for each of the three study sites. This was the intended sampling schedule, with less frequent monitoring when it was anticipated that spawning would not be occurring and a more consistent sampling schedule being carried out when it was projected that clam spawning might be expected.

During 1999, clams were collected on a weekly basis between June 22 and August 31 at Oates Point, the sole sampling site in 1999. This was the intended sampling schedule as it coincided with a seed collection trial at the same site (see Chapter 5). The sampling regimen data for all three years of study is summarized in Appendix A.

### 2.3.3 Sample size

Twenty clams were collected at each of the three sites per sampling trip in 1997. Clams were grouped into shell length categories. Ten clams were collected between 20 - 49.99 mm and ten clams were collected between 50 - 75 mm in length.

The 1998 sample size was thirty clams per site visit within a shell length range of 20 - 75 mm. Within this range, ten clams were collected from each of the following size classes: 20 - 34.99 mm, 35 - 49.99 mm, and 50 - 74.99 mm in shell length. The sample size of ten clams per size class was chosen to remain consistent with the sampling design established by the PEI Department of Fisheries and Tourism in 1997. Again, collection was performed at each visit and at all three sites over the duration of the 1998 study.

In 1999, an attempt to collect ten clams with shell lengths surpassing 50 mm was made during each site visit. However, as it was not always possible to find enough clams of this length, clams as close as possible to 50 mm were collected during five of the sampling weeks. In any of the given five weeks, no more than five clams were less than the 50 mm size requirement and all clams exceeded 41 mm in length. Additionally, for all eleven weeks sampled, an additional clam with a shell length surpassing 50 mm was collected and preserved, so that a total of eleven clams was available for processing.

### 2.3.4 Sample collection

As soft shell clams live within the bottom substrate, various methods were used to dig them from shallow water regions of the research sites for histological evaluation. Digging by hand, using a round point shovel, or applying pressure to a plunger in order to burrow a deep hole into the sediment were among the most successful techniques used where distinct siphon holes were present in the substrate surface. All sampling was scheduled to occur during low tides.

When clams were first located at each of the study sites, an effort was made to collect all samples from that general area. As the sampling was opportunistic in nature, the first intact clams with the appropriate shell lengths were those sampled for histological analysis in all three years of the study. For any given date of sampling, clams were collected from within the smallest area possible.

Due to harvest techniques, some of the clams collected had cracked or broken shells. In order to avoid complications in assessing reproductive staging, these clams were excluded from the study. It was thought that any damage to the gonad region of the clams could alter the histologic evaluation, as clams might spontaneously release gametes when a trauma occurred to the body tissue.

In 1997, shell length measurements were taken at the study site with vernier calipers to the nearest millimeter (mm) on their longest axis. Shell lengths were re-measured and recorded, using electronic calipers (Model CD-8"C, Mitutoyo Corporation, Tokyo, Japan, 1997), in the laboratory. In 1998 and 1999, electronic

calipers (Model CD-8"C, Mitutoyo Corporation, Tokyo, Japan, 1997) were used at the study site to measure and record clam shell lengths to the nearest 0.01 mm along the longest axis, anterior-posterior.

### 2.3.5 Environmental temperature

A conductivity meter (YSI model 30, YSI Incorporated, Yellow Springs, Ohio, USA, 1998) was utilized to collect water temperature data in 1997, 1998 and 1999. This parameter was measured at each site just below the surface of the water during each visit over the duration of the study. In addition, VEMCO Minilog-TR version 2.08 (VEMCO Limited, Shad Bay, Nova Scotia, Canada, 1998) temperature and depth data loggers were deployed at each site each year to monitor and record hourly water temperatures for the duration of the study.

### 2.3.6 Tissue preparation

In 1997, the clams collected for histology were transported back to the laboratory in a cooler with ice. In 1998 and 1999, clams underwent tissue preparation on site as it was thought that transporting the specimens intact could induce spawning. By preserving the tissues on site, a more credible depiction of the clam population could be captured. Following on site preservation, the 1998 and 1999 samples were transported to the laboratory.

Clams were measured for shell length, and the soft tissue was removed from the shells using a disposable scalpel as described by Howard and Smith (1983). The siphon and mantle tissue were trimmed away from the rest of the specimen. The remaining tissue, including the foot, digestive gland, and reproductive organ were preserved in ten percent buffered seawater formalin (Howard & Smith 1983). Pre-labeled plastic jars containing the fixative and information such as location, date, and size range of clams found in the container were used for all clam samples.

After a minimum of 48 hours post fixation, a final trimming of the tissue was performed by making two transverse sections (5 mm) through the clams, cutting into kidney, digestive gland, gonad, gills and foot. These cross-sections were then placed in labeled cassettes and preserved in ten percent buffered seawater formalin. Standard histological processing as described by Rosenblum and Niesen (1985) provided 5 micron (μm) sections of the tissue which were subsequently placed on microscope slides. Hematoxylin and eosin (Shandon™ Instant Hematoxylin and Eosin Y) stain was added to the slides to enhance differences between the major tissues and then the slides were cover slipped. Each slide held anywhere from 1 to 4 clam specimens, depending on the size of the tissues.

### 2.3.7 Histological analysis

Examination of the slides was performed using light microscopy. Clam gonadal tissue was examined for gender and ranked into one of five stages of

gonad development as described by previous investigators (Coe and Turner 1938, Shaw 1963, Ropes and Stickney 1965) and elaborated on by many (Brousseau 1978, Rosenblum and Niesen 1985). As discussed previously, the five main stages are inactive, active, mature, partially spent, and completely spent. For specimens in which reproductive stage was not clearly discerned due to poor quality of the tissue in histologic preparation of the section, a category referred to as unknown was established. In these cases, male or female gametes were not visible.

Approximately sixty of the histological slides containing clam specimens were selected to assess the repeatability of the staging technique by reclassifying the reproductive stages. Each site and each year of study was represented in the sampling. In all cases, two readers performed blinded readings of the clam tissues.

Photographs of representative stages of the male and female reproductive cycle were taken with a light microscope under the same magnification with a 10x objective using a digital camera.

### 2.3.8 Statistical analysis

Data collected over the three-year period were entered and stored in a computer spreadsheet (Quattro® Pro version 8, Corel® Corporation Limited, Ottawa, Ontario, Canada, 1998). All data was examined and manually checked for errors against the original data entry sheets. The gonadal evaluations were grouped by stage on a monthly basis, at each site and for each year. Standard

deviations were calculated, and graphic representations were made using Quattro® Pro 8 program.

The data were analyzed using the statistical software STATA™ version 7.0 (Stata Corporation, College Station, Texas, USA, 1984 - 2002). Random effects logistic regression was used to model the effect of month, temperature, shell length grouping and gender on histological stages dichotomized between mature clams versus clams that were partially spent. Each year of sample collection was analyzed separately. Sampling date was included as a random effect. Variables were removed using a backward elimination procedure. Categorical variables were tested as a group using a likelihood ratio test. For all analyses, differences were considered significant when $P \leq 0.05$.

## 2.4 Results

### 2.4.1 Gonad development in soft shell clams on Prince Edward Island

#### 2.4.1.1 Male clam

Histologic examination of the inactive male gonad of the soft shell clam revealed shrunken alveoli, which were largely empty with the exception of follicular cells containing basophilic staining pyknotic cells, or degenerative cells with a single nucleus, and cysts of multinucleated non-pyknotic cells (Figure 2). Both of these are products of spermatogenesis (Ropes and Stickney 1965). Very few or no mature gametes were present, as no active spermatogenesis occurs at this stage.

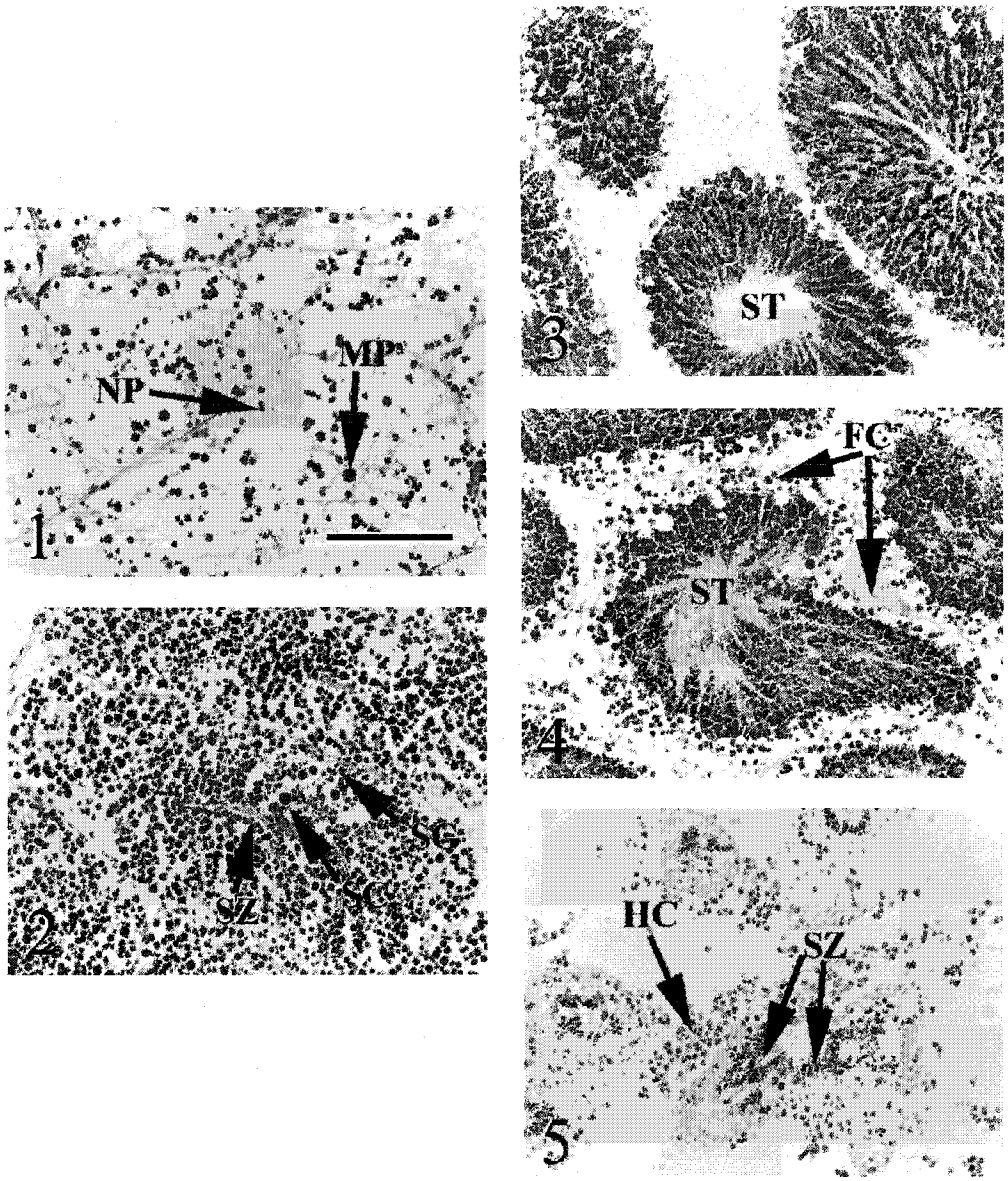


**Figure 2.** Representative stages of the male reproductive cycle in soft shell clam, *Mya arenaria,* gonads. Slides were stained with hematoxylin and eosin and were photographed under the same magnification with a 10x objective. Scale bar shown in photo 1 is equal to 100 μm. (1) Inactive stage showing multinucleated pyknotic cysts (MP) and non-pyknotic cells (NP). (2) Active stage of reproductive cycle with spermatogonia (SG), spermatocytes (SC) and spermatozoa (SZ). (3) Mature stage alveoli containing spermatozoa oriented with tails (ST) inward and heads outward. (4) Partially spent alveoli with empty follicle cells (FC) and a reduced lumen containing spermatozoa. (5) Spent stage with very few spermatozoa remaining and the presence of hemocytes (HC).

Connective tissue containing hemocytes was noted, as hemocytes reabsorb any unspawned materials.

Active male gonads clearly showed the stages of spermatogenesis occurring (Figure 2). The entire process of spermatogenesis occurs in this stage. Primary spermatogonia were proliferating at the base of the alveolar membrane. These spermatogonia differentiated into spermatocytes, then spermatids and ultimately spermatozoa. The spermatocytes were located between the spermatogonia and the lumen of the alveoli. The presence of a few spermatozoa migrating toward the lumen between follicular cells was also apparent.

Examination of mature male gonads showed eosinophilic or pink spermatozoal tails arranged in radial columns pointing toward the center of the lumen (Figure 2). The spermatozoal heads were oriented outward to the base of alveoli. Each alveolus was full of mature gametes.

Partially spent male gonads contained fewer spermatozoa than the active stage (Figure 2). Empty rows of follicular cells were present along the alveolar membrane, with fewer spermatogonia present, while the central cells in the alveoli were still undergoing spermatogenesis. The presence of pyknotic cells was recognizable within the some of the follicular cells.

Spent male gonads showed hemocyte infiltration occurring to reabsorb the remaining spermatozoa (Figure 2). Alveoli contained a reduced lumen with very few spermatozoa. No signs of active spermatogenesis were occurring at this time.

Numerous follicular cells contained multinucleated non-pyknotic cysts and pyknotic cells.

#### 2.4.1.2 Female clam

Examination of the inactive female gonad revealed numerous follicular cells with eosinophilic spherical products of cytolysis called inclusions (Figure 3). Inclusions, present at this stage and others, are foreign bodies or nonliving masses such as starch grains or droplets of fat, in the cytoplasm or nucleus of a cell (Ropes and Stickney 1965). Phagocytes, which are cells in the bloodstream and tissues, engulf and ingest unwanted substances from the follicular cells such as unspent oocytes. Very few, if any, oocytes were present at this stage.

Examination of the active stage of female gonads showed a commencement of gametogenesis. Small alveoli were present, containing peripheral oocytes adjacent to the alveolar membrane and were attached by stalks. Some oocytes were noted beginning to grow between the follicular cells. These oocytes had broad bases and were irregular in shape until more growth occurred. As the oocytes became larger and round in shape, a more constricted base developed. Very few larger oocytes were seen free within the lumen of the alveoli.

Fully mature ova were present with a well defined nucleus and nucleolus in the mature stage (Figure 3). A very slender stalk connected some of the oocytes to alveolar walls, while large numbers of mature ova were completely round and

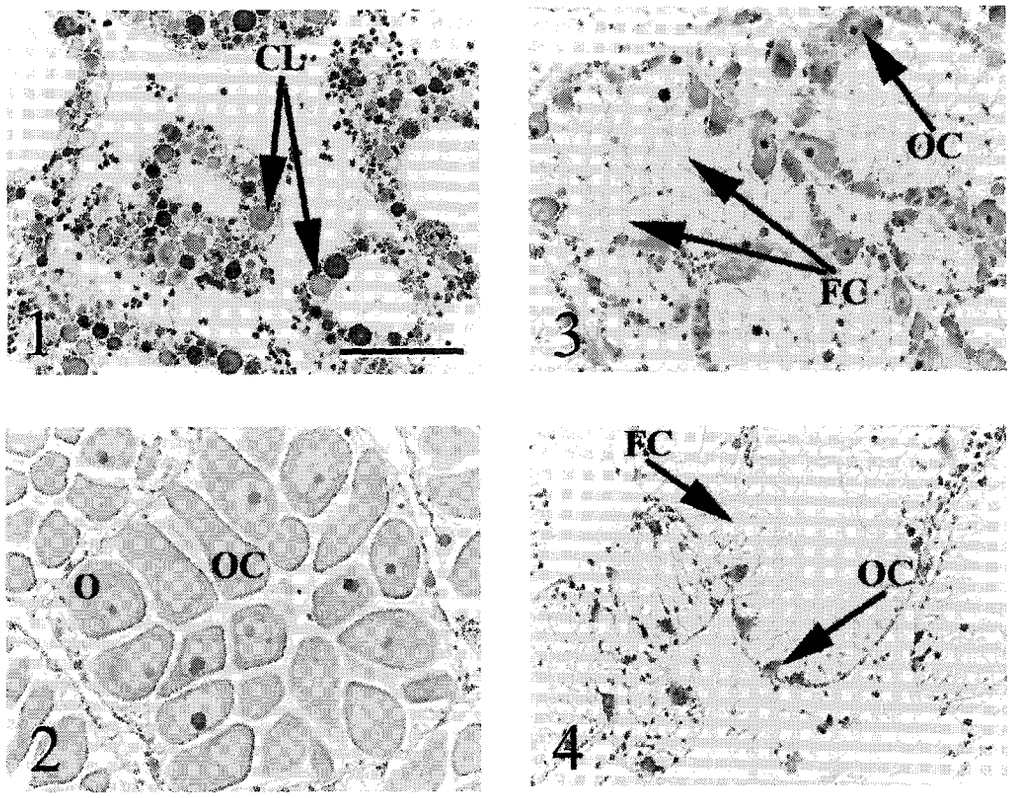


**Figure 3.** Representative stages of the female reproductive cycle in soft shell clam, *Mya arenaria,* gonads. Slides were stained with hematoxylin and eosin and were photographed under the same magnification with a 10x objective. Scale bar shown in photo 1 is equal to 100 μm. (1) Inactive stage showing spherical products of cytolysis (CL) and lack of gametes. (2) Mature stage with an increasing number of oocytes (OC) and much larger alveoli. Alveoli also contain mature ova (O). (3) Partially spent alveoli with reduced number of oocytes and empty follicle cells (FC). (4) Spent stage with very few gametes in alveoli and products of cytolysis. Note that any remaining oocytes are reduced in size.

unattached. These large ova filled the lumen and were more numerous than those seen in the active phase. Alveoli were greatly increased in size at this point and the number of female gametes present also increased compared to the active stage. Within this stage, no gametes were released.

Partially spent female gonads contained decreasing numbers of oocytes attached by stalks along the periphery of the alveolar membrane (Figure 3). Smaller oocytes were present, imbedded in follicular cells. An absence of mature oocytes in many of the alveoli and the cessation of oogenesis in all alveoli was indicative of the partially spawned condition.

The spent stage of the female clam reproductive system showed reabsorption of the few remaining darkly stained oocytes (Figure 3). Inclusions were present in the follicular cells. In the female clam, spent individuals were distinguished from those in which no gametogenesis had taken place at all by the presence of a few unspent oocytes in early stages of cytolysis.

### 2.4.2 Histological analysis

All specimens collected in 1997 and 1999 were processed into slides for histologic evaluation. Specimens collected and preserved in 1998 at the Barsway and Gascoigne Cove sites were not processed into slides for histologic evaluation due to budget constraints. Of the specimens collected and preserved from Oates Point in 1998, only the two larger size ranges were processed as female clams in

the southern Gulf of St. Lawrence typically reach sexual maturity at shell lengths between 35 – 40 mm (Fisheries and Oceans Canada 1996). Therefore, there was no need to process clams ranging in shell length from 20 to 34.99 mm.

A total of 1012 clam specimens was evaluated for reproductive stage by a single reader to ensure overall consistency and accuracy. Of this number, 522 were identified as female, 454 were male and 36 were labeled unknown.

Sixty slides containing anywhere between 1 and 4 clam specimens were selected from the 1012 samples to assess the repeatability of the staging technique by having the original reader and a second reader conduct blinded classifications of the specimens. Agreement was noted over eighty-five percent of the time among the readers doing the reproductive reassessment. The only reproductive stage that readers disagreed on was the active stage in the male clam, as it was classified by one party as partially spent.

#### 2.4.2.1 Histologic classification of reproductive stages in 1997

In 1997 at Oates Point, 60.00 percent of the clams sampled in the month of May were found to be in the mature stage of the reproductive cycle. Large proportions of partially spent clams were found in the 1997 Oates Point sampling within the months of June, July and August, at 57.35, 65.43, and 69.23 percent respectively (Figure 4). The percentage of mature stage clams decreased as the percentage of partially spent clams increased over time. By September, all clams

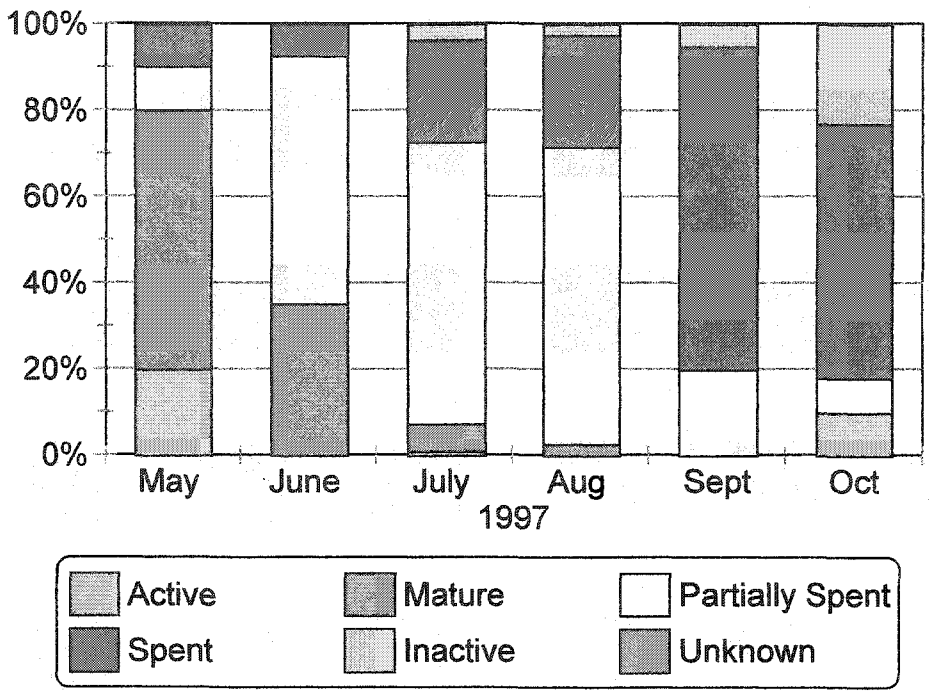


**Figure 4**. Stage of reproduction in gonads of soft shell clams (in percentage) at Oates Point, collected from May to October 1997.

sampled were in the partially spent, completely spent or inactive stage of reproduction.

At the Barsway site in 1997, 84.81 percent of the clams sampled in the month of June were in the mature stage of the reproductive cycle; this decreased to 12.50 percent by July. Spawning, indicated by the appearance of partially spawned clams, increased to 30.00 percent during the month of July, from 6.33 percent in the month of June (Figure 5). Of the clams sampled in the month of July, 20.00 percent of them were completely spent and an additional 25.00 percent were classified as inactive. In September and October of 1997 at Barsway, there were no clams in the mature stage of the reproductive cycle. The percentage of clams reaching the inactive stage of their reproductive cycle increased throughout the months sampled, from 2.53 percent in June to 57.89 percent of all clams sampled at that site in October.

The histologic examination of clams at Gascoigne Cove in 1997 indicated that the percentage of mature stage clams in the months of May, June and July, were 68.42, 77.33, and 58.59 percent, respectively (Figure 6). By August, the proportion of clams in the mature stage declined to 20.00 percent, and in September and October, there were no clams in the mature stage. The percentage of clams reaching the inactive stage of their reproductive cycle increased from 0 percent in May and June up to 53.85 percent of clams sampled in October, and then decreased to 35.00 percent of all clams sampled in November.

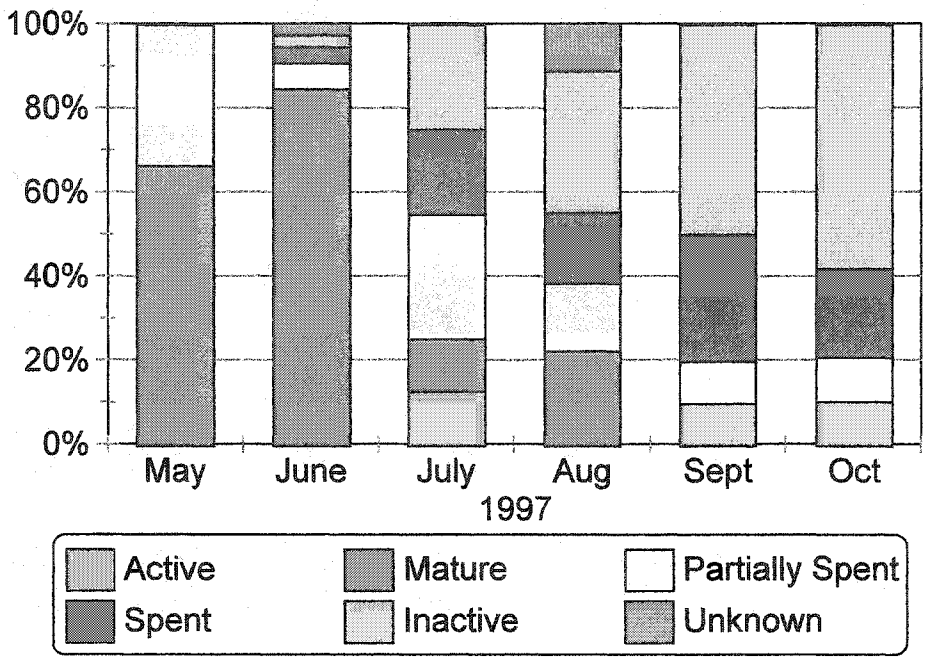


**Figure 5.** Stage of reproduction in gonads of soft shell clams (in percentage) at Barsway, collected from May to October 1997.

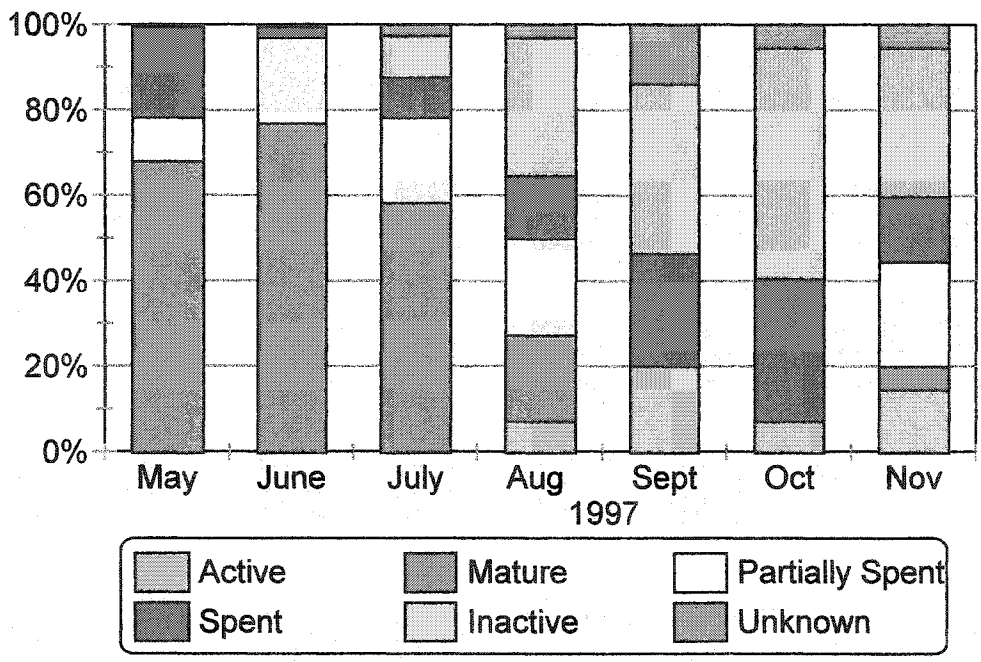


**Figure 6.** Stage of reproduction in gonads of soft shell clams (in percentage) at Gascoigne Cove, collected from May to November 1997.

The percentage of mature clams over the weeks sampled at Oates Point in 1997 indicated that spawning commenced following June 18, as 50.00 percent clams were mature on that date, and ended by July 11 with no mature stage clams (Figure 7).

When examining the percentage of mature clams over the weeks sampled at Barsway in 1997; 95.00 percent of the clams were in the mature stage on June 26, and this declined to 10.00 percent by July 2 (Figure 8). Therefore, it appeared that a large spawning event had occurred after June 26, with spawning continuing through to July 2.

When examining the percentage of mature clams over the weeks sampled at Gascoigne Cove in 1997, it appeared that a large spawning event occurred commencing following June 25, as 100 percent of the clams were mature on June 25 (Figure 9). The percentage of mature clams decreased gradually for two months, reaching a low of 5.00 percent on August 25.

#### 2.4.2.2 Histologic classification of reproductive stages in 1998

From the sampling regimen in 1998 at Oates Point, no mature stage clams were present in the month of April and 60.00 percent of the clams in April and May were partially spent (Figure 10). Moreover, 30.00 percent of the clams sampled in the month of April were in the active stage of reproduction. However, by June,

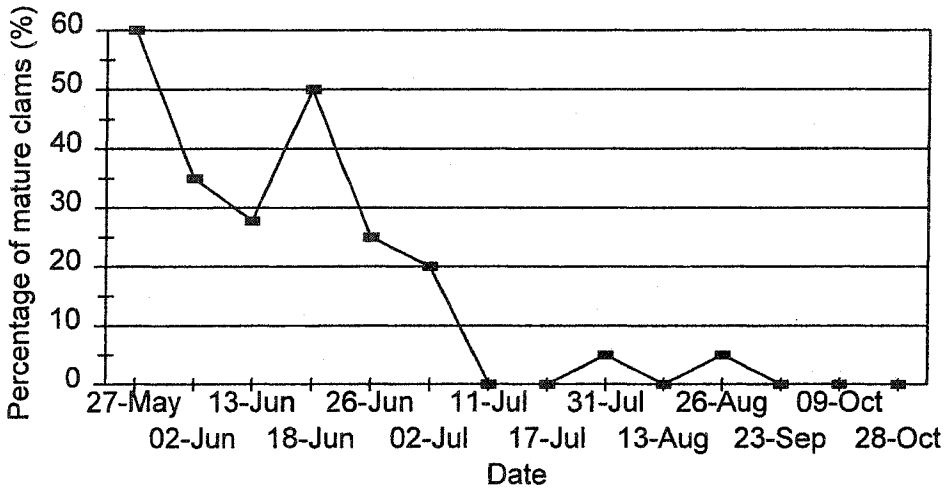


**Figure 7**. Percentage of mature clams over time at Oates Point in 1997.

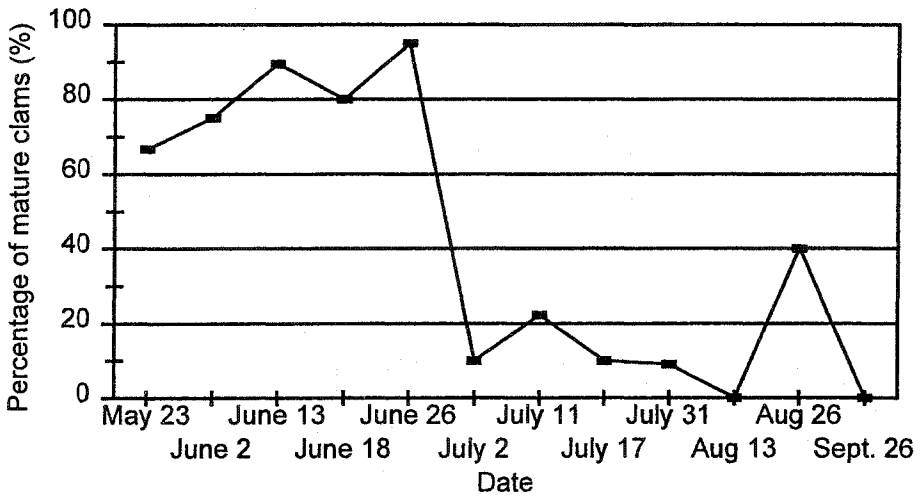


**Figure 8.** Percentage of mature clams over time at Barsway 1997.

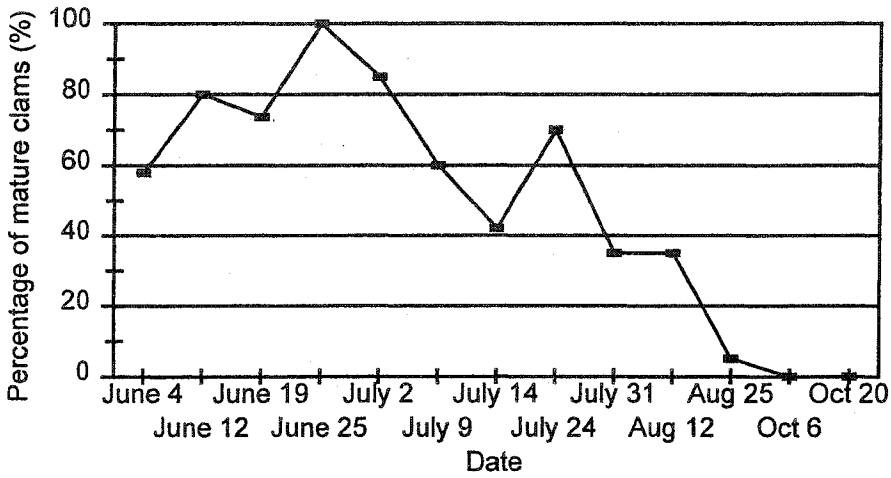


**Figure 9.** Percentage of mature clams over time at Gascoigne Cove 1997.

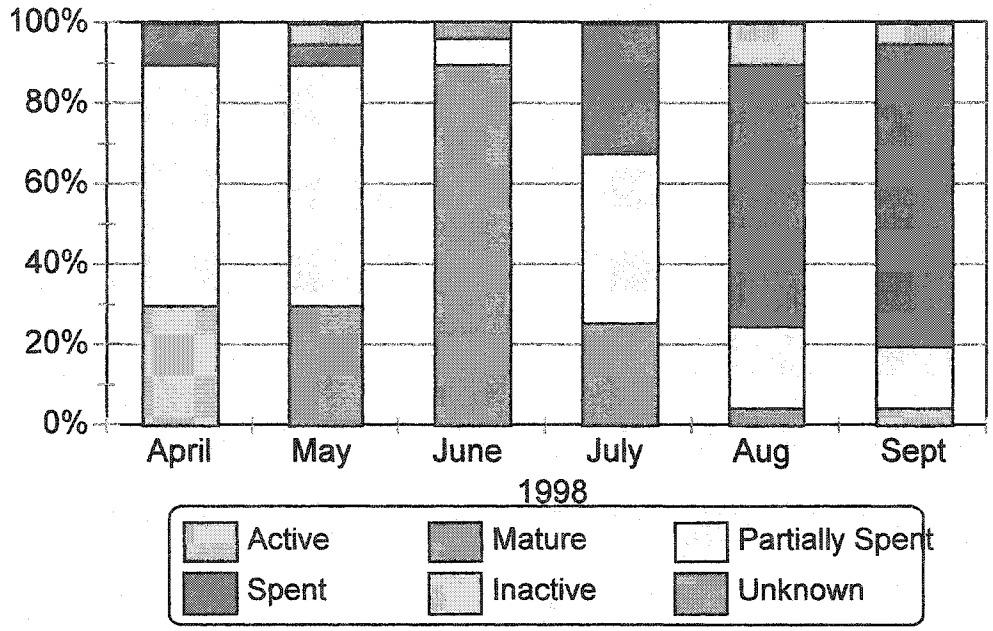


**Figure 10**. Stage of reproduction in gonads of soft shell clams (in percentage) at Oates Point, collected from April to September 1998.

90.00 percent of the clams sampled were in the mature stage. This decreased to 26.00 percent of mature stage clams by July.

When examining the percentage of mature stage clams at Oates Point in 1998, there was a decline from 90.00 percent on June 8 to 10.00 percent on July 2 (Figure 11). This would indicate that a spawning event had occurred within this time frame at Oates Point.

#### 2.4.2.3 Histologic classification of reproductive stages in 1999

The 1999 Oates Point sampling indicated that no mature stage clams were present in the months of June or July. In August, 1.75 percent of the clams were classified as mature. Between 82.46 and 90.91 percent of the clams sampled throughout the field season were either partially spent or completely spent (Figure 12).

### 2.4.3 Environmental temperature

All three study areas were near shore where low tide sampling water depths ranged from approximately 0 to 110 cm. Water temperatures were not retrieved from the Oates Point collection site for 1997 as the remote data logger was lost during the study. For the same year, temperatures ranged between 13.5°C in June and 21.6°C in August at the Barsway site, while the Gascoigne Cove site had water temperatures ranging from 6.55°C in October to 20.59°C in July (Figure 13).

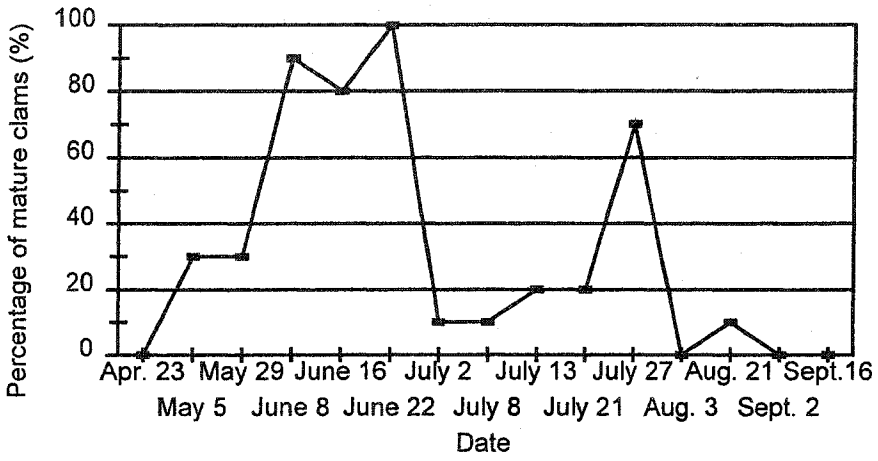


**Figure 11.** Percentage of mature clams over time at Oates Point 1998.

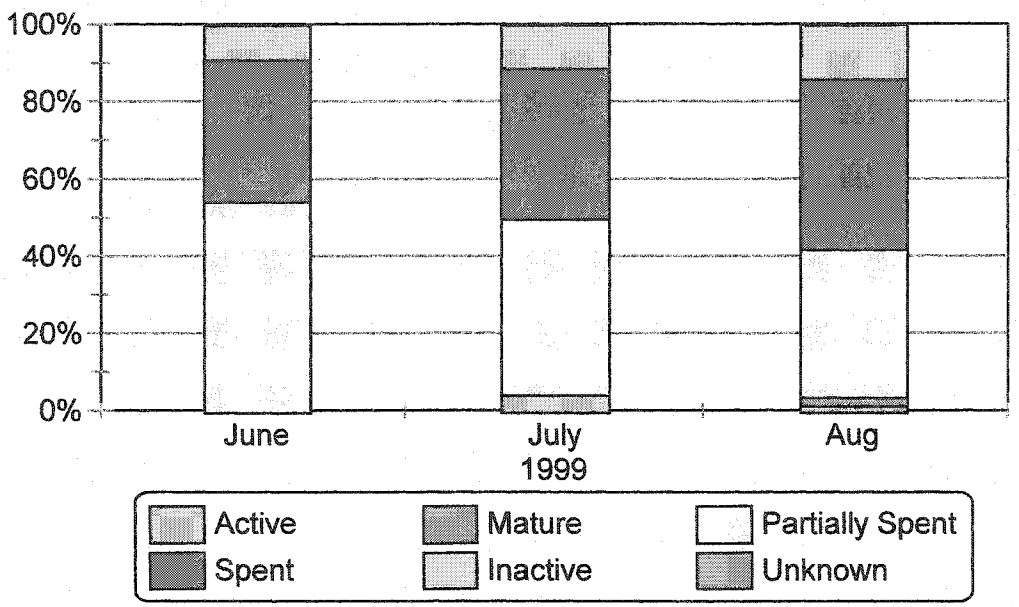


**Figure 12.** Stage of reproduction in gonads of soft shell clams (in percentage) at Oates Point, collected from June to August 1999.

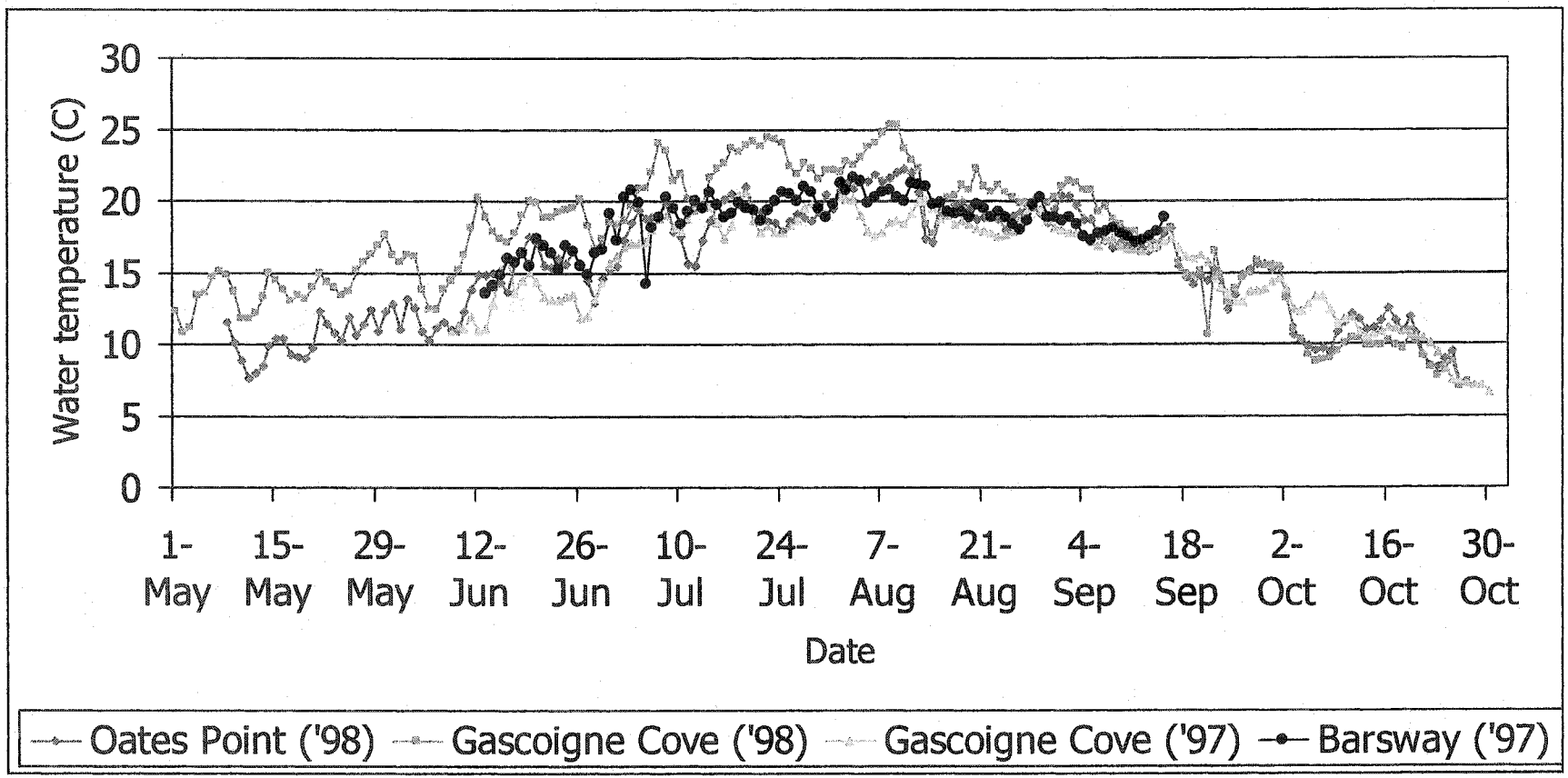


**Figure 13.** Water temperature over time at Gascoigne Cove and Barsway in 1997 and at Gascoigne Cove and Oates Point in 1998.

In 1998, water temperature at Oates Point ranged between 7.27°C in October and 22.22°C in August (Figure 13), while the data logger from Barsway was not recovered from the site. At the Gascoigne Cove site, temperatures ranged from 7.35 in October to a maximum of 25.28°C in August (Figure 13).

In the 1999 study, temperatures ranged between 17.60°C and 22.20°C for the months of June to August at Oates Point. The temperature data for all three years of study is summarized in Appendix B.

### 2.4.4 Predictors of spawning

Of the 1012 clams histologically examined, 123 of the specimens were not tested statistically as they were obtained in the 1999 study year. In that year, spawning had already occurred prior to the commencement of the field sampling, so it was impossible to predict spawning from the data. For the remaining 889 clams evaluated (1997-1998), 33 of them were discarded as the gender was unidentifiable. In most of these cases, reproductive stage was also undeterminable. Therefore, statistical analyses were conducted on the remaining 856 specimens.

For the 1997 data collected at all three sites, temperature was determined to be the only significant influence of spawning when compared to mature and partially spent clams ($P \leq 0.01$). For each one degree Celsius increase in water temperature, the probability of spawning increases by a factor of 1.503. The effect of temperature on spawning can be seen in Figure 14.

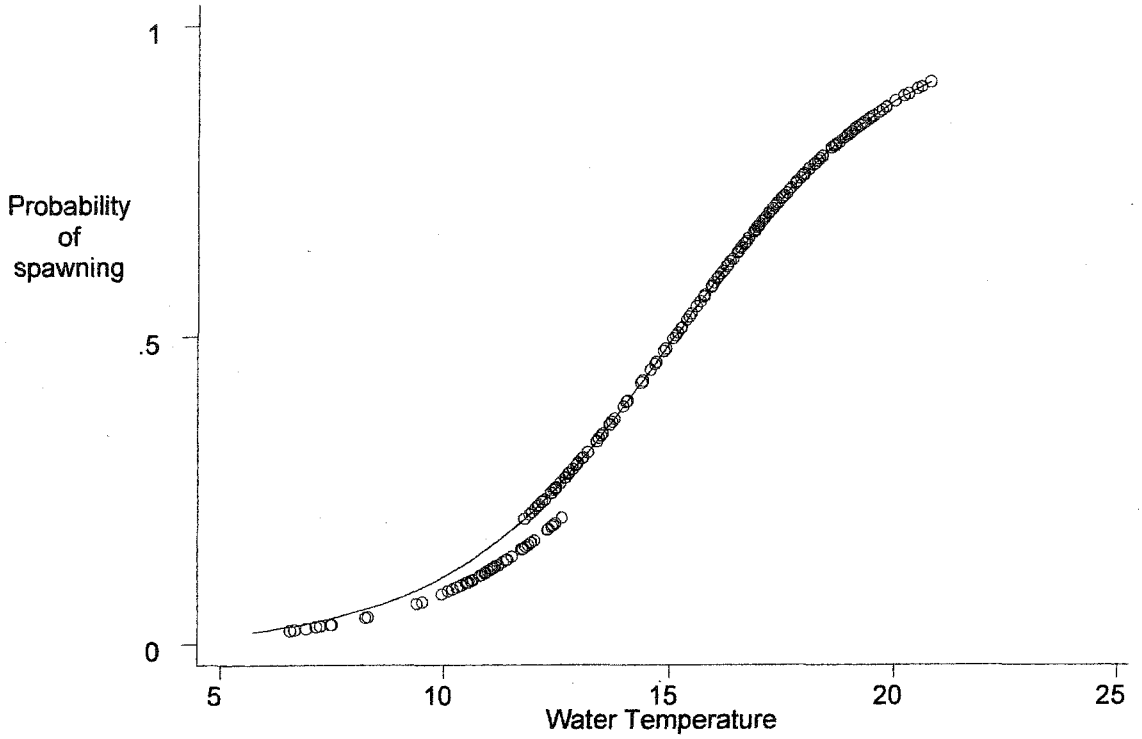


**Figure 14.** Predictive probabilities for spawning to occur versus water temperature, in degrees Celsius, for the study sites in 1997.

The effect of water temperature on the 1998 data was insignificant in 1998. For the 1998 data collected at Oates Point, month was determined to be the only significant influence on spawning events when compared to mature and partially spent clams ($P \leq 0.01$). Month of year had a large predictive effect, indicating that the probability of spawning in July was 24 times greater than spawning in June (Figure 15).

## 2.5 Discussion

One of the concerns in assessing reproductive stages in bivalves is that spawning can inadvertently be induced by artificial conditions in a study. There was a concern that clams from the 1997 study may have commenced spawning while in transit to the laboratory. The change for the clams, from warm water to being chilled on ice and jostled around for approximately a one hour drive, may have affected the results. Therefore in subsequent field seasons (1998 - 1999) clams were preserved on site. Even with this precaution, clams in the 50 -74.99 mm size range collected from Oates Point on July 13, 1998 appeared to spontaneously release gametes when they were placed in fixative. This may have altered the histologic staging for those specimens from a mature to partially spent stage of reproduction. Therefore, it is important to interpret the data with a degree of hindsight from the previous sampling.

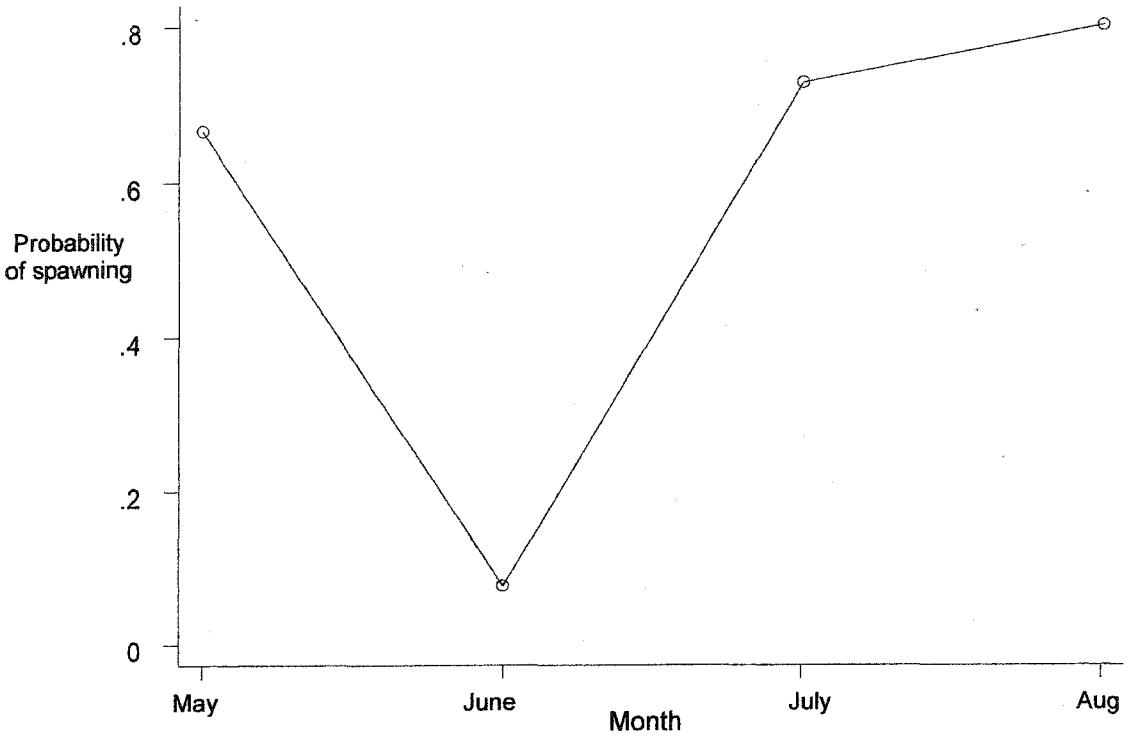


**Figure 15.** Predictive probabilities of spawning versus month of year for study sites in 1998.

The ranking of clam gonads in order to determine their reproductive stage may be considered a subjective analysis. The same individual evaluated all histological preparations so that direct comparisons could be made between sampling dates and study sites. In very few cases, the reproductive stage was not identifiable. Reasons for this would include poor sectioning of specimens prior to histologic tissue processing. In this case, gender was often also undeterminable.

In assessing the repeatability of the staging technique, the discrepancies noted between readers with respect to male clams in the active stage were not seen as critical to the project work. As this study focused on the actual spawning event, characterized by the mature stage of reproduction, the results were not impacted by the differences.

In 1997, the dates of soft shell clam spawning were found to be similar, regardless of site, using histological analysis of clam gonads. The peak of mature stage clams was in mid to late June in 1997. This corresponded to the peak of mature clams at Oates Point in 1998, early June, as well. The first date sampled in 1999 was June 22, as the sampled coincided with another facet of the overall study, and by then, spawning had already occurred.

As the clams in all three study sites spawned within a week of each other, this information would indicate that factors in the natural environment contributed to the spawning activities in 1997. Water temperature was found to be significantly associated with the onset of spawning in 1997, but as this factor was not found to

influence spawning in 1998. The actual role of temperature in the timing of gamete release remains unclear. A critical temperature has been hypothesized as a potential trigger, as this is common in other species such as the American oyster. The rate change of temperature over a short period of time has also been speculated as being crucial to triggering spawning events. In this case, the difference between water temperature of covered clam beds at high tide and the temperature of interstitial water on exposed mudflats at low tide may be may be a potential factor in the onset of spawning.

Month of year was found to be the only statistically significant factor influencing spawning in 1998. The potential biological and physical covariates of month of year, such as day length changes over the field season, are not fully understood, nor were they investigated in this study, and these may have contributed to month being a factor.

With varying results between years, it appears that neither temperature nor month of year is the sole predictor of spawning. Instead, the information indicates that there is likely more than one factor involved in soft shell clam spawning. The evaluation additional parameters would be recommended for future analysis in this field of study.

In 1997 at Gascoigne Cove, the duration of spawning was approximately 2 months as the majority of clams examined were in the mature or partially spent stage between late June and mid August. This contrasts to the Barsway site in the

same year in which spawning occurred over a one-week window between late June and early July. The Oates Point site spawning duration lasted approximately 3 weeks with a large percentage of partially spent clams between mid June and mid July. In 1998, the Oates Point site clams spawned from early June into early July. Spawning occurred prior to the commencement of sampling at Oates Point in 1999.

From histological evaluation of gonads, it appears that the frequency of soft shell clam spawning on PEI occurred once in all three sites in 1997 as well as in the site monitored in 1998. During the field season in 1998, there was one spawning event as indicated in the specimens collected at the Oates Point research site. With approximately 60 percent of the clams already partially spent in April and May, it appears that there may have been an additional spawning event prior to the first date of sampling at Oates Point in 1998. With only one sampling date in the latter part of April, it seems more likely that spawning occurred earlier in the month of April, rather than clams remaining in a partially spent stage throughout the winter. However, there is no data to verify this spawning event.

With the shorter time frame monitored at the study site in 1999, the data shows evidence of a single spawning event. Prior to the commencement of sampling on June 22, 1999, there was a complete spawning of all the clam specimens collected at the Oates Point study site as 100 percent of the clams sampled in June were partially spent, completely spent or inactive.

The methodology for measuring clams changed between 1997 and subsequent years due to a problem in achieving the correct number of clams in each size range. With the Vernier plastic caliper measurements, it appeared that there were enough clams for each size range. Sand particles also interfered with the correct operation of the calipers. Once clams were measured with the electronic calipers, it was found that there were shortages in some groups and surpluses in others. This was resolved by measuring clams on site with the more accurate tool.

Although the Oates Point site had a high density of clams, it was challenging to collect the sample size of ten clams over 50 mm in shell length in 1999. In that summer, high mortalities were noted within a short time span, making it difficult to find clams for the sampling regimen. Histologic evaluation of clams from this site for an independent study carried out by Fisheries and Oceans Canada in Moncton, New Brunswick found that the prevalence of haemic neoplasia, or leukemia, ranged from 9 to 80 percent of the clams sampled at any given time (McGladdery *et al.* 2001). Therefore, this disease outbreak, and subsequent mass mortalities of clams, affected the ability to collect enough clams within the pre-determined shell length range. McGladdery *et al.* (2001) also examined histology samples from the same study site for 1998, and found only background levels, in the order of 5 percent, of the haemic neoplasia disease.

## 2.6 Conclusions

The frequency of spawning appears to be similar throughout the study period; regardless of site, in that one spawning event occurs each year. The only site where this may be in question is the Oates Point area in 1998, as there may have been a spawning event in the spring months prior to the start of sampling. In the year that data was analyzed for all three study sites (1997), dates of spawning were found to be similar. The 1997 dates of spawning were similar to the spawning date at Oates Point in 1998.

Neither temperature nor month of year alone appears to be the sole predictor of clam spawning events on PEI. Instead, it is highly probable that the combination of more than one factor is involved in the onset of clam spawning. Future studies should be focused on evaluating a greater number of environmental conditions and testing their likelihood to predict spawning in a multivariate model. The accurate prediction of soft shell clam spawning is an important piece of information required for the development of aquaculture on PEI.

# 3. EVALUATION OF CONDITION INDICES AS INDICATORS OF SOFT SHELL CLAM (*MYA ARENARIA*) SPAWNING ON PRINCE EDWARD ISLAND

## 3.1 Introduction

Condition indices measure the meat content relative to the total size of shellfish (Hawkins and Rowell 1987). These indices have been used extensively for many years to assess *Mytilus edulis* (Seed and Suchanek 1992) and *Crassostrea virginica* (Crosby and Gale 1990) species, both in scientific research and in the commercial fishery. Various methods for measuring and calculating condition index in bivalves were reviewed by Crosby and Gale (1990). Condition indices may be used to reflect different stages of the reproductive cycle, as large amounts of the visceral mass may be occupied by the gonads (Robert 1981). Condition indices are known to be affected by seasonality (Hawkins and Rowell 1987, Crosby and Gale 1990, Seed and Suchanek 1992). A dramatic decline in condition index may coincide with spawning events in the population (Seed and Suchanek 1992).

As any bivalve prepares itself for gamete production and spawning, it puts most of its energy into reproductive development (Crosby and Gale 1990). As the reproductive cycle reaches the mature phase, the gonad becomes larger, resulting in the tissue component of the animal weighing more relative to the shell. As the clam spawns, tissue weight decreases as compared to the shell weight. It is a normal phenomenon that the body condition, or flesh weight, of an animal changes

over the year, as the shell weight remains relatively constant (Zwarts, 1991).

Histologic analysis of gonad tissue is an accepted tool for monitoring clam reproductive development and spawning as it has been used by numerous researchers over the last century. It is the standard reference procedure to indicate spawning events. However, it is costly, labour intensive and requires specialized training for interpretation.

Three types of condition indices were used in this study to evaluate soft shell clam spawning at three sites in PEI; steam meat yield, a gravimetric condition index, and a shell condition index. Numerous condition indices exist for measuring condition of bivalves. Of the various condition indices used in shellfish culture, steam meat yield is the industry standard on PEI, therefore it was chosen for further evaluation. The gravimetric index and shell index were selected as part of this study as they were the recommended condition indices in previous work done by Crosby and Gale (1990). These researchers concluded that the gravimetric index was easy to use, had fewer measuring errors and that it should be accepted as the standard method for determining bivalve condition index (Crosby and Gale 1990). The shell index was ranked second by Crosby and Gale (1990) and was described as being an index that focuses on somatic and gametic processes and not on nutritive status of the specimens.

## 3.2 Objectives

The primary objective of this study was to determine the date(s), frequency

and duration of soft shell clam spawning under natural conditions in different regions of PEI by calculating and monitoring condition indices. Another objective was to determine if condition indices can be effective predictors of spawning when compared against histologic analysis, which is described in Chapter 2.

## 3.3 Materials and methods

### 3.3.1 Site selections

All research took place on PEI (Canada). The three study sites, as described in section 2.3.1, were employed in the evaluation of condition indices for soft shell clam spawning. The clam sites were chosen for this study were Barsway, Oates Point and Gascoigne Cove. All of the study areas were in the intertidal shoreline where low tide water depths ranged from approximately 0 to 110 cm. during sampling.

### 3.3.2 Sampling schedule

In 1997, clams at Gascoigne Cove were collected for steam meat yield on a weekly basis from May 27 to July 31 and then bi-weekly until November 7. Clams were collected at Barsway and Oates Point on a weekly basis from May 27 until July 17, then bi-weekly through to August 26, and then monthly until October 28, 1997. In 1998, soft shell clams were collected for steam meat yield, gravimetric index and shell condition index calculations bi-weekly from April 1 through May 31, and weekly from June 1 through September 16 for all three clam sites.

### 3.3.3 Sample size

Sixty clams were collected at each site per sampling trip in both 1997 and 1998. The sixty clams were divided into two size ranges of thirty clams: 20 - 49.99 mm and 50 - 74.99 mm in 1997, and 35 – 49.99 mm and 50+ mm in 1998.

The size range sampled for the larger clams in both years commenced at a shell length of 50 mm. This shell length was chosen as it represents the minimum legal size for soft shell clam harvesting on PEI; therefore, the study assumed that this size range would encompass the major contributors to spawning events. As it was uncommon to find clams of a shell length greater than 70 mm, the size range in the second year only specified that clams had to be greater than 50 mm in length. The size range of clams which were less than 50 mm in shell length for each year was used to determine if these clams also contribute to spawning events on PEI.

In 1997, approximate shell length measurements were taken on site using plastic vernier calipers. In 1998, electronic calipers (Model CD-8"C, Mitutoyo Corporation, Tokyo, Japan, 1997) were used on site to measure clam shell lengths to the nearest 0.01 mm along the anterior-posterior axis. Each week, specimens were collected from a different area within that selected site. However, in any given week, clams were collected from the same area within the smallest possible distance. The first clams collected with the appropriate shell lengths were those sampled for steam meat yield in both years sampled.

### 3.3.4 Calculation of condition indices

Clams were transported in a cooler with ice to the laboratory, where whole live clams were weighed and their shell lengths re-measured and recorded with electronic calipers (Model CD-8"C, Mitutoyo Corporation, Tokyo, Japan, 1997). In both years, the clams were steamed in lots of 30 using 150 ml of water at medium-high heat on a stove burner for approximately 8 -10 minutes. The clam shells and tissue were then weighed separately to calculate steam meat yield. In 1998, tissue was then dried in a gravitational oven at 100 °C for 24 hours following steaming and the tissue was re-weighed. The data collected from this procedure was used in calculating gravimetric and shell condition indices. Condition indices were calculated for the 1997 and 1998 sampling period as follows:

Steam meat yield = [steam meat wt (g) / steam shell wt (g) + steam meat wt (g)] x 100 (Bernard 1993).

Gravimetric condition index = [dry meat wt (g) / whole wt (g) – shell wt (g)] x 1000 (Drinnan and Henderson 1959, Medcof 1961).

Shell condition index = [dry meat wt (g) / dry shell wt (g)] x 1000 (Walne and Mann 1975).

### 3.3.5 Statistical analyses

Data collected were entered and stored in a computer spreadsheet

(Quattro® Pro version 8, Corel® Corporation Limited, Ottawa, Ontario, Canada, 1998). Each conditional index was evaluated in a logistic regression univariate model as a predictive variable with the mature and partially spent stages of reproduction as outcome variables using Stata™ statistical software, version 7.0 (Stata Corporation, College Station, Texas, USA, 1984 - 2002). Logistic regression analysis was used to determine if the condition indices could detect spawning events in the clam populations. A spawning event was characterized as the change in clams from the mature stage to the partially spent stage of reproduction (Chapter 2). For this study, differences were considered significant when $P \leq 0.05$.

## 3.4 Results

### 3.4.1 Steam meat yield - 1997

Steam meat yield was calculated for clams 50 mm and greater in shell length for the Barsway site in 1997. Due to a shortage of smaller clams on site, clams between 20 – 50 mm in length were collected only on two occasions. This did not provide sufficient information to evaluate steam meat yield over the season, so the Barsway clams with shell lengths between 20 – 50 mm were omitted from the study. For the steam meat yield of clams 50 mm in shell length and greater at Barsway, a large decrease in tissue weight occurred between June 13 and July 2 (Figure 1). Steam meat yield decreased from 47.7 percent to 33.4 percent over that 3 week period (Figure 1). From July 2 onward to October 28, steam meat yields never

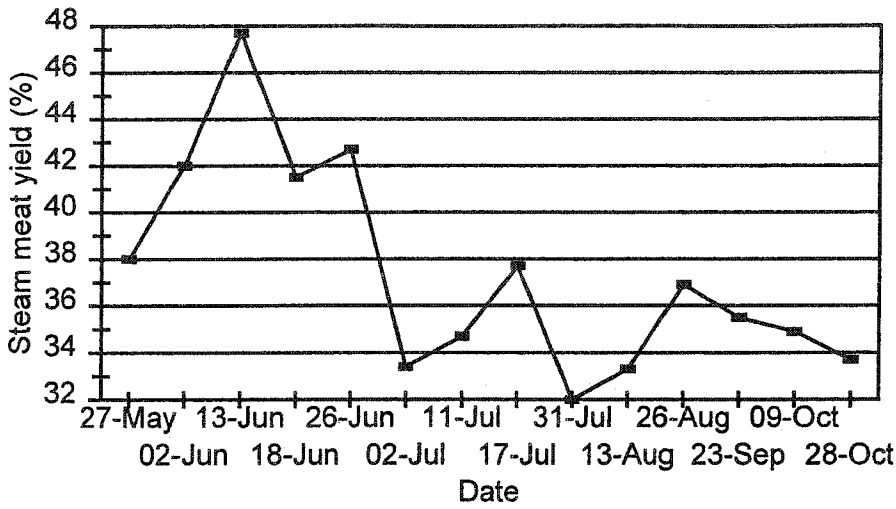


**Figure 1.** Mean steam meat yield in soft shell clams 50+ mm in shell length over time at Barsway in 1997.

exceeded 37.7 percent (Figure 1).

Steam meat yield at Oates Point in 1997 remained relatively high throughout the field season for clams between 20 – 50 mm in shell length. Steam meat yields ranged from 39.2 percent to 44.9 percent (Figure 2). Steam meat yield for clams 50 mm and greater in shell length at Oates Point indicate a drop in tissue weight relative to the rest of the animal between June 26 and July 2, when steam meat yield decreased from 56.2 percent to 38.6 percent (Figure 2).

The steam meat yield index calculated for Gascoigne Cove appeared to be similar between the two size ranges of clams collected. The clams ranging from 20 – 50 mm in shell length had a steam meat yield peak of 53.7 percent on June 19, which dropped to 32.8 percent by June 25 and remained at levels lower than that for the remainder of the field season (Figure 3). In the clams of 50 mm and greater shell length, steam meat yield peaked at 61.6 percent on June 19, and decreased to 37.9 percent on June 25 (Figure 3). Again, steam meat yields remained low for the remainder of the field season. For complete results of the steam meat yield index for sites in 1997, see Appendix C.

### 3.4.2 Steam meat yield - 1998

The steam meat yield index for the Barsway clams in the 35 – 50 mm size range did not fluctuate much from May 29 to August 6, 1998 (Figure 4). In this time, steam meat yields ranged from 47.41 percent to a high of 53.46 percent. Following August 6, steam meat yield continually decreased to the end of the field season on

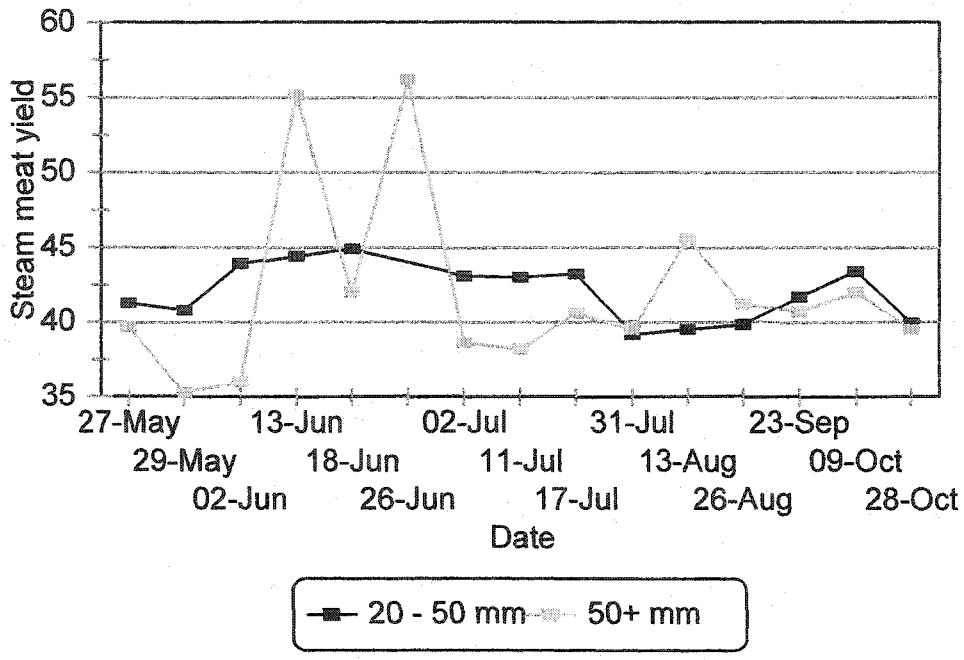


**Figure 2 .** Mean steam meat yield in soft shell clams between 20 – 50 mm and 50+ mm in shell length over time at Oates Point in 1997.

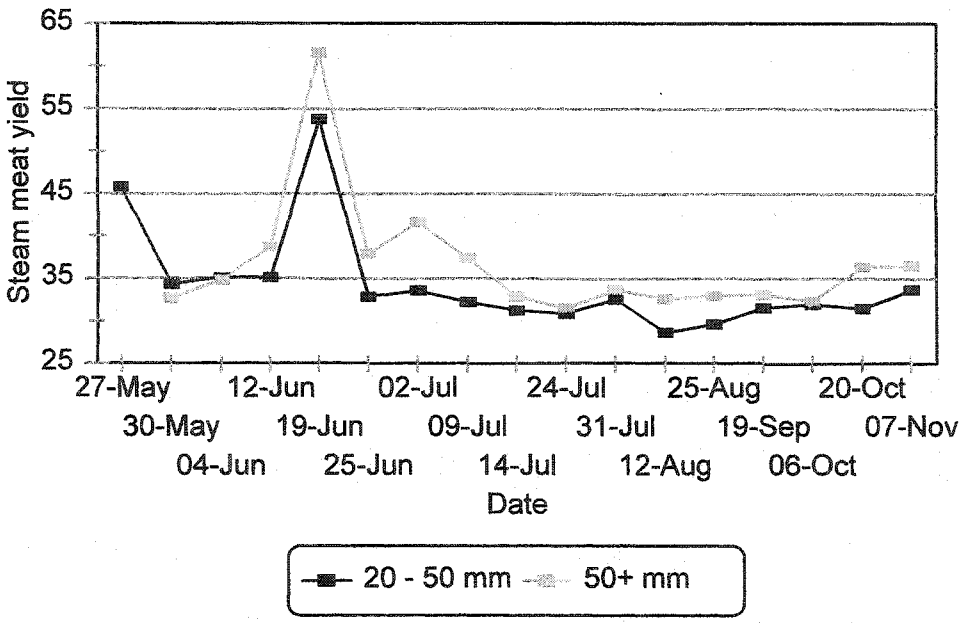


**Figure 3.** Mean steam meat yield in soft shell clams between 20 – 50 mm and 50+ mm in shell length over time at Gascoigne Cove in 1997.

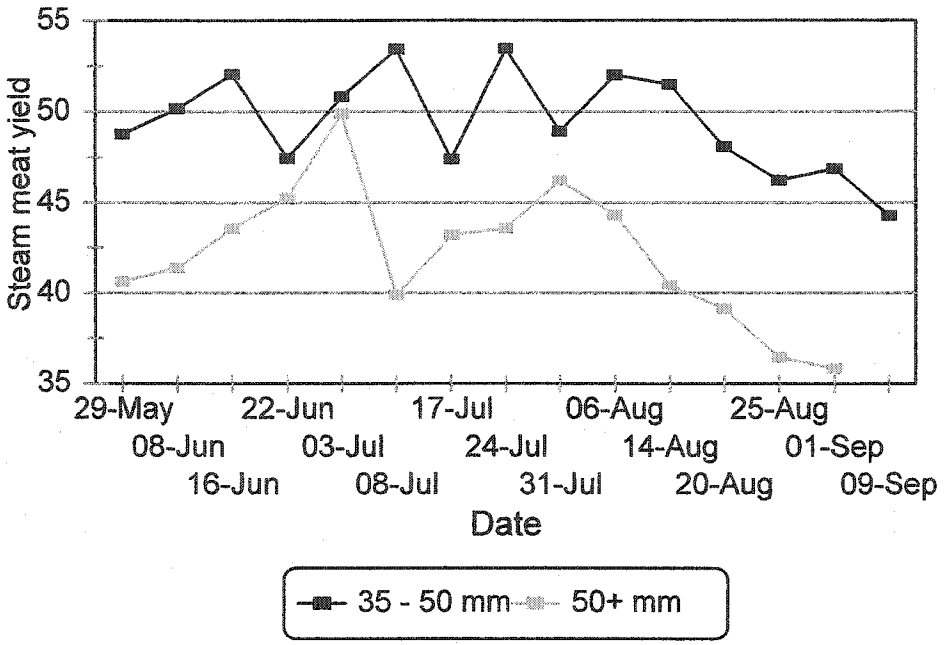


**Figure 4.** Mean steam meat yield in soft shell clams between 35 - 50 mm and 50+ mm in shell length over time at Barsway in 1998.

September 9, at which time yields were calculated to be 44.27 percent (Figure 4).

In the Barsway samples of clams 50 mm and greater in shell length, steam meat yields reached a peak of 49.88 percent on June 16, with a drop to 39.89 percent on June 22. However, steam meat yields rebounded and reach a second peak on July 31 of 46. 20 percent, at which time yields continually declined to 35.83 percent by the end of the field season on September 9 (Figure 4). For the results of conditional indices at Barsway for 1998, see Appendix D.

At Oates Point, steam meat yields remained relatively high throughout the field season for clams between 35 – 50 mm in shell length. Steam meat yields ranged from 38.52 percent to 43.94 percent (Figure 5). Steam meat yield for clams 50 mm and greater in shell length at Oates Point indicate a drop in tissue weight relative to the rest of the animal between July 21 and August 21, when steam meat yield decreased from 44.01 percent to 33.15 percent (Figure 5). Yields then rebounded to 44.12 percent by September 2 (Figure 5). For the results of conditional indices calculation at Oates Point in 1998, see Appendix E.

The steam meat yields at Gascoigne Cove for clams in the 35 – 50 mm size range continually decreased following a peak around June 5 of 45.81 percent (Figure 6). Steam meat yield dropped to 32.89 percent by August 19 (Figure 6). In clams 50 mm and greater in shell length, a peak steam meat yield of 42.96 percent was noted on June 9 (Figure 6). From this point onward in the season, decreases in meat yield were recorded, with a low of 32.85 percent reached on September 26 (Figure 6). For the results of conditional indices calculations at

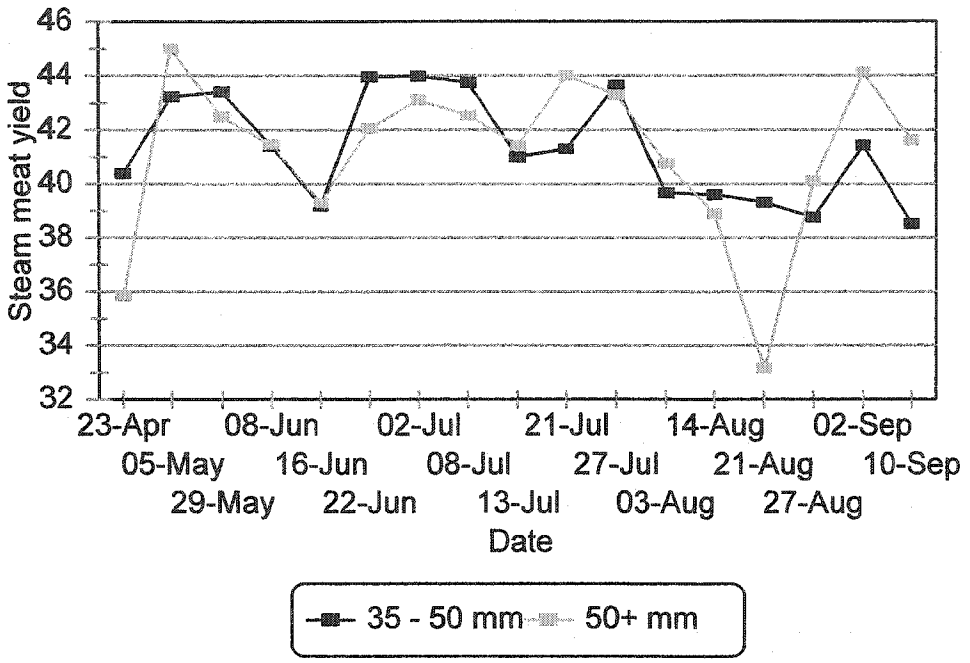


**Figure 5.** Mean steam meat yield in soft shell clams between 35 - 50 mm and 50+ mm in shell length over time at Oates Point in 1998.

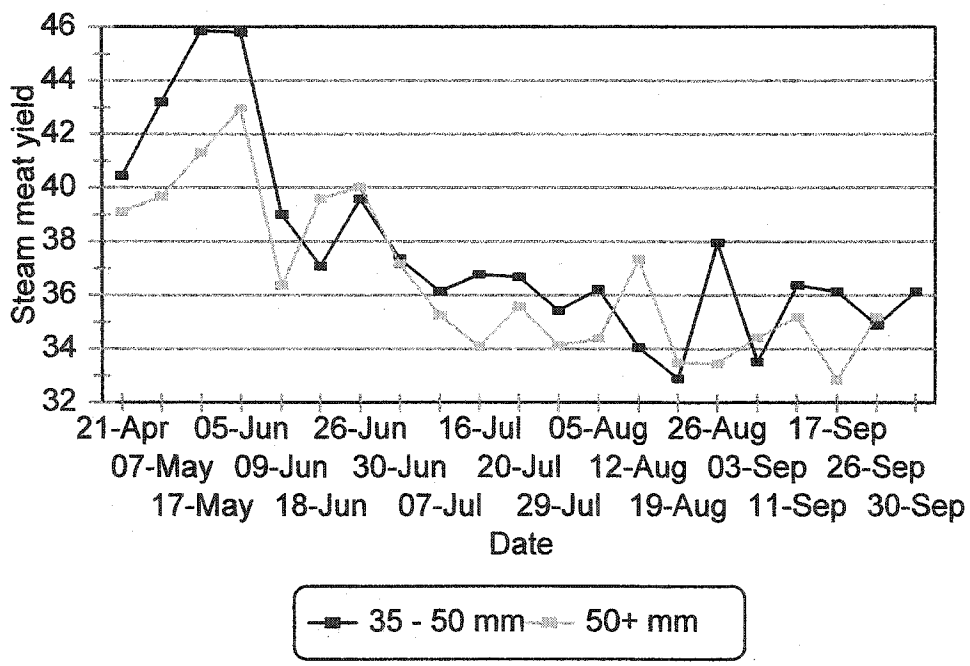


**Figure 6.** Mean steam meat yield in soft shell clams between 35 - 50 mm and 50+ mm in shell length over time at Gascoigne Cove in 1998.

Gascoigne Cove for 1998, see Appendix F.

### 3.4.3 Shell condition index - 1998

Shell condition index at Barsway for clams between 35 – 50 mm in length remained relatively high up until and including July 3 (Figure 7). Following July 3, shell condition index continued to decrease throughout the remainder of the field season. Shell condition index dropped from 243.01 on July 3 to 190.90 on September 9 (Figure 7). The 50 mm and greater size range of clams at Barsway reached a peak shell condition index of 205.77 on June 16 (Figure 7). Following this date, shell condition index fluctuated greatly until August 6. After August 6, shell condition dropped 55 points on the index with a low shell condition of 141.66 on September 1 (Figure 7).

The shell condition index for Oates Point clams in both size ranges varied greatly over the field season. For the 35 – 50 mm size range of clams, condition ranged from 138.41 to a high of 199.79 (Figure 8). The 50 mm and greater shell length of clams ranged from 122.28 to 207.52 (Figure 8).

Shell condition in Gascoigne Cove for clams between 35 – 50 mm in length remained high up to June 30, when the index was 162.66 (Figure 9). Then the shell condition index started to decline and decreased to 104.57 by August 12 (Figure 9). Following this, the shell condition index started to rebound. For the Gascoigne clams 50 mm and greater in shell length, a peak of 164.61 in shell condition index was reached on June 26 (Figure 9). Subsequently, shell condition index declined

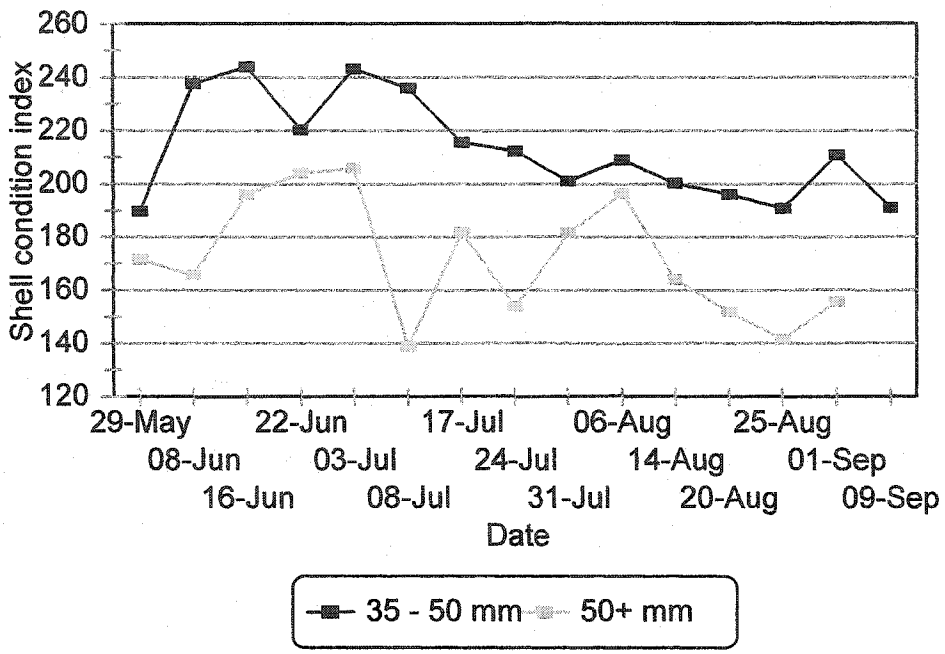


**Figure 7.** Mean shell condition index in soft shell clams between 35 – 50 mm and 50+ mm in shell length over time at Barsway in 1998.

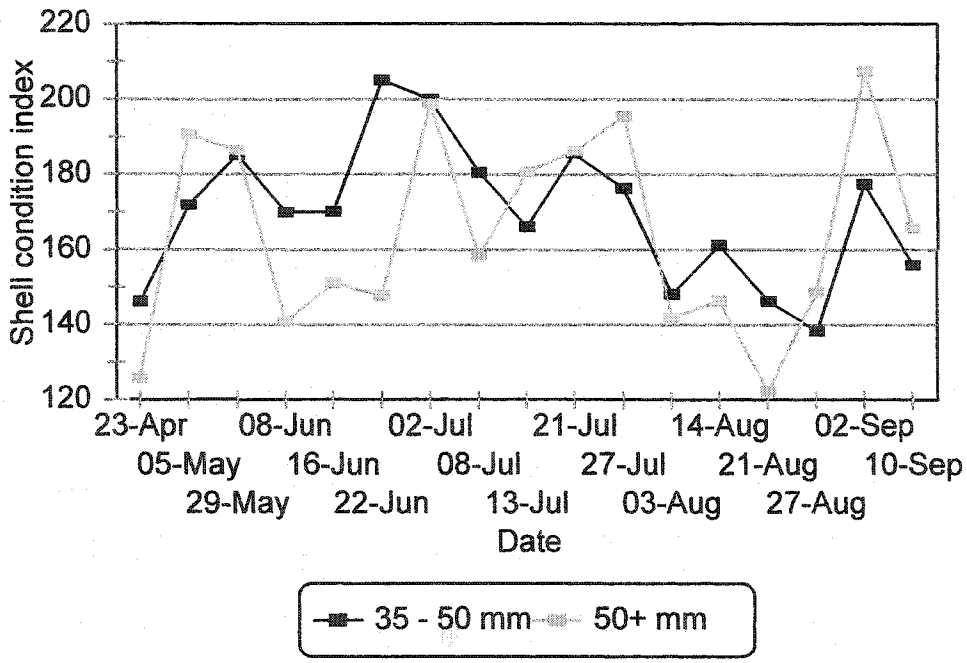


**Figure 8.** Mean shell condition index in soft shell clams between 35 - 50 mm and 50+ mm in shell length over time at Oates Point in 1998.

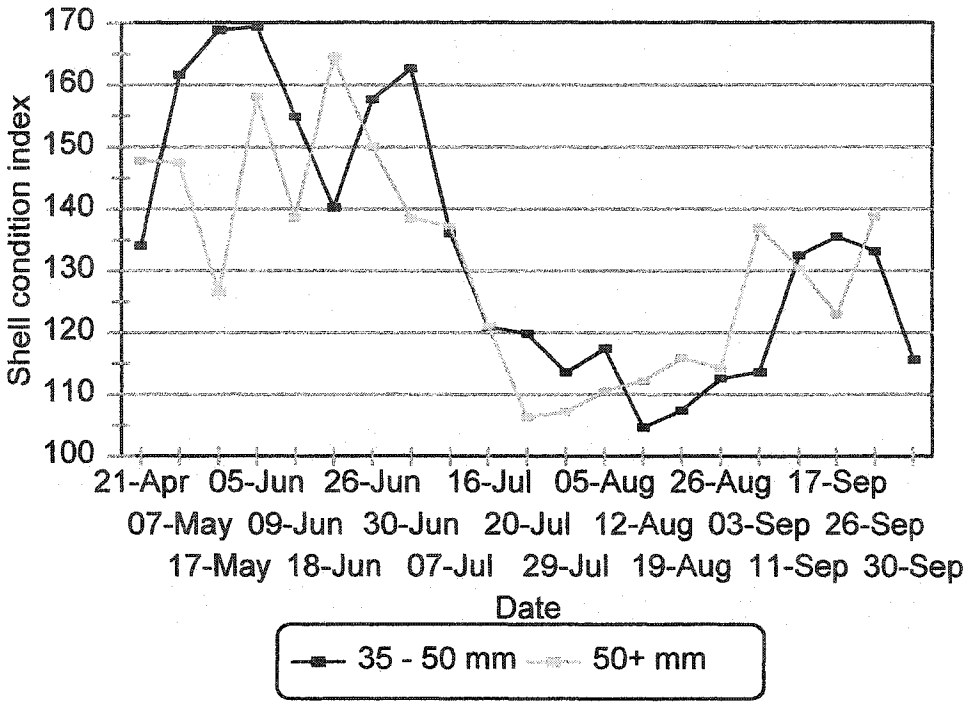


**Figure 9.** Mean shell condition index in soft shell clams between 35 - 50 mm and 50+ mm in shell length over time at Gascoigne Cove in 1998.

to a low of 106.31 on July 29, before rebounding (Figure 9).

### 3.4.4 Gravimetric condition index - 1998

The gravimetric condition index at Gascoigne Cove for clams between 35 – 50 mm in length indicates a large decline in values following June 30 (Figure 10). The gravimetric condition index on June 30 was 71.83, and it dropped to 47.46 by August 19 (Figure 10). For the clams with shell lengths of 50 mm and greater, gravimetric condition index peaked at 72.59 on June 9, and then it decreased to a low of 43.83 by September 3 (Figure 10).

The gravimetric condition index decreased steadily over the field season in Barsway for both size ranges of clams. Gravimetric condition for the clams between 35 – 50 mm in length reached its peak on June 8 with an index of 79.44, and dropped to a low of 60.41 by August 14 (Figure 11). For clams 50 mm and greater in shell length at Barsway, gravimetric condition was its highest on May 29, with an index of 88.65 (Figure 11). Subsequently, gravimetric condition index declined to 58.13 by September 1 (Figure 11).

In Oates Point, the gravimetric condition index calculated for clams between 35 – 50 mm in length shows relatively high values for condition up to and including June 22, when values reached 77.69 (Figure 12). Following this date, gravimetric condition index decreased to a low of 55.68 on August 27 (Figure 12). For clams 50 mm and greater in length at Oates Point, the gravimetric condition fluctuated until July 27, when the index was calculated to be 78.14 (Figure 12). A decrease in

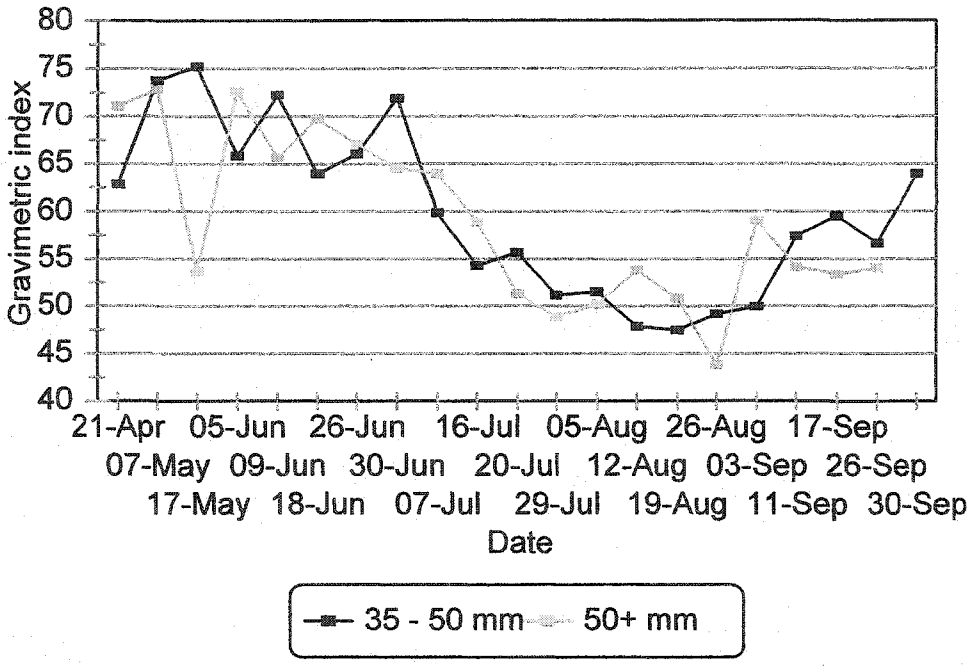


**Figure 10.** Mean gravimetric condition index in soft shell clams between 35 - 50 mm and 50+ mm in shell length over time at Gascoigne Cove in 1998.

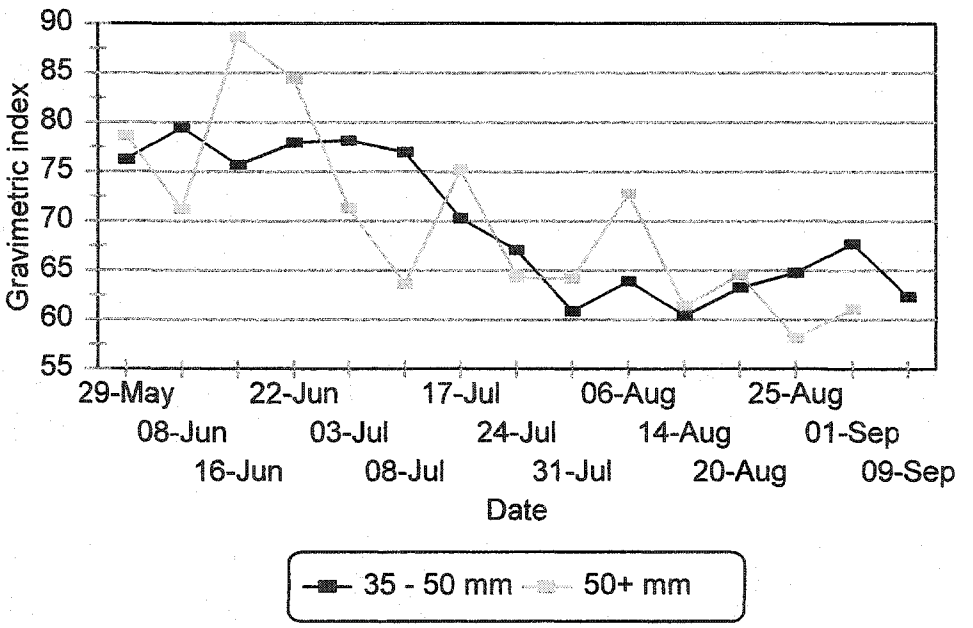


**Figure 11.** Mean gravimetric condition index in soft shell clams between 35 – 50 mm and 50+ mm in shell length over time at Barsway in 1998.

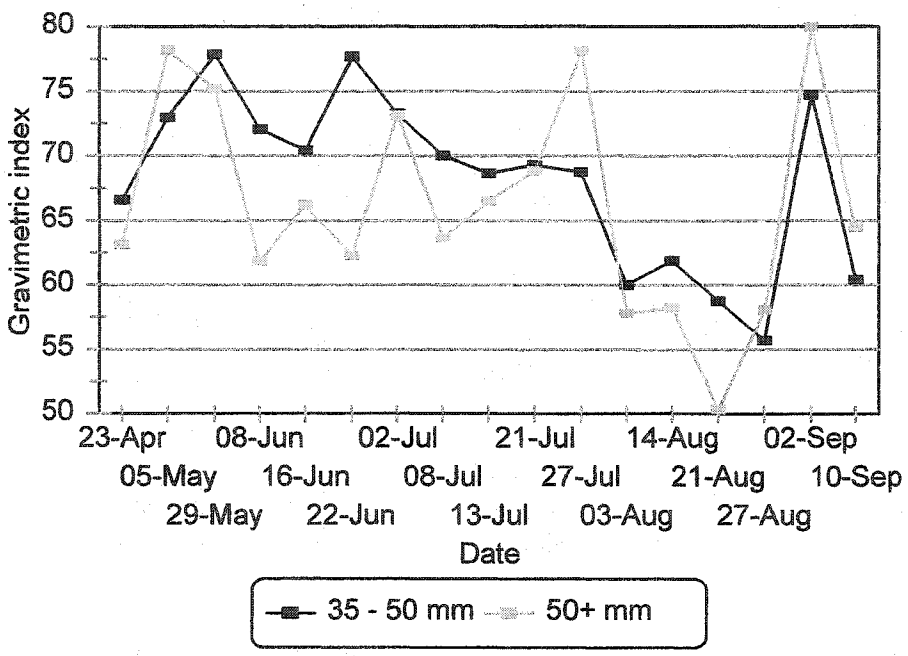


**Figure 12.** Mean gravimetric condition index in soft shell clams between 35 - 50 mm and 50+ mm in shell length over time at Oates Point in 1998.

gravimetric condition then occurred, reaching a low of 50.32 by August 21 (Figure 12).

### 3.4.5 Histology results

Regression analysis revealed that there was no significant association between the various condition indices and spawning. As discussed in Chapter 2, the histology data collected at each site can be represented over time by the percentage of mature clams. The results, as reported in Chapter 2, are briefly described here. For Barsway in 1997, spawning occurred between June 26 and July 2. Similarly at Oates Point in 1997, spawning lasted from June 18 to July 11. In Gascoigne Cove, spawning commenced following June 25 and lasted until August 25. At Oates Point in 1998, clams spawned between June 8 and July 2.

## 3.5 Discussion

### 3.5.1 Steam meat yield - 1997

Between June 13 and July 2, there appeared to be large spawning events in all clams 50 mm and greater in shell length at all three sites in 1997. The results from the 20 – 50 mm size range of clams were less conclusive. The Barsway site did not have enough samples for an in depth analysis and the Oates Point site clams did not fluctuate in steam meat yields over time. The smaller size range of clams at the Gascoigne Cove site followed the pattern of the larger sized clams.

Although shell length was measured to ensure that all clams fit within the

appropriate size range, these shell lengths were unfortunately not recorded. Within the 20 – 50 mm grouping, there is a potential gap of 30 mm between shortest and longest clam. Therefore, it is possible that the majority of clams sampled at Gascoigne Cove fell in the higher ranges of the 20 – 50 mm size class. Female clams in the southern Gulf of St. Lawrence typically reach sexual maturity at shell lengths between 35 – 40 mm (Fisheries and Oceans Canada 1996). This could explain why a similar pattern was noticed between these clams and those clams in the 50 mm and greater size class at the same study site.

The site with no definitive decrease in steam meat yield, Oates Point, may have had most or all of its clams in the lower ranges of the 20 – 50 mm size class. These immature clams would not have a decrease in steam meat yield, as they are not building up and subsequently releasing any gametic materials. Instead these clams may have been putting their energy into shell and tissue growth.

### 3.5.2 Steam meat yield - 1998

Large decreases in steam meat yield of clams with shell lengths 50 mm and greater were noted for all three sites. At Barsway, a small spawning event may have occurred following June 16, and a much larger spawning event appears to have taken place between July 31 and September 9. Oates Point had a similar spawning event between July 21 and August 21. As these two sites are close in proximity to each other, it is not surprising that they have the same spawning dates.

At the Gascoigne Cove site, clams 50 mm and greater in shell length

appeared to spawn continually throughout the field season, starting after June 9. This situation was also seen in the smaller size range of clams (35 – 50 mm) at the same site, with spawning starting around June 5 and continuing into September.

The 35 – 50 mm size range of clams at Barsway appeared to have a similar spawning to the larger clams sampled at that site. Spawning activities seem to have occurred following August 6 and were recorded until September 9. The clams between 35 – 50 mm at Oates Point did not appear to spawn as meat yields remained relatively high throughout the field season.

### 3.5.3 Shell condition index - 1998

At Barsway, spawning events appear to have occurred in both size ranges. In the 35 – 50 mm size ranges, shell condition declined following July 3, whereas in the 50 mm and greater size range, condition decreased following August 6. At Oates Point, shell condition fluctuated greatly in both size ranges of clams, so that no conclusions may be made from these calculations.

The 35 – 50 mm sized clams at Gascoigne Cove appear to have spawned at a similar time to their counterparts in Barsway. A decline in shell condition was noted following the sampling on June 30. The 50 mm and greater sized clams at Gascoigne Cove also spawned in late June as shell condition decreased following June 26.

### 3.5.4 Gravimetric condition index - 1998

Spawning events appear to have occurred in both size ranges of clams at all three project sites. In Barsway, the 35 – 50 mm clams dropped in condition following June 8, and a spawning event likely occurred at that time. Similarly, the 50 mm and greater sized clams appear to have spawned following May 29.

In Oates Point, spawning likely occurred in the 35 – 50 mm clams between June 22 and August 27, while spawning appears to have occurred in the 50 mm and greater clams between July 27 and August 21.

Spawning occurred in the 35 – 50 mm sized clams at Gascoigne Cove following June 30, and following June 9 in the 50 mm and greater sized clams at that site.

### 3.5.5 Histology and condition indices

Although the condition index data was not found to be statistically significant when compared to histologic analysis, some similarities between the two were evident in the results presented. For 1997, the steam meat yield index was similar to the percentage of mature clams at all three study sites, indicating a large decrease in both of these values, steam meat yield and percentage of mature stage clams, in late June.

The gravimetric condition index for both size classes of clams at Gascoigne Cove in 1998 were similar in that a large decrease in index was noted between June and August. Similarly, the shell condition for both size classes of clams at

Gascoigne Cove decreased in the month of June. The steam meat yield for both size ranges of clams also indicated a decline in values in the month of June, though the steam meat yield decreased earlier in the month than the other two types of indices.

At Barsway in 1998, a sharp decrease in gravimetric index, shell condition index, and steam meat yield for clams 50 mm and greater in shell length was apparent in early June. Although the 35 – 50 mm size class of clams for gravimetric and shell condition indices at this site also showed a decline in index value, this was not noted until early July. The steam meat yield for Barsway clams between 35 – 50 mm in shell length did not decrease until early August.

In 1998, the changes in percentage of mature clams at Oates Point indicate that spawning between early June and the beginning of July. Within the 50 mm and greater size range of clams, the gravimetric index and shell condition index at this site fluctuated throughout the field season. However, the 35 – 50 mm size range of clams at Oates Point for gravimetric index and shell condition index did decrease over time, but this decline in the indices did not commence until the end of June. The steam meat yield for clams 50 mm and greater in shell length showed a sharp decline, but this did not occur until late in July. The 35 – 50 mm size range of clams used to calculate steam meat yield at Oates Point did not show any indication of spawning throughout the field season. Therefore, no correlations were found between condition indices and percentage of mature clams at Oates Point in 1998.

The condition indices and percentage of mature clams, as tracked by histology, should have a relationship. When a bivalve spawns, its tissue weight decreases, and this change in body weight relative to the entire animal should be discernable in a calculation of condition index. As the histology portion was visual in nature, it is more representative of actual spawning events. The rationale for this is that as the percentage of mature clams decreases, the percentage of partially spent clams is likely to increase. Biologically, the percentage of mature clams should replicate an accurate condition index, as body condition and percentage of mature clams should both decrease when spawning events occur.

The clams sampled for the condition indices and histology were collected together in the same area and on the same sampling trips to each site. Therefore, there should not be any difference in reproductive development between the two groups. Thirty clams were sampled for the calculation of each condition index on each sampling trip. This should be a sufficient number of soft shell clams to evaluate an area for the occurrence of spawning events.

In order to determine if a more accurate monitoring of spawning events is possible in future studies, it may be beneficial to decrease the range of the size classes sampled, so that shell lengths of clams being used to calculate indices are more closely matched. As well, a higher cut-off shell length should be established to ensure immature clams are not being sampled. Landry *et al.* (1999) found that quahaugs, *Mercenaria mercenaria,* on PEI became sexually mature at shell lengths between 25 – 30 mm. Female clams in the southern Gulf of St. Lawrence typically

reach sexual maturity at shell lengths between 35 – 40 mm (Fisheries and Oceans Canada 1996). This type of information is needed for male soft shell clams. In future studies, the individual shell lengths should be recorded so that when each clam is tracked from that point forward, condition index can be directly matched to each clam sampled to determine if there are any outliers.

It may possible that condition indices are not accurate monitors of spawning events versus the histological staging of gonads specifically for soft shell clams. In a study completed on PEI pertaining to quahaug spawning, researchers found that variation in the shell condition index was small (Landry *et al.* 1999). This was equated to the oyster which tastes different after spawning, and typically exhibits dramatic changes in condition index, whereas quahaugs taste the same year round. They concluded that the quahaug likely spawns gradually throughout the field season and this would result in the minute changes in condition index (Landry *et al.* 1999). As soft shell clams and quahaugs are both in the clam family of bivalves, and no change in taste occurs in soft shell clams after spawning, it can be speculated that soft shell clams also spawn gradually. This would explain why no statistical significance could be determined when comparing the various condition indices to mature stage clams. In staging the specimens viewed for histology, once there is any indication of spawning, the clam is labelled as partially spent. However, this protocol did not account for the degree to which the clams were spent. As long as there was some number of viable gametes in the gonad, the animal remained labelled as partially spent. Therefore, a soft shell clam could theoretically be

partially spent, in varying degrees, for several weeks.

Future studies should also determine if any condition indices have been successfully used in other clam species and incorporate the use of those condition indices into a study foe evaluation. Steam meat yield is widely used on PEI, but it has been used solely for mussels and oysters. Similarly, the recommended indices from the Crosby and Gale (1990) study were only tested on oysters.

### 3.6 Conclusions

Within this study, steam meat yield, shell condition index or gravimetric condition index were not proven to be reliable management tools used in consistently detecting the date of clam spawning at PEI sites. Although steam meat yield appeared to be similar in 1997 to the results of histology, this was not the case in 1998. The gravimetric and shell condition indices appear to detect changes in body condition at the same level of sensitivity, and therefore the results of these indices were very similar. None of the condition indices tested in this study are recommended for use as a sole indicator of spawning events.

Presently, the information may only be beneficial in providing secondary information or a confirmation of spawning when used in conjunction with other methods of identifying a spawning event, such as histologic analysis. Of the three indices evaluated, the steam meat yield index was the least difficult to calculate in that less processing of the clams is required. This index also appeared to be more accurate in its detection of spawning events, from the 1997 study.

Future studies should include determining the shell length at which male soft shell clams reach sexual maturity so that only mature clams are used in calculating condition indices. In addition, histologic specimens of the clam gonads should incorporate a more stringent evaluation, in terms of calculating the relative percentage of gonad in the tissue section. These determinations may be made using a computer with a specified software package. By doing this, the degree to which the clam is partially spent may be taken into account. Once these measures are in place, evaluating these or other indices may finally prove whether or not one or more of the indices are accurate monitors of spawning when compared to histology.

# 4. LARVAL MONITORING OF SOFT SHELL CLAM (*MYA ARENARIA*) SPAWNING TO PREDICT SEED SETTLEMENT ON PRINCE EDWARD ISLAND

## 4.1 Introduction

Spatfall or larval settlement predictors are important tools for correctly timing seed collector deployment. Seed and Suchanek (1992) define settlement as the process whereby larval animals come in contact with, and attach to, any structure. Monitoring larval activities to estimate the date of larval settlement is commonly practiced in Prince Edward Island (PEI) for oyster (Medcof 1961, Woo and McIver 1974, Drinnan and Stallworthy 1979) and mussel (Bernard 1991-1997) aquaculture.

Larval development in oysters lasts approximately 3 weeks (Medcof 1961, Woo and McIver 1974, Burrell 1985). Oyster larvae attach or set at shell lengths between 320 µm (Doiron 1997) and 335 µm (Sullivan 1948) in eastern Canada. Mussel larvae develop over a period of up to 4 weeks (Lutz 1985, Scarratt 1993) and set at shell lengths between 315 µm (Doiron 1997) and 320 µm (Sullivan 1948). The soft shell clam larval period lasts approximately 4 weeks in the southern Gulf of St. Lawrence (Fisheries and Oceans Canada 1996). Clams set at a shell length of approximately 235 µm (Sullivan 1948, Doiron 1997). Sullivan (1948) found that soft shell clam larvae were present in Malpeque Bay, PEI from late May until the end of August, with peak levels achieved throughout the month of June.

Most predictions of larval settlement are derived from plankton tows and

plankton pump sampling. Plankton tows for the collection of bivalve larvae are commonly used (Sullivan 1948, Drinnan and Stallworthy 1979, Quayle and Newkirk 1989) and may be conducted by hauling the plankton net a set distance through the water using a boat. The second approach uses a small pump to run a measured volume of water through a mesh screen.

A plankton net has a funnel-shaped design with a metal hoop at its widest part and a small detachable basket at its smallest end, which is where the plankton is collected (Quayle and Newkirk 1989). The area from the hoop to the basket is enclosed with fine meshed silk or nylon cloth with a special weave so the apertures remain an exact size (Quayle and Newkirk 1989). The metal hoop of the plankton net keeps the net open and it is attached a towing bridle. The plankton net is used by towing, or dragging, it behind a boat in order to collect a sample of the water column. The net is towed over a set distance and the time it takes to cover that distance is also recorded. The net is towed behind a boat whose speed is just enough to keep the net below the surface of the water. The usual tow duration may vary, with recommendations noted between 5 minutes (Quayle and Newkirk 1989) and 10 minutes (Drinnan and Stallworthy 1979). The net is then drawn to the boat, lifted out of the water and allowed to drain. The sides of the net are then washed down with water and the contents of the bucket are drained into a labelled sample jar. The contents of the sample jar are preserved by adding alcohol (Quayle and Newkirk 1989) and a sub-sample of the sample is examined under a microscope to identify bivalves.

The plankton tow is an imprecise field measure that is well accepted and used by many (Sullivan 1948, Drinnan and Stallworthy 1979). It is difficult to make an accurate estimate of the amount of water filtered in a plankton tow because the efficiency of the net or the rate at which it collects can vary greatly between the beginning and the end of the tow due to clogging of the meshes with plankton organisms or silt (Quayle and Newkirk 1989). This type of sampling is really only qualitative (Drinnan and Stallworthy 1979) and notes the presence or absence of a particular larva or whether it is rare or abundant (Quayle and Newkirk 1989).

The plankton pump method is operated with a 12 volt battery connected to a submersible bilge pump with a hose, or tubing, attached with hose clamps. A weight is fastened to the bottom of the hose to keep the sample pump submerged in the water (Woo and McIver 1974). It is important to calculate the flow rate of the water using the pump. This can be done by using a flow meter, or by timing how long it takes to fill a container of a known capacity (Woo and McIver 1974). Prior to and at the time of sampling from the near-surface or near-bottom depth, it is very important that there is minimal sediment disturbance. The station should be approached slowly, ideally with boat engine cut off as the boat is coasting into position, and then the anchor gently lowered. The sample should be taken as far away from any points of possible disturbance such as the anchor, engine or other sampling activities. The recommended depth at which the near-bottom sample would be taken using a bilge pump is 0.3 m above the bottom (Sigua *et al.* 1998).

Of the two methods used for collecting plankton, the pump sampling

approach is more feasible in providing information that spawning has occurred and in predicting the intensity of larval settlement to be expected (Drinnan and Stallworthy 1979). Drinnan and Stallworthy (1979) noted that both plankton tows and pump samples provide predictions for oyster seed settlement. However, they concluded that the pump samples were more reliable in capturing larvae. For an accurate determination of the number of larvae per unit volume of water, it is necessary to know the actual volume of water passing through a mesh screen (Quayle and Newkirk 1989). The most direct method is to pump a known volume of water through a small mesh screen. The amount of water can be calculated by timing how long it takes to fill a container of a known volume. This water volume variable is accounted for in the plankton pump sampling protocol.

## 4.2 Objectives

The purpose of the current study was to infer the date of soft shell clam spawning from larval monitoring activities and to predict the date of clam settlement under natural conditions on PEI. Specific objectives included verifying that spawning had occurred and identifying clam larvae in plankton samples to determine the specific date of spawning in the adult population, and to predict the date of larval settlement.

## 4.3 Materials and Methods

The study site used for this field trial was Oates Point and the work was

carried out in 1998. Water column samples were collected at the study site between July 8 and September 28, 1998. Specific site location and information are described in section 2.3.1. Water column samples were collected using two methods.

Two samples were collected in the same general area per site visit using a plankton net with a 50 µm screened basket. Two landmarks, of a known distance, identified the path of travel while timed records indicated the extent of plankton net collection from the water column. The plankton net, towed in the opposite direction to the tidal movement at a constant boat speed, was lowered and raised throughout the water column to achieve a random sample.

The second method employed in this study was the plankton pump method, and it was performed once in each of two sampling stations per site visit during the study. Sampling stations were located approximately 350 meters (m) from each other. A battery powered bilge pump generated 41.38 liters of water through a 64 µm mesh screen per minute. The pump was lowered and raised through the water column, sampling at a depth between 2 and 3 m of water.

In both collection methods, the collected plankton samples were rinsed into labeled sample jars with previously filtered seawater. Samples were preserved on site with 10 percent buffered seawater formalin.

Training for the identification of soft shell clam larvae from other plankton centred around existing bivalve identification programs. Staff of the PEI Department

of Fisheries, Aquaculture and Environment and New Brunswick Department of Agriculture, Fisheries and Aquaculture routinely differentiate soft shell clam larvae from other bivalve larvae using light microscopy; both parties provided training on larval identification. In addition, published descriptions and photographs of larval stages of bivalves were employed in identification (Sullivan 1948, Lutz *et al.* 1982, Doiron 1997). Preserved soft shell clam larvae, at various stages of development, were shipped to the Atlantic Veterinary College from the New Brunswick Department of Agriculture, Fisheries and Aquaculture hatchery in Shippigan, New Brunswick. The hatchery specimens were identifiable using a Wild Heerbrugg stereoscope.

Sub-samples, and in some cases, entire contents of the preserved seawater samples were examined using a Wild Heerbrugg stereoscope and later using a Nikon Alphashot YS light microscope as described by Bernard (1997). Some samples were centrifuged onto glass slides using a cytocentrifuge (Shandon Cytospin 2), stained with Wright – Giemsa stain, cover slipped and examined using light microscopy.

## 4.4 Results

Plankton tows were encumbered by high algal content at the study site. The plankton tows ranged from 1 minute to approximately 5 minutes in duration. Normally plankton tows would be conducted over a 5 (Quayle and Newkirk 1989)

to 10 minute duration (Drinnan and Stallworthy 1979). Bilge pump samples also collected massive amounts of algae. Sample collection time ranged from approximately 1 minute to 3 minutes. The volumes of water sampled, between 41.38 liters (l) and 124.14 l, corresponded with those of Bernard (1991 -1997) who sampled 50 l per minute over similar durations.

Samples were collected on July 8, August 3, 7, 11, 21, 27, and on September 2, 10, 28 in 1998. No soft shell clam larvae were identified in any of the plankton tow or plankton bilge pump samples viewed using the stereoscope and light microscope. No clam larvae were identified using light microscopy after the centrifuging and staining technique.

## 4.5 Discussion

The methods described for plankton tows and plankton pump samples are successful in providing predictions of settlement for blue mussels (Bernard 1991-1997), American oysters (Woo and Bernard 1974, Drinnan and Stallworthy 1979) and many other bivalve species (Burnell 1991). In this experiment, soft shell clam larvae were not identified in plankton tows or plankton pump samples. The dates on which larvae appear in the water column differ from year to year and from location to location (Sullivan 1948).

According to the histologic evaluation (Chapter 2) for Oates Point in 1998, the clam population spawned on two occasions during the summer season. The first event was prior to the commencement of field sampling and the second event

occurred in the month of July. According to steam meat yield and gravimetric condition indices (Chapter 3) for Oates Point in 1998, there was a spawning event which started between July 21 - 27 and continued until August 21. Plankton tow and plankton pump samples were collected in the months of August and September. Given that there is approximately a 4 week window from spawning to settlement, it appears that larvae would have been in the water during the time frame sampled. However, low numbers of clam seed were collected at the end of August that year (Chapter 5).

Soft shell clam larvae were noted by Sullivan (1948) to be present in the water column continually from late May to the end of August within Malpeque Bay, PEI, possibly due to continual small spawning events. Low numbers of spawning adult clams within the study site at any given time would lessen the chances of capturing clam larvae in the sampling regimen as the volume of water needed to collect a sample depends largely on the concentration of larvae present (Woo and McIver 1974).

The water depth chosen for plankton pump sampling may not have been ideal for clam larvae. The water sampled was at depths between 6 and 9 feet of water. Separate plankton samples could have been collected for each foot depth of water using the plankton pump method to determine if soft shell clam larvae are confined to a particular vertical distribution within the water (Quayle and Newkirk 1989). This method should be considered for future studies.

There was a large amount of phytoplankton in the samples collected. This

plant material likely reduced the overall efficiency of the plankton tows as the basket was continuously clogged with materials. With a larger mesh size in the basket, a greater volume of water could be sampled in the same time frame, and the basket would not plug up as rapidly, thereby increasing the duration of the plankton tows. This study used a 50 µm mesh size screened basket, but soft shell clams do not set until a shell length of 235 µm. Increasing the mesh size of the basket to a level between these two values could improve the quality of testing in future studies.

In this study, it would have been preferable to examine the samples within hours or days of field collection, so that adjustments in the sampling locations, or size of mesh used in the plankton net to increase the duration of sampling could be modified to attempt to successfully capture soft shell clam larvae. However, examination of the preserved samples was delayed several months until training in clam larvae identification was completed. One sampling date occurred in July, while the sampling frequency was weekly for the month of August and first half of September. Weekly sampling dates should have commenced earlier, around the end of June, and additional sampling stations within the study site could have been added to the program.

## 4.6 Conclusions

Within this study, larval monitoring was not an effective method to predict the date of soft shell clam settlement. Several techniques may be assessed should future larval monitoring of soft shell clams be conducted. The plankton tows and

plankton pump sampling should be re-evaluated, with additional sampling stations being monitored throughout the estuary and an earlier start date for sampling. Separate plankton samples should be collected for each foot depth of water using the plankton pump method to determine if soft shell clam larvae are confined to a particular vertical distribution within the water. Samples should be examined for the presence or absence of any clam larvae immediately following collection in future studies so that sample collection methods can change if required. Future testing would verify if these methods are appropriate for capturing soft shell clam larvae.

# 5. SOFT SHELL CLAM *(MYA ARENARIA)* SEED COLLECTION TRIALS ON PRINCE EDWARD ISLAND

## 5.1 Introduction

Seed collection may be the most important activity an aquaculturist undertakes, as without something to grow, the aquaculture site will fail (Scarratt 1993). While the existing clam fishery is not in collapse in the southern Gulf of St. Lawrence, resource abundance is well below historical values. The soft shell clam capture fishery on Prince Edward Island (PEI) is a commercial enterprise which has increased from 345 tonnes in 1997 and 420 tonnes in 1998, to 450 tonnes in 1999 (Fisheries and Oceans Canada 2002a). However, wild stock production cannot be sustained at increasingly higher harvest levels. Driven by a continued high demand for soft shell clams (Gray *et al.* 1998) and a market value that increases annually (Fisheries and Oceans Canada 2002a), PEI soft shell clam fishers became interested in the concept of aquaculture site enhancement techniques. This could expand the present wild capture fishery to include cultured product reared on private aquaculture sites.

Commencing in 1993, soft shell clam developmental aquaculture sites were awarded on PEI (Fisheries and Oceans Canada 2002b). While soft shell clam seed is a required component to develop commercially viable clam aquaculture sites on PEI, only two main sources of this seed exist. One option is to purchase clam seed reared in a hatchery, and the second is to collect seed during spawning of wildstock

clam populations. Capturing the set of natural seed can be far less expensive than spawning adults and rearing hatchery seed. To capture the natural set of seed, an artificial substrate in the form of mesh filled-bags hung in the water column are used. This design is commonly referred to as a Japanese collector. The great success with this seed collector design has established this method as commonplace in the sea scallop (*Placopecten magellanicus*) (Naidu 1991, Pouliot *et al.*1995), bay scallop (*Argopecten irradians*), icelandic scallop (*Chlamys islandica*), and Japanese scallop (*Patinopecten yessoensis*) industries (Ventilla, 1982).

Because of its availability, durability and better performance over other types of filling, monofilament gillnetting has become the preferred substrate for collectors (Naidu 1991). In order to harvest the clam seed, a collector has to be deployed in the water at the time of, or just prior to, the naturally occurring spawning event. The collector design used in this project was based on the model used in the southern Gulf of St. Lawrence region for sea scallop seed collection (MacLean and Gillis 1996). The sea scallop collector is comprised of an onion sack filled with fish netting such as monofilament. The collector is fastened to a longline with a length of rope so that the collector is suspended in the water (Scarratt 1995). The purpose of a collector is to collect larvae from the water column and provide an accommodating environment by avoiding a bottom substrate to increase overall larval survival. This bottom substrate can smother the seed and contains benthic, or bottom, dwelling predators. The larvae enter the mesh bag and attach

themselves to a section of the monofilament, or netting material, with secretions of byssus, or a mass of strong silky, threads. The clam seed continue to feed and grow inside the onion sack.

Collectors are also used in the mussel (*Mytilus edulis*) and oyster (*Crassostrea virginica*) industries on PEI. In the case of mussels, seed settle on frayed rope or plastic collectors and when the seed grows to about 2 cm in length, it is placed into tube shaped mesh socks. Large numbers of these socks are hung on a longline and remain attached to the sock until harvest time (Scarratt 1993). Within the oyster industry, it is common to coat materials in concrete to use as collectors in order to provide an attachment surface for the oyster seed. These concrete-dipped collectors are then hung on a longline near the surface of the water. When a minimum growth has been attained, the juvenile oysters are removed from the collectors and seeded on aquaculture sites or placed in bags for grow out (Medcof 1961).

Two methods of collecting wild seed for clam culture aquaculture sites are commonly used on Prince Edward Island. The first method consists of mesh bag collectors deployed in estuaries with established wild clam populations. The goal is to capture seed that can grow on the collector for several months before being transported back to the aquaculture site for grow-out and harvest. At the time of transfer to the aquaculture site, seed shaken free from the collector is spread on the estuary bottom. This procedure may have to be repeated for a number of years to achieve a successful clam aquaculture site with its own spawning adults to

replenish the area. This is due to the amount of time it takes for seed to mature to adult size, noted as approximately 5 – 6 years on PEI (Robert 1981) to 6 - 8 years in eastern Canada (Fisheries and Oceans 1997b), and to establish several separate year classes of clams. Unfortunately, the collection of wild seed may not provide the consistent supply desired for clam bed recruitment.

The second option is to relay juvenile and adult clams from prolific clam producing areas to the aquaculture site. Areas that are currently closed to the public fishery due to high levels of bacteria are typically those used for relaying purposes. The relayed stock is then spread on the estuary floor within the aquaculture site in an attempt to establish a population with all size and age classes. Because the clams have been moved to clean water aquaculture sites, the shellfish are able to depurate themselves prior to any harvest that might occur. Although this method yields much faster results than its counterpart, it is not always successful. Clams are sensitive to transport and will not always survive and reproduce in a new area.

Advantages to using seed which is collected in nature versus hatchery-reared clam seed include a lower seed cost, low level of technology required and, as seed is native to the area, higher percentage yields of juvenile clams surviving for future uses on an aquaculture site. Hatchery set-up and operation is a technically challenging and costly endeavour regardless of the species being reared (Pouliot *et al.* 1995). Difficulties in consistently conditioning and spawning *Mya arenaria* in the hatchery include adult broodstock not performing optimally due to the

non-sediment environment (Hidu and Newell 1989). Presently, clam harvesters on PEI seek a simple and inexpensive means of acquiring clam seed for growout.

The percent survival of natural setting soft shell clams to the settlement stage of development is approximately 0.1 percent (Hidu and Newell 1989). The settlement stage of the larval life cycle seems to be key to survival since mortality is greatest up to this stage. Under natural conditions, most mature larvae die because they fail to find a place to settle (Medcof 1961). Reducing this heavy loss of larvae by providing seed collectors will increase productivity. By targeting the settlement stage, and offering the young clams an alternative to bottom substrate, the result may be the overall increase in the numbers of clam seed surviving to juvenile and adult life stages.

Several practical reasons exist for using mesh bag collectors, including limited effort and time needed for preparation, rapid deployment and retrieval, and the use of inexpensive materials to make the collectors. In addition, the collector design is easy to construct, not harmful to the environment in its finished format, and has been proven to be successful with other shellfish species. Collectors capture clam larvae and provide a hospitable settling environment for clam seed in order to optimize survival. Additionally, food is easier to access, as the clams are filter feeders. Since the water is warmer near the surface than it is on the bottom, this also helps to promote clam growth. Clams are most susceptible to predators at shorter shell lengths, so the increased growth rate seen with clams in collectors decreases the duration in which predators are problematic. The elevation of the

collectors in the water column and the actual structure of the collectors also aid in protecting clams from predation.

## 5.2 Objectives

The primary objective of this study was to assess the feasibility of capturing quantities of soft shell clam seed by deploying seed collectors on a longline. The second objective was to describe the distribution and abundance of seed collection in a temporal fashion to determine the optimal timing for seed collector deployment to capture viable soft shell clam seed for grow out. This study will provide information on the quantity of soft shell clam seed available at a specific site and the growth of juvenile clams during the first summer season. This is the first step in understanding juvenile clam production potential. A final objective was to monitor water temperature and salinity at the Oates Point study site.

## 5.3 Materials and methods

### 5.3.1 Site selection and description

The Oates Point study site was chosen as the area for the seed collection field trials; this study site is described in Chapter 2. The site contained areas with shallow and deep water for adult sampling and seed collector deployment, respectively. The area was sheltered enough to protect clam seed in the collectors from adverse environmental effects such as heavy wave action. A scientific permit for the research was granted by the Department of Fisheries and Oceans (DFO) for

the 1998 and 1999 seed collection field trials.

### 5.3.2 Sampling schedule

Seed collectors were deployed on a weekly basis in two consecutive field seasons, 1998 and 1999. Commencing on July 17, 1998, collectors were deployed in lots of 3. For the 1999 season, collectors were hung in lots of 4, with the exception of the first date, June 22, when 8 collectors were deployed. The last deployment of seed collectors was on September 2 for the 1998 field season and on August 31 for the 1999 field trial. All collectors were left in the water for the duration of the study; the retrieval date for all collectors was September 28 in both years.

### 5.3.3 Collector deployment and retrieval strategy

Onion mesh bags measuring 81 centimeters (cm) in length by 46 cm in width and having a mesh size of 3 millimeters (mm) were used in the collector design. A single type of nylon monofilament netting, of a uniform thickness, was placed in the onion bag with a small cement block. The amount of monofilament used in each bag ranged from approximately 169 grams (g) to 798 g, with a mean weight of 374 g. The cement blocks had a mean weight of 467 g. A total of 24 collectors were set out in 1998, while 48 collectors were deployed in 1999. Using a boat to reach the site, bags were tied to a floating longline and hung at a depth of approximately 91 cm from the surface by tying a 122 cm length of rope around the top of each bag

and securing it to the longline (Figure 1). Tagged collectors were deployed in sequential order down the longline approximately 31 cm apart. In 1998, plastic containers were used for buoyancy at every sixth seed collector bag. In 1999, single polystyrene buoys were attached to the longline at every second seed collector in order to float the longline and to keep the collectors in an organized linear arrangement.

As collectors were retrieved, they were placed inside individual plastic bags in order to ensure that any clams which might fall from the collector in transport could still be associated with a specific bag. The plastic bags were stored in a freezer unit at -20 degrees Celsius (°C) until they could be processed at the laboratory.

### 5.3.4 Environmental parameters

A conductivity meter (YSI model 30, YSI Incorporated, Yellow Springs, Ohio, USA, 1998) was used to record salinity data upon each visit to the study site over the two year study. The same meter was used to record water temperature data in 1999. Both salinity and temperature of the water column were measured approximately 91-122 cm below the water surface at the seed collection site during the two-year study. In addition, temperature data loggers (VEMCO Minilog-TR version 2.08, VEMCO Limited, Shad Bay, Nova Scotia, Canada, 1998) were deployed at the site each year to monitor and record hourly water temperature for

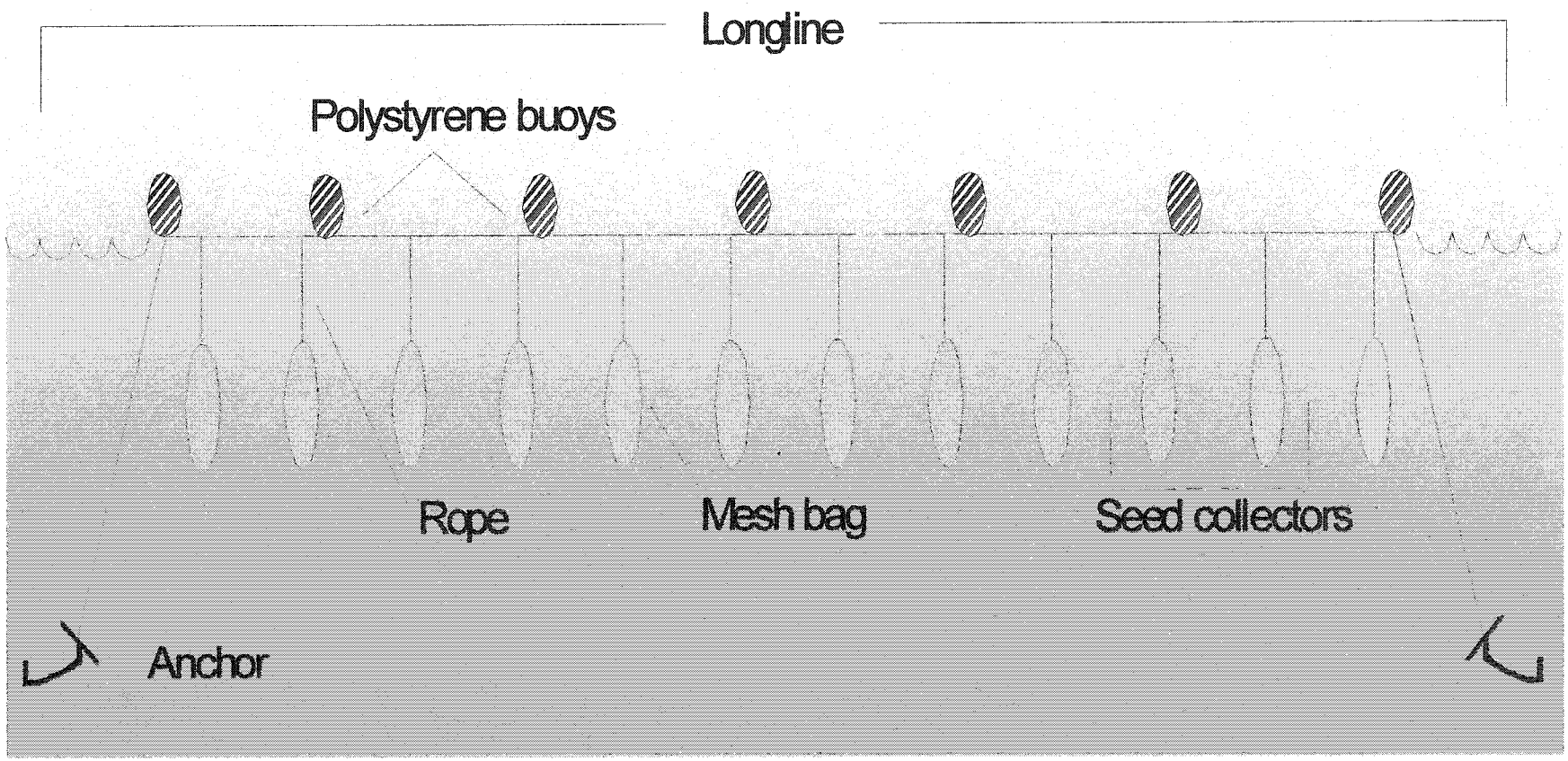


**Figure 1.** Longline and seed collector design used for soft shell clam seed collection study at Oates Point in 1999.

the duration of the study. These parameters were monitored and recorded to have baseline data on file should future studies determine that either temperature or salinity plays a role in clam settlement.

### 5.3.5 Enumeration and shell length

In the laboratory, the contents of each collector were scrubbed and rinsed using the method described by MacLean and Gillis (1996) for sea scallops, with the exception that a 64 micron (μm) mesh screen was employed in this study. For each collector, clams were sorted from the other organisms captured, enumerated, and shell lengths recorded to the nearest 0.1 mm using electronic calipers (Model CD-8"C, Mitutoyo Corporation, Tokyo, Japan, 1997). Collector bag condition and content were recorded, noting any siltation as well as recording any other marine species found in or attached to the outside of the collector.

In the 1999 study, the amount of monofilament used in each collector was recorded and compared to the number of seed captured in each collector.

### 5.3.6 Species identification

Soft shell clams were identified directly or with the aid of a magnification lamp or dissecting microscope and differentiated from other bivalve species such as gem shell, *Gemma gemma*, false angel wing, *Petricola pholadiformis*, and blue mussel, *Mytilus edulis*. Identification employed many techniques, including

hatchery-reared samples of soft shell clam seed, wildstock clam seed samples, photographs and descriptions of clams and other bivalves (Sullivan 1948, Doiron 1997).

### 5.3.7 Statistical analysis

Data collected over the two-year period were entered and stored in a Corel® Quattro® Pro 8 spreadsheet (Quattro® Pro version 8, Corel® Corporation Limited, Ottawa, Ontario, Canada, 1998). All data was examined and manually checked for errors against the original caliper printouts. Error checking was also conducted by comparing the descriptive statistics, including means, medians, minimums and maximums for each collector in the spreadsheet versus the caliper mini processor printout equivalent. The mean shell length was calculated on a per collector basis as well as on the total number of clam seeds in all collectors for any given sampling day. The mean number of clam seed captured was calculated using the total abundance of clam seed in all collectors for any given sampling day.

Statistical analyses on the quantity and shell length of clam seed collected, and weight of the filling used in the collectors were conducted using the Stata™ 7.0 program (Stata Corporation, College Station, Texas, USA, 1984-2002). As the data was not distributed normally, the natural log of the total number of clams was calculated to normalize the outcome. Analysis included descriptive statistics, linear regression, one-way analysis of variance, and Bonferroni adjustments for

categorical variables. For all analyses, differences were considered significant when $P \leq 0.05$.

The VEMCO Minilog-TR temperature data logger for the 1998 field season was downloaded to a University of Prince Edward Island (UPEI) mainframe computer using the Minilog-PC interface. Daily temperatures were graphed over the duration of the study.

## 5.4 Results

### 5.4.1 Quantity

The number of clams captured in the 1998 bags was significantly fewer than those retrieved from the 1999 study bags (Tables I and II). Individual collectors had seed quantities between 0 and 222 seed in 1998 and between 7 and 1565 seed in 1999. In 1998, the quantity of seed captured decreased throughout the duration of the study. For details on sampling protocols, see Appendices G and H.

For the 1998 field season, mean number of clam seed per collector ranged from approximately 2 for the three collectors deployed on August 21 to 189 for the three collectors deployed on July 17 (Table I). In 1999, the mean number of seeds per collector ranged from 22 for the four collectors deployed on August 31 to 636 seeds for the four collectors deployed on August 10 (Table II). The overall mean number of clam seed per collector was 32 in 1998, in contrast to 298 in 1999.

Over 74 percent of the clam seed captured in the 1998 trial came from the July 17 collectors, with the remaining 201 clams being captured between July 24

**Table I.** Deployment date and number of weeks collectors were in water, total and mean number of soft shell clams collected per sampling trip, and mean length of clams measured following collector retrieval, 1998. Standard deviation in parentheses.

| Deployment Date | Weeks in Water | Total Number Seed in Bags[1] | Mean Number Seed per Bag | Mean Shell Length (mm) |
|---|---|---|---|---|
| Jul 17 | 10.5 | 567 | 189.00 (43.18) | 9.01 (2.68) |
| Jul 24 | 9.5 | 143 | 47.67 (15.54) | 6.84 (3.00) |
| Aug 3 | 8 | 10 | 3.33 (1.25) | 7.70 (3.87) |
| Aug 7 | 7.5 | 14 | 4.67 (1.25) | 4.50 (1.40) |
| Aug 11 | 7 | 14 | 4.67 (3.30) | 3.86 (1.99) |
| Aug 21 | 5.5 | 5 | 1.67 (1.25) | 3.00 (1.10) |
| Aug 27 | 4.5 | 8 | 2.67 (0.94) | 3.50 (2.50) |
| Sept 2 | 3.5 | 7 | 2.33 (0.47) | 3.71 (2.25) |
| AVERAGE | | | 32.00 (63.24) | 8.27 (3.07) |

[1] based on a weekly deployment of three collector bags

**Table II.** Deployment date and the number of weeks collectors were in water, total and mean number of soft shell clams collected per sampling trip, and the mean length of clams measured following collector retrieval, 1999. Standard deviation in parentheses.

| Deployment Date | Weeks in Water | Total Number Seed in Bags[1] | Mean Number Seed per Bag | Mean Shell Length (mm) |
|---|---|---|---|---|
| Jun 22 | 14 | 5058* | 632.25 (129.47) | 12.46 (3.41) |
| Jun 29 | 13 | 355 | 88.75 (18.39) | 10.19 (5.63) |
| Jul 6 | 12 | 418 | 104.50 (32.48) | 5.82 (2.98) |
| Jul 13 | 11 | 292 | 73.00 (14.30) | 8.29 (4.20) |
| Jul 20 | 10 | 301 | 72.25 (28.68) | 6.79 (3.42) |
| Jul 27 | 9 | 1129 | 281.00 (60.11) | 5.77 (2.20) |
| Aug 3 | 8 | 1172 | 293.00 (135.24) | 6.34 (2.20) |
| Aug 10 | 7 | 2544 | 636.00 (561.61) | 5.65 (1.73) |
| Aug 17 | 6 | 2532 | 632.75 (467.20) | 5.85 (1.38) |
| Aug 24 | 5 | 418 | 104.50 (58.93) | 5.35 (1.51) |
| Aug 31 | 4 | 87 | 21.75 (9.60) | 4.18 (1.65) |
| AVERAGE | 9 | | 298.04 (334.23) | 8.34 (4.17) |

[1] based on a weekly deployment of four collector bags
*based on a deployment of eight collector bags on June 22, 1999

and September 2 (Figure 2). In 1999, the number of seed captured in the collectors was high on June 22 but began to decline until the week of July 27. At this point, the seed quantities increased weekly with a peak of settlement between August 10 -17 (Figure 3).

The weight of the monofilament in the 1999 study had no significant effect on the log total number of clams collected when evaluated in a one-way analysis of variance. The most monofilament used, by weight, was in the June 22 collectors, whereas the most seed was captured in the August 10 collector (Figure 4). With the exception of the June 22 collectors, the onion sacks had a relatively consistent amount of mesh material in them, as per Appendix H.

### 5.4.2 Shell length

There was considerable similarity in growth between the two years sampled, as the mean shell length of the clam seed was 8.27 mm in 1998 and 8.34 mm in 1999 (Tables I and II). Individual clam shell lengths ranged from 1 mm to 20 mm in 1998 and from 1.42 mm to 26.59 mm in 1999.

In evaluating the 1999 seed trial, the peak of seed collection was noted on the August 10 - 17 collectors. The seed in these collectors had mean shell lengths of 5.65 and 5.85 mm, respectively (Table II). With this information, this study established a cut-off shell length of 6.0 mm, thereby assuming that the clams captured on the August collectors would be less feasible in terms of survival on aquaculture sites. Therefore, for the purposes of this study, those clams not

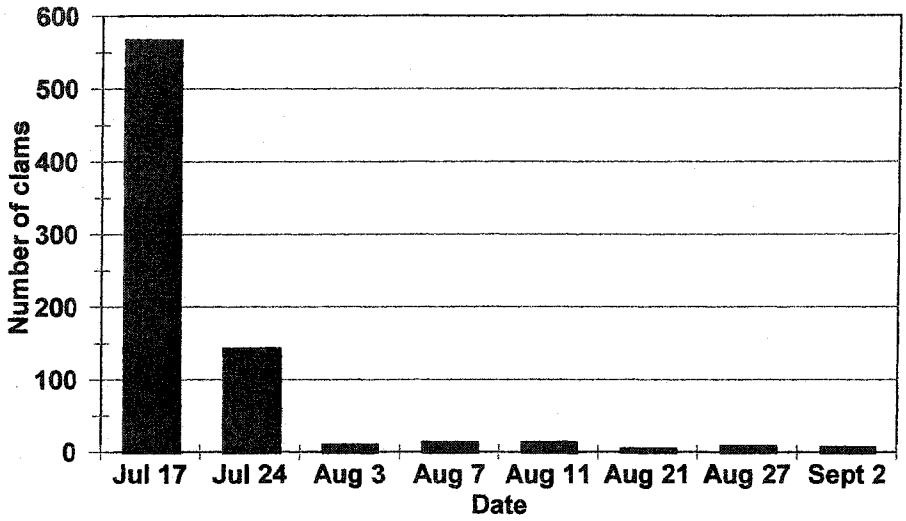


**Figure 2.** Clam seed abundance in collectors over time during the 1998 collector trial.

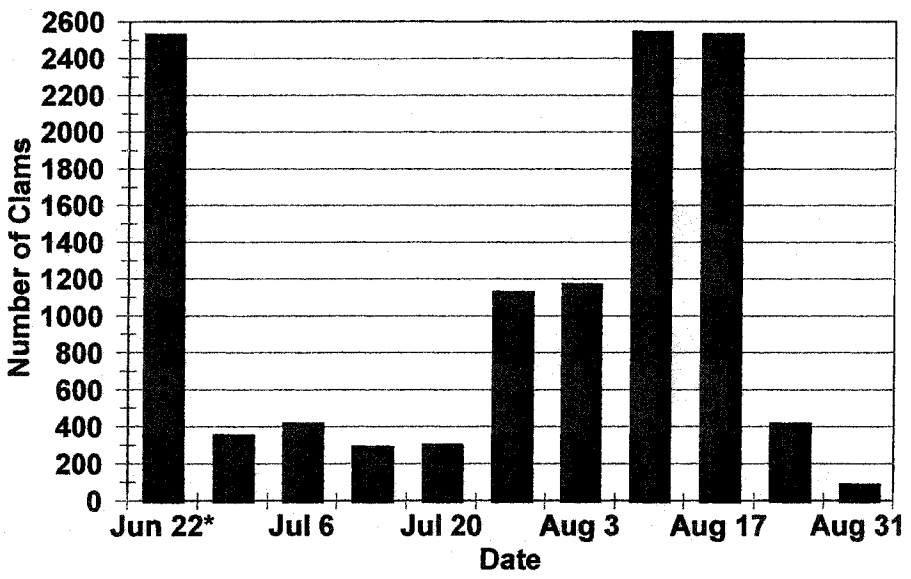


**Figure 3.** Clam seed abundance in collectors over time during the 1999 collector trial. (*Total clam abundance on June 22 was 5058 clams in 8 collectors; this number was divided in half to average the abundance for 4 collectors.)

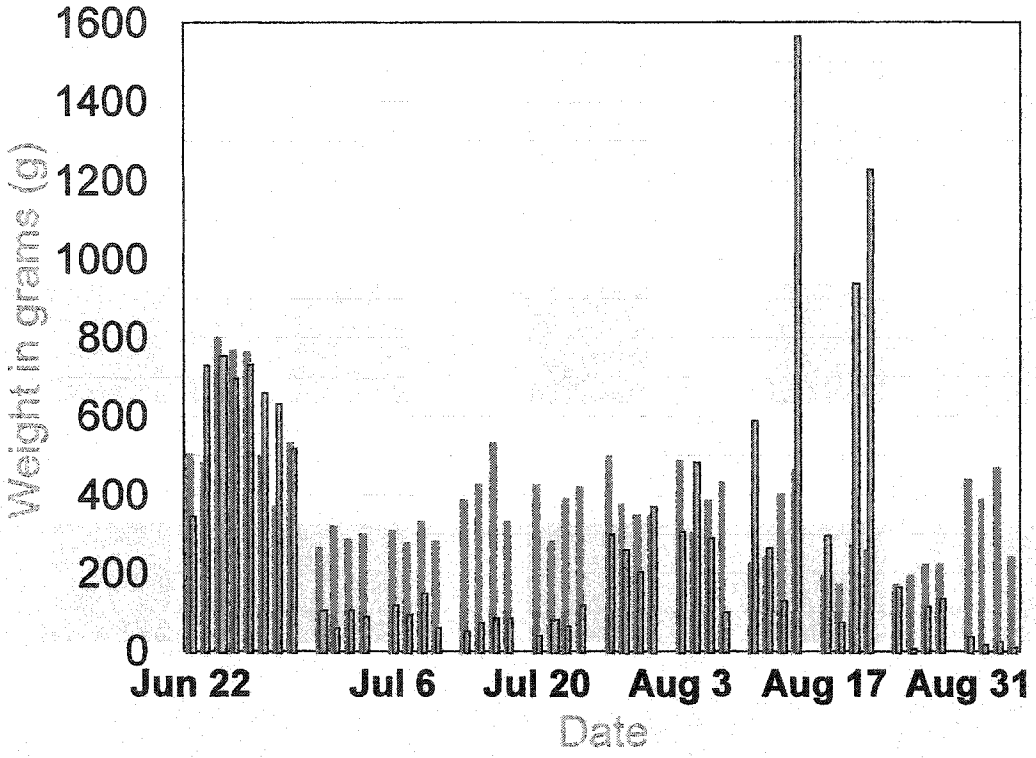


**Figure 4.** Weight of monofilament (red bars), measured in grams, used in collector design versus the abundance of clam seed (grey bars) captured over time in the seed collectors during the 1999 soft shell clam seed collector study.

reaching 6.0 mm would not be considered as viable in their ability to survive handling prior to bottom seeding, predation following bottom seeding and the harsher temperatures and conditions of the winter months on PEI. The aim in seed collection is to capture as many clams as possible of the longest possible shell length.

Approximately 90 percent of the clam seed captured on or after August 7 failed to grow to 6.0 mm in shell length during the 1998 field study (Figure 5). Prior to this date, more than 50 percent of the clams were able to achieve this shell length. In the 1999 seed collection study, 60 percent of the clam seed captured on or after August 10 failed to grow to 6.0 mm in shell length (Figure 6). Of all the clams surpassing 6.0 mm in length in 1999, 55 percent of them were found in the June 22 collectors.

As expected, the mean shell length decreased over time. In the 1998 study, mean shell length significantly decreased between the clam seed collected on July 17 and the seed collected from August 10 onward (Figure 7). In the 1999 study, mean shell length of the clams significantly decreased between June 22 and July 6 (Figure 8). Mean shell length collected on June 22 was also significantly different than mean clam shell length collected from all sampling dates following July 6. The June 29 mean shell length was significantly different than the clam mean shell length on the July 6 collectors and than the mean shell length of all collectors from July 20 onward.

Deployment date was significantly associated with the log number of clams

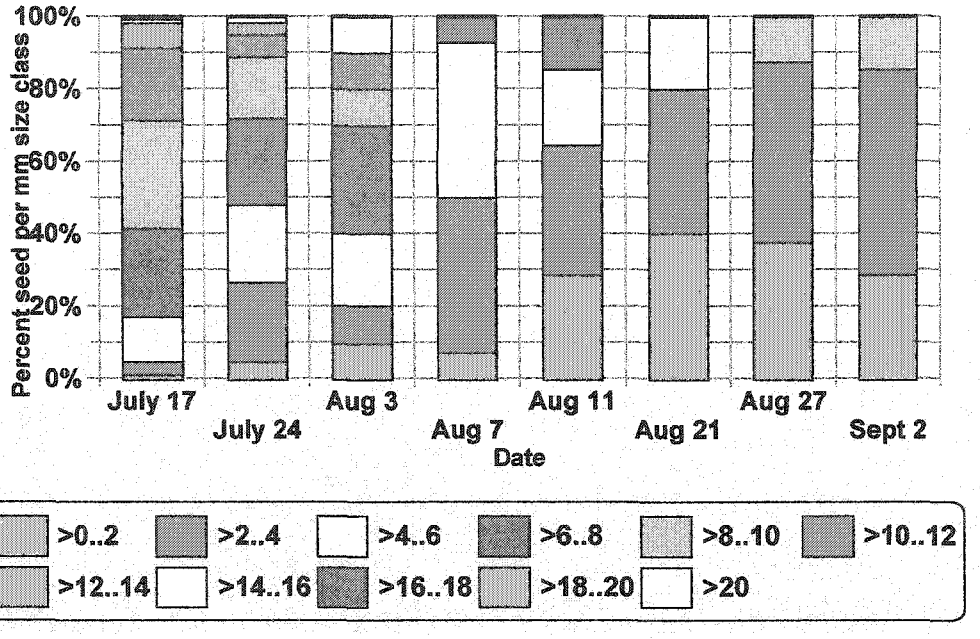


**Figure 5.** Percentage of clam seed in each size range, measured in mm, for the 1998 seed collector trial.

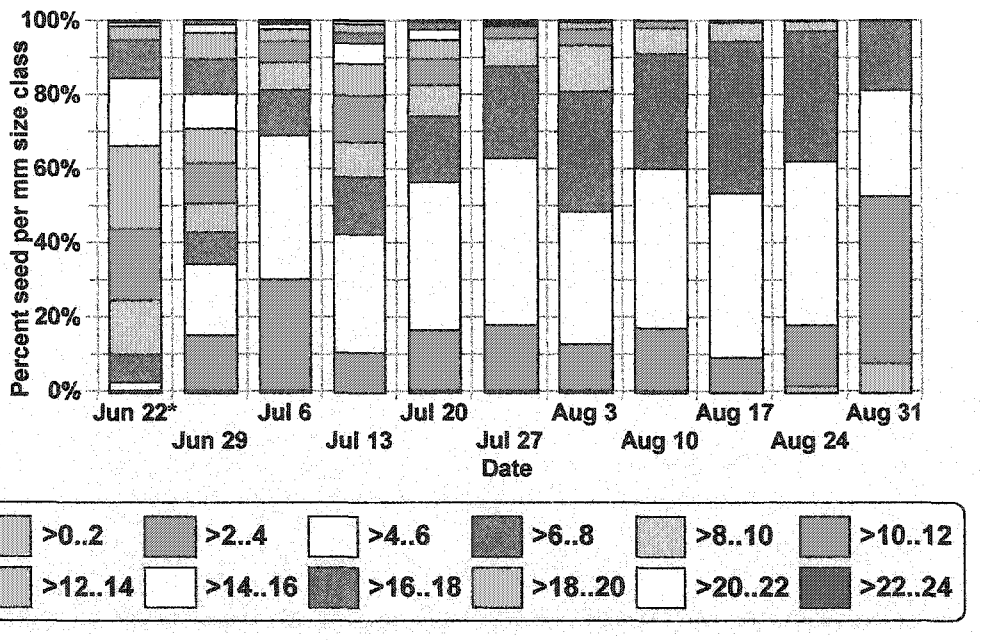


**Figure 6.** Percentage of clam seed in each size range, measured in mm, for the 1999 seed collector trial (* based on eight collectors).

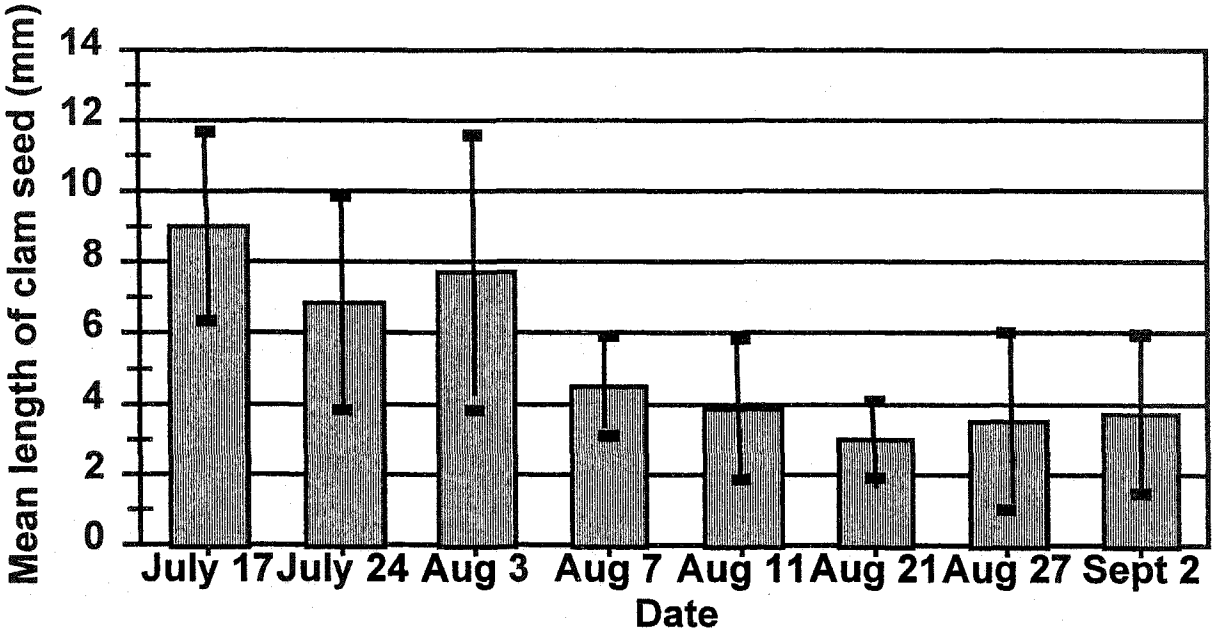


**Figure 7.** Mean shell length of clam seed, plus or minus standard error of the mean, per week in the 1998 sampling season.

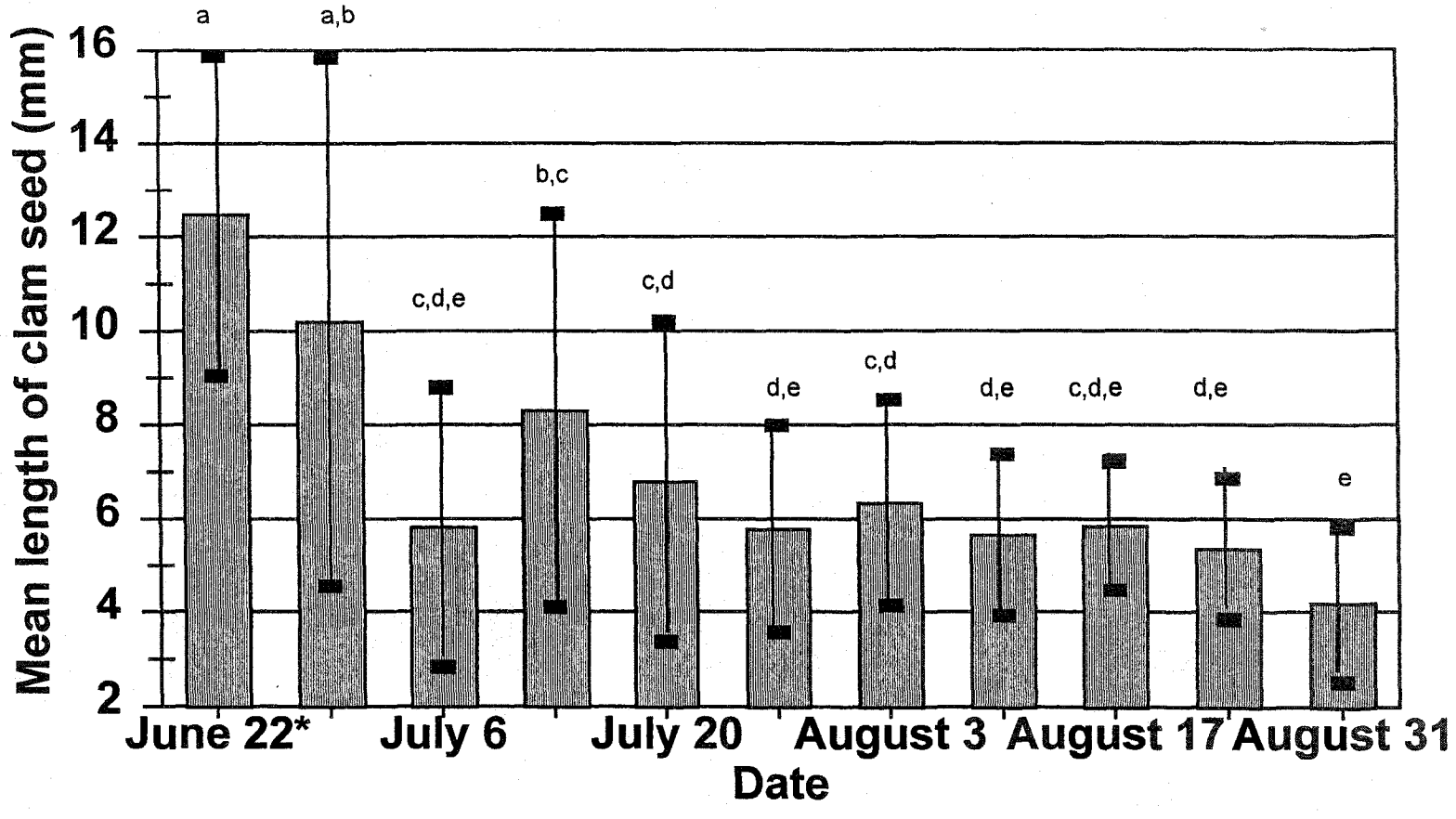


**Figure 8.** Mean shell length of clam seed, plus or minus standard error of the mean (SEM), per week in the 1999 sampling season (* based on eight collectors). A one way analysis of variance was used to determine that there was statistical significance between the groups in terms of mean shell length. The superscript letters indicated above each mean shell length and SEM indicate between which groups statistical significance was found. Significance was determined between specific groups using the Bonferroni calculation. Means with a superscript in common are not statistically different from each other whereas mean shell lengths with different superscripts were found to be statistically different ($P \leq 0.05$).

recorded at the end of the study. Differences between mean log total number of clams collected, using the Bonferroni method to detect differences, indicates that significance may be found. For example, the August collectors would have had less duration in the water than the June collectors, and the seed captured in August would therefore have shorter shell lengths than those captured in June. This is demonstrated in Figures 7 and 8. The June 22 collectors had the lowest percentage of seed in the 2 - 4 mm, 4 - 6 mm, and 6 - 8mm size ranges, yet had the highest percentage of seed in the 8 -10mm, 10 -12 mm, 12 -14 mm, 14 -16 mm, and 16 -18 mm size ranges as compared to all other collectors in the 1999 trial (Figure 6).

### 5.4.3 Water temperature and salinity

Water temperature and salinity were monitored during the seed collector trials in 1998 and 1999 onsite with a handheld meter. Temperature was also recorded with a datalogger deployed on site. In 1998, the salinity varied from 24.2 parts per thousand (ppt) on September 2 to 27.2 ppt on July 20 while the water temperature ranged from 17.75 degrees Celsius (°C) on August 14 to 25.28 °C on August 8 (Figure 9). Although the temperature datalogger for 1999 was no longer attached to the longline on September 28, water temperature, as recorded from the handheld meter, ranged from 17.6 on August 10 to 22.2 °C on July 20 and July 27 (Figure 10). In 1999, the salinity varied from 23.9 on August 24 to 26.9 ppt on July

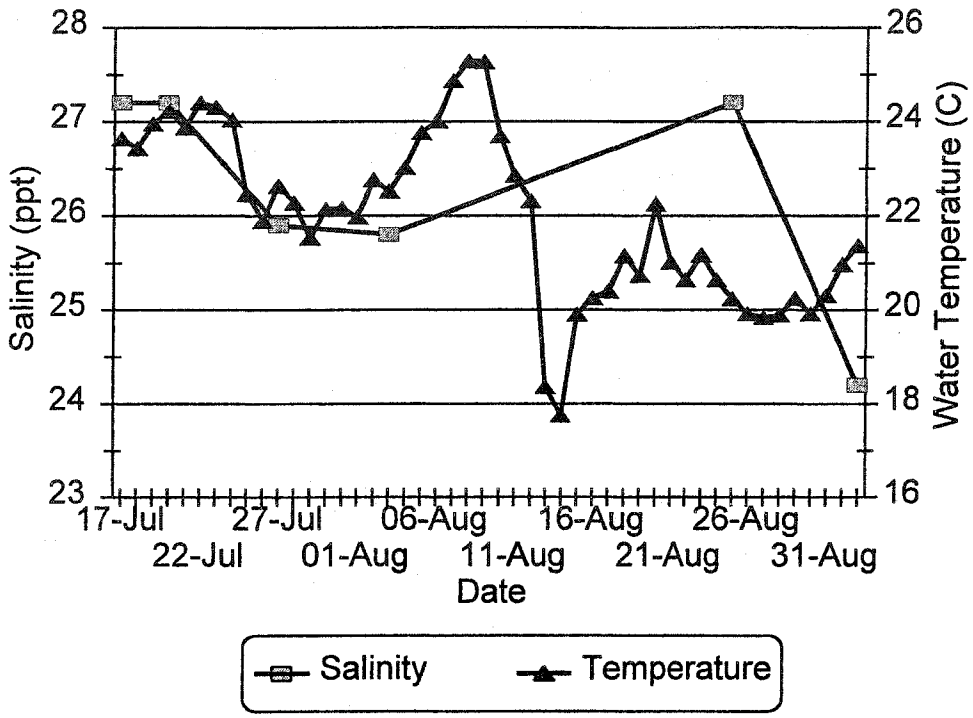


**Figure 9.** Environmental parameter monitoring of Oates Point during the 1998 clam seed collector trial.

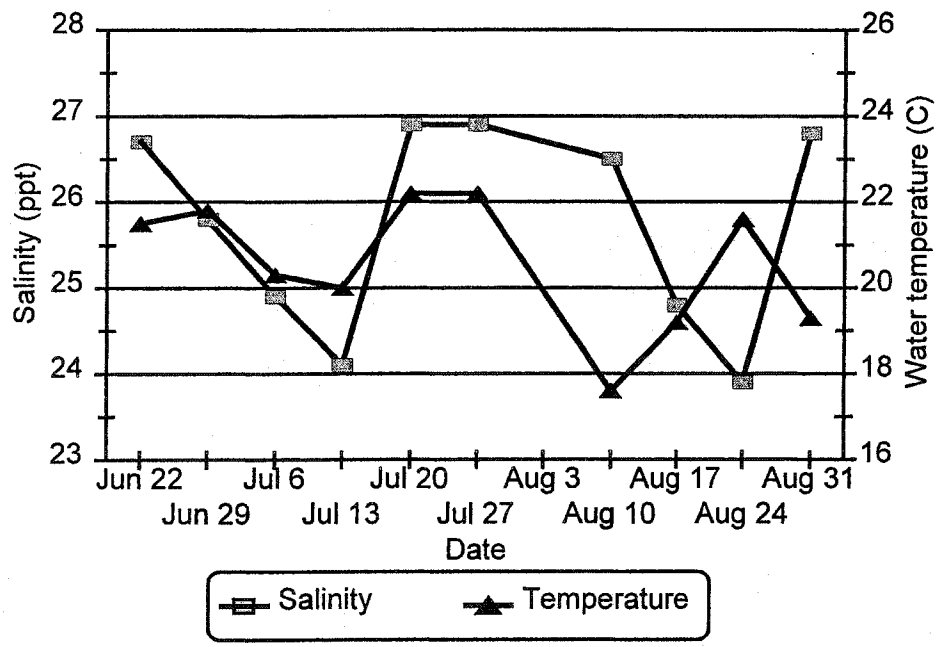


**Figure 10.** Environmental parameter monitoring of Oates Point during the 1999 clam seed collector trial.

20 and July 27.

## 5.5 Discussion

Collectors were deployed with an earlier start date in 1999; counts from that year might have been influenced, to some extent, by this factor. In 1998, the amount of seed collected may have been influenced by the sediment-filled collector bags since the longline was not adequately buoyed. Moore (1897) found that young soft shell clams setting on a very soft substrate are liable to suffocate in the mud. Upon retrieval, it was discovered that most of the seed collectors had been resting in the benthos, or bottom.

Since the 1999 collectors were deployed with an earlier start date than in 1998, some of the seed had more time to grow as compared to seed in 1998. This may also explain why the mean shell length was slightly higher in the 1999 collector trial.

Seed counts were less for later collector deployments in the 1998 season; it appears that the earlier the collector was set, the higher the number of seed collected. Clams were sampled for histology in 1998 at the Oates Point site within the same time frame (Chapter 2). The histological analysis indicates that a large spawning event occurred on site within the last week of June. This would lead to settlement of clam larvae on collectors in late July. This concurs with the findings of the seed collectors since the most seed was captured in July.

The single peak in clam numbers captured in 1998 differs from the 1999

season in that there were two main peaks of seed collection. The first peak was at the beginning of the field season, June 22, while the second peak was from August 10 -17. In 1999, the latter dates of peak seed collection correspond well with a spawn in mid July, as settling would be approximately 4 weeks later. As the June 22 and mid August collector dates indicated, it can be speculated that there were two spawning events in 1999.

One of the spawning events could have been from the clams in the area, as histology indicates that all clams had spawned sometime prior to the start of sampling on June 22. The seed for the second spawning event may have come from spawning clams in another geographic region as larvae can be carried long distances with the currents. A spring spawn may have occurred in mid May in addition to the mid July spawning event, as the clams captured on the June collectors are longer in shell length as compared to the August seed.

Clams were sampled for histology at this site in the same time frame (Chapter 2). As sample collection did not commence until June 22, it can not be confirmed as to whether or not a mid May spawning event took place. However, according to the histology results for the months of June, July and August, between 85 to 90 percent of the clams sampled were already at the partially spent or completely spent stages of reproduction (Chapter 2). This data supports an earlier spawn, but also points to the lack of a spawning event in mid July on the site of the seed collectors. Therefore, the large quantities of seed captured in mid August are likely from a spawning population of clams that is geographically removed from the

site as larvae are able to travel large distances in their free swimming stages.

In this study, there appears to be no direct correlation between the total weight of monofilament used in the onion sacks versus the actual number of seed captured by the sack. This does not agree with the work of Naidu *et al.* (1981) who observed that there was a positive relationship between the amount of monofilament in the collector up to approximately 454 g and the quantity of seed it is capable of capturing. Several factors may have influenced the varying results including the target species for the study in that Naidu *et al.* were collecting sea scallops. Another consideration is that locations were different for the studies, and likely varied in terms of currents, tides, wind and wave action.

Since the mean seed shell length decreased over the duration of the field season, it provided evidence of when the collectors actually captured the seed. The large quantity of seed captured on June 22 collectors in 1999 does not appear to be an effect of the collectors capturing seed through the duration of the study as the majority of the clam seed was between 8 - 16 mm in length. In contrast, the August 10 and 17 collectors had large quantities of seed in which the majority fell between 4 - 8 mm in length.

In the 1998 and 1999 seed studies, the majority of clam seed captured on or after the first week of August failed to grow to 6.0 mm in shell length. This leads to two practical conclusions. First, collectors are not likely to be as useful in terms of achieving clams of the set shell length if deployed after this date. Second, these clams were likely from a later spawn, as the majority of the seed is smaller than the

shell length cut off.

Of the clams surpassing 6.0 mm in length in 1999, the majority of them originated from the June 22 collectors. This is an expected result as these clams had more weeks in the water, and therefore more time to grow. The optimal timing for collector deployment is the earliest time frame in which large quantities of clams can be captured.

Because seed that settles earliest in the season grows largest before autumn, it is recommended to place seed collectors in the water just before the first heavy set is expected (Medcof 1961). If collectors are placed too early, they will foul and lose their efficiency before the larvae are ready to settle (Medcof 1961).

## 5.6 Conclusions

The primary objective of this chapter was to assess soft shell clam seed collection and early growth using scallop collectors; this objective was achieved as clam seed was captured, enumerated, and measured in both years of the study. Secondary objectives included describing the distribution and abundance of seed collection in a temporal fashion in order to ascertain the optimal time frame for collector deployment in order to maximize the quantity of seed captured, and to monitor the environmental parameters of the study site. While the distribution and abundance of seed collection was described, it was difficult to fully accomplish the goal of knowing when to deploy the collectors given the variability seen in the two years studied. It appears that the peak of clam larvae numbers may be at a

different time each year, as peak settlement varied between 1998 and 1999. The site was successfully monitored for water temperature and salinity.

One of the major limitations of this study was the poor longline design in the 1998 seed trial. Had the collectors been more effectively buoyed, perhaps there would have been a marked increase in the quantity of seed collected. However, in a three-month span, the soft shell clam seed grew a mean of 8.0 mm in length in both years of the study. Therefore, when designed properly, seed collectors are an effective method to capture commercial quantities of clam seed for grow-out purposes and from this study, the documented early survival of the soft shell clam seed in the first summer is promising.

Future studies focusing on determining the factors that influence clam spawning so that spawning dates can be predicted or the successful monitoring of soft shell clam larvae would provide beneficial information for the deployment of seed collectors at the optimal time. In lieu of this information, results of the present study suggest that seed collectors be deployed on a weekly basis throughout the months of June and July. Although the earliest collectors deployed in this study were in late June, large numbers were captured and growth of the clams was maximized. Therefore, an earlier start and end date for collector deployment would be worth further investigation. Another element that requires evaluation in the future is the survival rate of juvenile clams over the first winter. This study could test several methods of overwintering the juvenile clams from the collectors and growth rates could be determined as well.

# 6. GENERAL DISCUSSION

## 6.1 Clam reproductive cycle

The assessment of male and female clams for stage of reproductive development using histologic examination indicated that the inactive reproductive stage primarily occurred in late summer and autumn of each year. However, as clams were not collected for sampling in the winter months, it could not be verified whether this stage occurs in that time frame as well. This could be evaluated in future studies.

Active stage clams within the reproductive cycle were noted in small percentages throughout the time frame sampled. These low percentages could be due to the timing of field sampling, suggesting that higher percentages of active clams are present in the winter and early spring months, but this remains to be determined.

Mature stage clams were present throughout the sampling seasons but were at the highest percentages in late spring and early summer. Clams in the partially spent stage of reproduction occurred in increasing percentages as mature clam percentages declined. The majority of partially spent clams were recorded during the summer months.

Completely spent clams were present in the summer and early autumn. These clams were noted until the end of the sampling period and may continue later into the year.

## 6.2 Triggers of spawning

From the data in the present study, neither temperature nor month of year alone appears to be the sole factor controlling the onset of soft shell clam spawning on PEI. The commencement of clam spawning is likely to be explained by a more complex multivariable model. Elements such as phytoplankton blooms, tides, and the tracking of storm events could be evaluated in the future along with temperature and salinity monitoring.

The findings in this study differ from that for oysters, *Crassostrea virginica* (Loosanoff and Davis 1951, Medcof 1961), and quahaugs, *Mercenaria mercenaria* (Eversole 1989), in that water temperature is known to be the sole factor influencing the onset of spawning events in those species. Although some relationship between spawning and water temperature in mussels, *Mytilus edulis*, seems evident, it does not appear to be the only contributing factor to spawning activities (Seed 1976). In a complex process like reproduction, the interaction of factors is to be expected and it can be less straightforward in determining what causes the onset of spawning (Seed 1976). This appears to be the case for soft shell clams on PEI as well.

Given the results of this study, it is anticipated that the timing of soft shell clam spawning events could vary from year to year as it is likely a combination of environmental factors that are influencing the commencement of spawning.

## 6.3 Date, duration and frequency of spawning

The date, duration and frequency of spawning were assessed by histologic examination of gonads, the calculation of several condition indices, and by larval monitoring. In 1997, the dates of soft shell clam spawning were found to be similar, regardless of site, using histological analysis of clam gonads. The peak of mature stage clams was in mid to late June in 1997 at all study sites, and this corresponds well with the peak of mature stage clams, noted on June 8, at the 1998 Oates Point site as well. The first date sampled in 1999 was June 22, and by then, spawning had already occurred.

In 1997 at Gascoigne Cove, the duration of spawning was approximately 2 months as percent of clams in the mature stage decreased gradually from late June to late August. This contrasts to the Barsway site for the same year, in which spawning occurred over a one-week window between late June and early July. The Oates Point site spawning duration for 1997 lasted approximately three weeks with a decrease in mature stage clams between mid June and early July. In 1998, the Oates Point site spawned for approximately four weeks from early June into the beginning of July. Spawning occurred prior to the commencement of sampling at Oates Point in 1999.

From histological evaluation of gonads, it appears that the frequency of soft shell clam spawning on PEI was noted to occur once in all three sites in 1997 as well as in the site monitored in 1998. With the shorter time frame monitored at the study site in 1999, the data shows evidence of a single spawning event.

As histology is expensive and labor intensive, efforts should be placed in developing new methods that could be beneficial in predicting spawning, such as the use of needle biopsy. This method has been successfully used in evaluating gametogenesis in Atlantic surf clams, *Spisula solidissima* (Schneider *et al.* 1997). Alternatively, another way to use the histologic specimens of the clam gonads would be to evaluate the relative percentage of gonad in the tissue section. In this way, clams that are in the partially spent stage may be analyzed in greater detail and this information might tie in to other aspects of monitoring reproduction such as in the verification of condition indices.

Although the data was not found to be significant, some similarities were noted in comparing condition indices to the percent of mature stage clams. The condition indices evaluated, steam meat yield, shell condition and gravimetric condition, were not conclusively shown to be reliable in determining the date of soft shell clam spawning on PEI. Steam meat yield was more consistent in detecting clam spawning than were the other condition indices tested. Shell condition appeared to be the least accurate of the indices evaluated. As numerous methods exist to measure condition index, there may be a method which is beneficial in detecting spawning events in clams. This determination is useful as when spawning is known to have occurred, seed collectors may be deployed to capture seed.

Plankton tows are routinely used to detect the presence of larvae in the water column. The larvae are then classified by age to determine when spawning occurred. The plankton tows and plankton pump sampling did not

yield results in this study but the techniques should be refined and re-evaluated. Future studies should incorporate additional sampling stations for larval monitoring throughout the estuary and larval monitoring should commence earlier in the season. Close monitoring of spawning activities using histology or needle biopsy technique, once verified, combined with steam meat yield, should also be conducted to verify if present larval monitoring techniques are appropriate for capturing clam larvae.

### 6.4 Clam seed collection

The soft shell clam seed collection, using scallop collectors, and early growth was monitored as clam seed was captured, enumerated, and measured in both years of the study. It was difficult to fully accomplish the goal of knowing when to deploy the collectors as the maximum yield of clams may occur at a different time each year. Timing for peak seed settlement is directly linked to the timing of clam spawning, which appears to commence in June, but its duration varies between sites and from year to year.

In spite of this setback, meaningful and beneficial information was gained concerning the ability to collect and grow clams using onion sack collectors. In a three-month time span, the soft shell clam seed collected in both years grew to a mean shell length of approximately 8 mm. Therefore, seed collectors are an effective method to capture commercial quantities of clam seed for grow-out purposes and the documented early survivorship of the seed is promising.

## 6.5 Study objectives

The major objectives for the research described in this thesis were to document the reproductive cycle of soft shell clams in different regions of PEI, determine best methods of monitoring clam spawning events, and identify possible factors influencing the onset of soft shell clam spawning. Another important objective was to assess clam seed collection potential and to determine the optimal window for collecting soft shell clam seed. All of these objectives were accomplished to some degree during these studies.

It was impossible to control for every aspect of the study due to the nature of field work; therefore, determining the factors responsible for clam spawning was an extremely difficult undertaking. Still, information was gained on the possible inclusion of temperature and/or month of year in a more complex model to predict soft shell clam spawning. Although challenging, future studies should focus on determining the factors influencing soft shell clam spawning, so that upcoming spawning events may be accurately predicted. This work would be most beneficial if started in early spring so that baseline information could be gathered on the environmental features being evaluated. Knowledge of the factors influencing spawning is key in the development of this species as an aquaculture resource.

The work accomplished and presented in this thesis represents an initial step in the direction of understanding the previously unknown biological information, in terms of reproduction, for soft shell clams on PEI. This, and the information obtained on seed collection, may be used in developing approaches

to improve soft shell clam aquaculture techniques for an emerging industry on PEI.

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**Appendix A.** Numbers of clams examined histologically at each study site, and for each sampling date by reproductive stage.

| *Collection date* | *Study site* | *No. examined* | *Inactive stage* | *Active stage* | *Ripe stage* | *Partially spent stage* | *Spent stage* | *Unknown stage* |
|---|---|---|---|---|---|---|---|---|
| 25/06/99 | A | 11 | 1 | 0 | 0 | 7 | 3 | 0 |
| 29/06/99 | A | 11 | 1 | 0 | 0 | 5 | 5 | 0 |
| 06/07/99 | A | 11 | 1 | 0 | 0 | 3 | 7 | 0 |
| 13/07/99 | A | 11 | 2 | 0 | 0 | 4 | 5 | 0 |
| 20/07/99 | A | 11 | 0 | 0 | 0 | 8 | 3 | 0 |
| 27/07/99 | A | 11 | 2 | 2 | 0 | 5 | 2 | 0 |
| 03/08/99 | A | 11 | 0 | 1 | 0 | 3 | 7 | 0 |
| 10/08/99 | A | 11 | 0 | 0 | 1 | 6 | 4 | 0 |
| 17/08/99 | A | 11 | 0 | 0 | 0 | 8 | 3 | 0 |
| 24/08/99 | A | 13 | 3 | 0 | 0 | 4 | 6 | 0 |
| 31/08/99 | A | 11 | 5 | 0 | 0 | 1 | 5 | 0 |
| 23/04/98 | A | 10 | 0 | 3 | 0 | 6 | 1 | 0 |
| 05/05/98 | A | 10 | 0 | 0 | 3 | 5 | 1 | 1 |
| 29/05/98 | A | 10 | 0 | 0 | 3 | 7 | 0 | 0 |
| 08/06/98 | A | 10 | 0 | 0 | 9 | 1 | 0 | 0 |
| 16/06/98 | A | 10 | 0 | 0 | 8 | 1 | 0 | 1 |
| 22/06/98 | A | 10 | 0 | 0 | 10 | 0 | 0 | 0 |
| 02/07/98 | A | 10 | 0 | 0 | 1 | 5 | 4 | 0 |
| 08/07/98 | A | 10 | 0 | 0 | 1 | 7 | 2 | 0 |
| 13/07/98 | A | 10 | 0 | 0 | 2 | 5 | 3 | 0 |
| 21/07/98 | A | 10 | 0 | 0 | 2 | 3 | 5 | 0 |
| 27/07/98 | A | 10 | 0 | 0 | 7 | 1 | 2 | 0 |
| 03/08/98 | A | 10 | 1 | 0 | 0 | 2 | 7 | 0 |
| 21/08/98 | A | 10 | 1 | 0 | 1 | 2 | 6 | 0 |
| 02/09/98 | A | 10 | 0 | 0 | 0 | 3 | 7 | 0 |
| 16/09/98 | A | 10 | 1 | 1 | 0 | 0 | 8 | 0 |
| 27/05/97 | A | 10 | 0 | 2 | 6 | 1 | 1 | 0 |
| 06/02/97 | A | 20 | 0 | 0 | 7 | 12 | 1 | 0 |
| 13/06/97 | A | 18 | 0 | 0 | 5 | 11 | 2 | 0 |
| 18/06/97 | A | 18 | 0 | 0 | 9 | 9 | 0 | 0 |
| 26/06/97 | A | 12 | 0 | 0 | 3 | 7 | 2 | 0 |
| 02/07/97 | A | 20 | 2 | 0 | 4 | 12 | 2 | 0 |
| 11/07/97 | A | 22 | 0 | 0 | 0 | 18 | 4 | 0 |
| 17/07/97 | A | 19 | 0 | 1 | 0 | 10 | 8 | 0 |
| 31/07/97 | A | 20 | 1 | 0 | 1 | 13 | 5 | 0 |
| 13/08/97 | A | 19 | 1 | 0 | 0 | 15 | 3 | 0 |
| 26/08/97 | A | 20 | 0 | 0 | 1 | 12 | 7 | 0 |
| 23/09/97 | A | 20 | 1 | 0 | 0 | 4 | 15 | 0 |
| 09/10/97 | A | 19 | 4 | 2 | 0 | 3 | 10 | 0 |
| 28/10/97 | A | 20 | 5 | 2 | 0 | 0 | 13 | 0 |
| 23/05/97 | B | 9 | 0 | 0 | 6 | 3 | 0 | 0 |
| 02/06/97 | B | 20 | 1 | 0 | 15 | 1 | 2 | 1 |
| 13/06/97 | B | 19 | 0 | 0 | 17 | 1 | 1 | 0 |
| 18/06/97 | B | 20 | 1 | 0 | 16 | 2 | 0 | 1 |
| 26/06/97 | B | 20 | 0 | 0 | 19 | 1 | 0 | 0 |

| Collection date | Study site | No. examined | Inactive stage | Active stage | Ripe stage | Partially spent stage | Spent stage | Unknown stage |
|---|---|---|---|---|---|---|---|---|
| 02/07/97 | B | 10 | 2 | 1 | 1 | 4 | 2 | 0 |
| 11/07/97 | B | 9 | 3 | 1 | 2 | 2 | 1 | 0 |
| 17/07/97 | B | 10 | 2 | 2 | 1 | 4 | 1 | 0 |
| 31/07/97 | B | 11 | 3 | 1 | 1 | 2 | 4 | 0 |
| 13/08/97 | B | 8 | 2 | 0 | 0 | 3 | 2 | 1 |
| 26/08/97 | B | 10 | 4 | 0 | 4 | 0 | 1 | 1 |
| 23/09/97 | B | 10 | 5 | 1 | 0 | 1 | 3 | 0 |
| 09/10/97 | B | 9 | 6 | 1 | 0 | 0 | 2 | 0 |
| 28/10/97 | B | 10 | 5 | 1 | 0 | 2 | 2 | 0 |
| 30/05/97 | C | 19 | 0 | 0 | 13 | 2 | 4 | 0 |
| 04/06/97 | C | 19 | 0 | 0 | 11 | 8 | 0 | 0 |
| 12/06/97 | C | 20 | 0 | 0 | 16 | 3 | 1 | 0 |
| 19/06/97 | C | 19 | 0 | 0 | 14 | 4 | 1 | 0 |
| 25/06/97 | C | 17 | 0 | 0 | 17 | 0 | 0 | 0 |
| 02/07/97 | C | 20 | 0 | 0 | 17 | 1 | 1 | 1 |
| 09/07/97 | C | 20 | 5 | 0 | 12 | 2 | 1 | 0 |
| 14/07/97 | C | 19 | 1 | 0 | 8 | 7 | 3 | 0 |
| 24/07/97 | C | 20 | 2 | 0 | 14 | 2 | 2 | 0 |
| 31/07/97 | C | 20 | 2 | 0 | 7 | 8 | 2 | 1 |
| 12/08/97 | C | 20 | 5 | 0 | 7 | 7 | 1 | 0 |
| 25/08/97 | C | 20 | 8 | 3 | 1 | 2 | 5 | 1 |
| 19/09/97 | C | 15 | 6 | 3 | 0 | 0 | 4 | 2 |
| 06/10/97 | C | 19 | 8 | 2 | 0 | 0 | 8 | 1 |
| 20/10/97 | C | 20 | 13 | 1 | 0 | 0 | 5 | 1 |
| 07/11/97 | C | 20 | 7 | 3 | 1 | 5 | 3 | 1 |

Note: A = Oates Point, B = Barsway, C = Gascoigne Cove.

**Appendix B.** Dates and water temperatures (°C) of soft shell clams collected from each study site between 1997 - 1999.

| *Collection date* | *Study site* | *Temperature* |
|---|---|---|
| 25/06/99 | A | -- |
| 29/06/99 | A | 21.80 |
| 06/07/99 | A | 20.30 |
| 13/07/99 | A | 20.00 |
| 20/07/99 | A | 22.20 |
| 27/07/99 | A | 22.20 |
| 03/08/99 | A | -- |
| 10/08/99 | A | 17.60 |
| 17/08/99 | A | 19.20 |
| 24/08/99 | A | 21.60 |
| 31/08/99 | A | 19.30 |
| 23/04/98 | A | -- |
| 05/05/98 | A | 13.48 |
| 29/05/98 | A | 16.84 |
| 08/06/98 | A | 14.39 |
| 16/06/98 | A | 17.06 |
| 22/06/98 | A | 18.91 |
| 02/07/98 | A | 18.55 |
| 08/07/98 | A | 23.55 |
| 13/07/98 | A | 19.72 |
| 21/07/98 | A | 23.86 |
| 27/07/98 | A | 23.63 |
| 03/08/98 | A | 22.52 |
| 21/08/98 | A | 21.02 |
| 02/09/98 | A | 21.37 |
| 16/09/98 | A | 18.07 |
| 23/05/97 | B | -- |
| 02/06/97 | B | -- |
| 13/06/97 | B | 13.50 |
| 18/06/97 | B | 16.30 |
| 26/06/97 | B | 15.40 |
| 02/07/97 | B | 20.20 |
| 11/07/97 | B | 19.20 |
| 17/07/97 | B | 19.10 |
| 31/07/97 | B | 19.70 |
| 13/08/97 | B | 21.00 |
| 26/08/97 | B | 18.00 |
| 23/09/97 | B | -- |
| 09/10/97 | B | -- |
| 28/10/97 | B | -- |
| 05/06/98 | C | 11.51 |
| 12/06/98 | C | 15.08 |
| 17/06/98 | C | 12.13 |
| 23/06/98 | C | 13.43 |
| 30/06/98 | C | 13.68 |

| *Collection date* | *Study site* | *Temperature* |
|---|---|---|
| 06/07/98 | C | 16.18 |
| 13/07/98 | C | 13.83 |
| 20/07/98 | C | 17.83 |
| 27/07/98 | C | 18.84 |
| 03/08/98 | C | 16.60 |
| 10/08/98 | C | 20.92 |
| 17/08/98 | C | 19.30 |
| 23/08/98 | C | 23.43 |
| 30/08/08 | C | 22.45 |
| 06/09/98 | C | 21.98 |
| 13/09/98 | C | 25.28 |
| 20/09/98 | C | 19.90 |
| 27/09/98 | C | 20.64 |
| 04/10/98 | C | 20.24 |
| 11/10/98 | C | 20.71 |
| 18/10/98 | C | 17.09 |
| 25/10/98 | C | 14.73 |
| 01/11/98 | C | 14.60 |
| 30/05/97 | C | -- |
| 04/06/97 | C | -- |
| 12/06/97 | C | 10.84 |
| 19/06/97 | C | 14.88 |
| 25/06/97 | C | 13.40 |
| 02/07/97 | C | 16.75 |
| 09/07/97 | C | 18.13 |
| 14/07/97 | C | 19.56 |
| 24/07/97 | C | 17.67 |
| 31/07/97 | C | 19.79 |
| 12/08/97 | C | 20.01 |
| 25/08/97 | C | 17.90 |
| 19/09/97 | C | -- |
| 06/10/97 | C | 13.28 |
| 20/10/97 | C | 10.53 |
| 07/11/97 | C | -- |

Note: A = Oates Point, B = Barsway, C = Gascoigne Cove.

**Appendix C.** Results of steam meat yield condition at sample sites in 1997.

| *STEAM MEAT YIELD (%) GASCOIGNE COVE* | *STEAM MEAT YIELD (%) OATES POINT* | *STEAM MEAT YIELD (%) BARSWAY* | *DATE* | *SHELL LENGTH (20 – 50 mm)* | *SHELL LENGTH (50 + mm)* |
|---|---|---|---|---|---|
| 45.80 | 41.30 | 40.80 | 27 May 1997 | √ | |
| Nd | 39.70 | 38.00 | 27 May 1997 | | √ |
| Nd | 40.80 | nd | 29 May 1997 | √ | |
| Nd | 35.30 | nd | 29 May 1997 | | √ |
| 34.30 | nd | nd | 30 May 1997 | √ | |
| 32.70 | nd | nd | 30 May 1997 | | √ |
| nd | 43.90 | 42.90 | 02 Jun 1997 | √ | |
| nd | 36.00 | 42.00 | 02 Jun 1997 | | √ |
| 35.10 | nd | nd | 04 Jun 1997 | √ | |
| 34.80 | nd | nd | 04 Jun 1997 | | √ |
| 35.10 | nd | nd | 12 Jun 1997 | √ | |
| 38.80 | nd | nd | 12 Jun 1997 | | √ |
| nd | 44.40 | nd | 13 Jun 1997 | √ | |
| nd | 55.20 | 47.70 | 13 Jun 1997 | | √ |
| nd | 44.90 | nd | 18 Jun 1997 | √ | |
| nd | 42.00 | 41.50 | 18 Jun 1997 | | √ |
| 53.70 | nd | nd | 19 Jun 1997 | √ | |
| 61.60 | nd | nd | 19 Jun 1997 | | √ |
| 32.80 | nd | nd | 25 Jun 1997 | √ | |
| 37.90 | nd | nd | 25 Jun 1997 | | √ |
| nd | 56.2 | 42.70 | 26 Jun 1997 | | √ |
| 33.50 | 43.10 | nd | 02 Jul 1997 | √ | |
| 41.60 | 38.60 | 33.40 | 02 Jul 1997 | | √ |
| 32.20 | nd | nd | 09 Jul 1997 | √ | |
| 37.10 | nd | nd | 09 Jul 1997 | | √ |
| nd | 43.00 | nd | 11 Jul 1997 | √ | |
| nd | 38.20 | nd | 11 Jul 1997 | | √ |
| 31.20 | nd | nd | 14 Jul 1997 | √ | |
| 32.90 | nd | nd | 14 Jul 1997 | | √ |
| nd | 43.20 | nd | 17 Jul 1997 | √ | |
| nd | 40.60 | 37.70 | 17 Jul 1997 | | √ |
| 30.80 | nd | nd | 24 Jul 1997 | √ | |
| 31.50 | nd | nd | 24 Jul 1997 | | √ |
| 32.50 | 39.20 | | 31 Jul 1997 | √ | |
| 33.70 | 39.50 | 32.00 | 31 Jul 1997 | | √ |
| 28.60 | nd | nd | 12 Aug 1997 | √ | |
| 32.60 | nd | nd | 12 Aug 1997 | | √ |
| nd | 39.50 | nd | 13 Aug 1997 | √ | |
| nd | 45.60 | 33.30 | 13 Aug 1997 | | √ |
| 29.60 | nd | nd | 25 Aug 1997 | √ | |
| 33.00 | nd | nd | 25 Aug 1997 | | √ |
| nd | 39.80 | nd | 26 Aug 1997 | √ | |
| nd | 41.20 | 36.90 | 26 Aug 1997 | | √ |
| 31.50 | nd | nd | 19 Sep 1997 | √ | |
| 33.10 | nd | nd | 19 Sep 1997 | | √ |
| nd | 41.70 | nd | 23 Sep 1997 | √ | |
| nd | 40.70 | 35.50 | 23 Sep 1997 | | √ |
| 31.90 | nd | nd | 06 Oct 1997 | √ | |
| 32.30 | nd | nd | 06 Oct 1997 | | √ |

| *STEAM MEAT YIELD (%) GASCOIGNE COVE* | *STEAM MEAT YIELD (%) OATES POINT* | *STEAM MEAT YIELD (%) BARSWAY* | *DATE* | *SHELL LENGTH (20 – 50 mm)* | *SHELL LENGTH (50 + mm)* |
|---|---|---|---|---|---|
| nd | 43.40 | nd | 09 Oct 1997 | √ | |
| nd | 42.00 | 34.90 | 09 Oct 1997 | | √ |
| 31.40 | nd | nd | 20 Oct 1997 | √ | |
| 36.40 | nd | nd | 20 Oct 1997 | | √ |
| nd | 39.90 | nd | 28 Oct 1997 | √ | |
| nd | 39.50 | 33.70 | 28 Oct 1997 | | √ |
| 33.60 | nd | nd | 07 Nov 1997 | √ | |
| 36.50 | nd | nd | 07 Nov 1997 | | √ |

nd = not done

**Appendix D.** Results of conditional indices testing at Barsway in 1998.

| *STEAM MEAT YIELD (%)* | *SHELL CONDITION INDEX* | *GRAVIMETRIC CONDITION INDEX* | *DATE* | *SHELL LENGTH (35 – 50 mm)* | *SHELL LENGTH (50 + mm)* |
|---|---|---|---|---|---|
| 40.62 | 171.66 | 78.60 | 23 Apr 1998 | | √ |
| 41.39 | 165.72 | 71.24 | 05 May 1998 | | √ |
| 48.74 | 189.66 | 76.20 | 29 May 1998 | √ | |
| 43.56 | 195.99 | 88.65 | 29 May 1998 | | √ |
| 50.15 | 237.49 | 79.44 | 08 Jun 1998 | √ | |
| 45.26 | 204.12 | 84.37 | 08 Jun 1998 | | √ |
| 52.04 | 243.87 | 75.68 | 16 Jun 1998 | √ | |
| 49.88 | 205.77 | 71.30 | 16 Jun 1998 | | √ |
| 47.41 | 220.11 | 77.93 | 22 Jun 1998 | √ | |
| 39.89 | 138.86 | 63.64 | 22 Jun 1998 | | √ |
| 50.80 | 243.01 | 78.17 | 03 Jul 1998 | √ | |
| 53.42 | 235.77 | 76.97 | 08 Jul 1998 | √ | |
| 43.21 | 181.70 | 75.22 | 08 Jul 1998 | | √ |
| 47.37 | 215.48 | 70.25 | 17 Jul 1998 | √ | |
| 53.46 | 212.26 | 67.04 | 24 Jul 1998 | √ | |
| 43.58 | 153.80 | 64.33 | 24 Jul 1998 | | √ |
| 48.91 | 200.87 | 60.84 | 31 Jul 1998 | √ | |
| 46.20 | 181.63 | 64.15 | 31 Jul 1998 | | √ |
| 52.00 | 208.84 | 63.87 | 06 Aug 1998 | √ | |
| 44.31 | 196.48 | 72.74 | 06 Aug 1998 | | √ |
| 51.49 | 200.08 | 60.41 | 14 Aug 1998 | √ | |
| 48.07 | 195.91 | 63.24 | 20 Aug 1998 | √ | |
| 40.43 | 163.97 | 61.34 | 20 Aug 1998 | | √ |
| 46.21 | 190.53 | 64.75 | 25 Aug 1998 | √ | |
| 39.13 | 151.93 | 64.55 | 25 Aug 1998 | | √ |
| 46.86 | 210.71 | 67.71 | 01 Sep 1998 | √ | |
| 36.45 | 141.66 | 58.13 | 01 Sep 1998 | | √ |
| 44.27 | 190.90 | 62.28 | 09 Sep 1998 | √ | |
| 35.83 | 155.68 | 61.13 | 09 Sep 1998 | | √ |

**Appendix E.** Results of conditional indices testing at Oates Point in 1998.

| *STEAM MEAT YIELD (%)* | *SHELL CONDITION INDEX* | *GRAVIMETRIC CONDITION INDEX* | *DATE* | *SHELL LENGTH (35 – 50 mm)* | *SHELL LENGTH (50 + mm)* |
|---|---|---|---|---|---|
| 40.38 | 146.18 | 66.60 | 23 Apr 1998 | √ | |
| 35.83 | 125.85 | 63.15 | 23 Apr 1998 | | √ |
| 43.22 | 171.83 | 72.98 | 05 May 1998 | √ | |
| 45.00 | 190.73 | 78.18 | 05 May 1998 | | √ |
| 43.39 | 185.05 | 77.81 | 29 May 1998 | √ | |
| 42.49 | 186.37 | 75.21 | 29 May 1998 | | √ |
| 41.40 | 169.81 | 72.05 | 08 Jun 1998 | √ | |
| 41.46 | 140.88 | 61.82 | 08 Jun 1998 | | √ |
| 39.17 | 170.03 | 70.41 | 16 Jun 1998 | √ | |
| 39.28 | 151.08 | 66.21 | 16 Jun 1998 | | √ |
| 43.94 | 205.07 | 77.69 | 22 Jun 1998 | √ | |
| 42.06 | 147.77 | 62.30 | 22 Jun 1998 | | √ |
| 43.97 | 199.79 | 73.26 | 02 Jul 1998 | √ | |
| 43.10 | 198.72 | 73.11 | 02 Jul 1998 | | √ |
| 43.74 | 180.49 | 70.04 | 08 Jul 1998 | √ | |
| 42.53 | 158.24 | 63.65 | 08 Jul 1998 | | √ |
| 40.98 | 166.15 | 68.64 | 13 Jul 1998 | √ | |
| 41.42 | 180.55 | 66.52 | 13 Jul 1998 | | √ |
| 41.28 | 185.58 | 69.28 | 21 Jul 1998 | √ | |
| 44.01 | 186.27 | 68.84 | 21 Jul 1998 | | √ |
| 43.63 | 176.23 | 68.74 | 27 Jul 1998 | √ | |
| 43.30 | 195.56 | 78.14 | 27 Jul 1998 | | √ |
| 39.66 | 148.23 | 60.02 | 03 Aug 1998 | √ | |
| 40.76 | 141.82 | 57.77 | 03 Aug 1998 | | √ |
| 39.59 | 161.19 | 61.89 | 14 Aug 1998 | √ | |
| 38.89 | 146.29 | 58.27 | 14 Aug 1998 | | √ |
| 39.30 | 146.17 | 58.76 | 21 Aug 1998 | √ | |
| 33.15 | 122.28 | 50.32 | 21 Aug 1998 | | √ |
| 38.76 | 138.41 | 55.68 | 27 Aug 1998 | √ | |
| 40.10 | 148.74 | 58.06 | 27 Aug 1998 | | √ |
| 41.42 | 177.45 | 74.70 | 02 Sep 1998 | √ | |
| 44.12 | 207.52 | 79.95 | 02 Sep 1998 | | √ |
| 38.52 | 155.78 | 60.41 | 10 Sep 1998 | √ | |
| 41.61 | 165.82 | 64.48 | 10 Sep 1998 | | √ |

**Appendix F.** Results of conditional indices testing at Gascoigne Cove in 1998.

| *STEAM MEAT YIELD (%)* | *SHELL CONDITION INDEX* | *GRAVIMETRIC CONDITION INDEX* | *DATE* | *SHELL LENGTH (35 – 50 mm)* | *SHELL LENGTH (50 + mm)* |
|---|---|---|---|---|---|
| 40.45 | 134.13 | 62.83 | 21 Apr 1998 | √ | |
| 39.10 | 147.77 | 71.04 | 21 Apr 1998 | | √ |
| 43.18 | 161.61 | 73.75 | 07 May 1998 | √ | |
| 39.67 | 147.50 | 72.82 | 07 May 1998 | | √ |
| 45.86 | 168.77 | 75.18 | 17 May 1998 | √ | |
| 45.81 | 169.39 | 65.81 | 05 Jun 1998 | √ | |
| 41.31 | 126.52 | 53.60 | 05 Jun 1998 | | √ |
| 39.00 | 154.78 | 72.20 | 09 Jun 1998 | √ | |
| 42.96 | 158.08 | 72.59 | 09 Jun 1998 | | √ |
| 37.07 | 140.29 | 63.93 | 18 Jun 1998 | √ | |
| 36.35 | 138.67 | 65.72 | 18 Jun 1998 | | √ |
| 39.57 | 157.71 | 66.02 | 26 Jun 1998 | √ | |
| 39.58 | 164.61 | 69.74 | 26 Jun 1998 | | √ |
| 37.35 | 162.66 | 71.83 | 30 Jun 1998 | √ | |
| 40.02 | 150.10 | 67.06 | 30 Jun 1998 | | √ |
| 36.13 | 136.11 | 59.84 | 07 Jul 1998 | √ | |
| 37.16 | 138.49 | 64.51 | 07 Jul 1998 | | √ |
| 36.76 | 120.81 | 54.25 | 16 Jul 1998 | √ | |
| 35.24 | 137.21 | 63.97 | 16 Jul 1998 | | √ |
| 36.67 | 119.77 | 55.67 | 20 Jul 1998 | √ | |
| 34.10 | 121.06 | 58.86 | 20 Jul 1998 | | √ |
| 35.42 | 113.52 | 51.11 | 29 Jul 1998 | √ | |
| 35.58 | 106.31 | 51.28 | 29 Jul 1998 | | √ |
| 36.21 | 117.44 | 51.45 | 05 Aug 1998 | √ | |
| 34.12 | 107.21 | 48.87 | 05 Aug 1998 | | √ |
| 34.06 | 104.57 | 47.84 | 12 Aug 1998 | √ | |
| 34.38 | 110.59 | 50.21 | 12 Aug 1998 | | √ |
| 32.89 | 107.34 | 47.46 | 19 Aug 1998 | √ | |
| 37.33 | 112.21 | 53.82 | 19 Aug 1998 | | √ |
| 37.95 | 112.47 | 49.14 | 26 Aug 1998 | √ | |
| 33.49 | 115.96 | 50.83 | 26 Aug 1998 | | √ |
| 33.52 | 113.59 | 49.95 | 03 Sep 1998 | √ | |
| 33.45 | 114.25 | 43.83 | 03 Sep 1998 | | √ |
| 36.36 | 132.48 | 57.38 | 11 Sep 1998 | √ | |
| 34.41 | 137.00 | 59.03 | 11 Sep 1998 | | √ |
| 36.13 | 135.53 | 59.50 | 17 Sep 1998 | √ | |
| 35.19 | 130.51 | 54.12 | 17 Sep 1998 | | √ |
| 34.89 | 133.14 | 56.59 | 26 Sep 1998 | √ | |
| 32.85 | 122.96 | 53.35 | 26 Sep 1998 | | √ |
| 36.13 | 115.65 | 63.92 | 30 Sep 1998 | √ | |
| 35.19 | 138.78 | 54.01 | 30 Sep 1998 | | √ |

**Appendix G.** Sampling Protocol for seed collection trial, 1998. Standard deviation is in parentheses.

| Deployment Date | Seed Bag ID # | Total # Clam Seed | Mean Shell Length (mm) | Min. Shell Length (mm) | Max. Shell Length (mm) |
|---|---|---|---|---|---|
| July 17 | 38583 | 222 | 8.87 (2.20) | 2 | 16 |
| | 38584 | 217 | 9.57 (2.64) | 5 | 20 |
| | 38585 | 128 | 8.31 (3.28) | 2 | 17 |
| July 24 | 38588 | 28 | 5.04 (2.73) | 2 | 10 |
| | 38589 | 66 | 7.14 (2.48) | 2 | 14 |
| | 38590 | 49 | 7.47 (3.45) | 2 | 16 |
| August 3 | 38504 | 5 | 8.20 (4.87) | 3 | 16 |
| | 38505 | 3 | 7.33 (1.15) | 6 | 8 |
| | 38506 | 2 | 7.00 (7.07) | 2 | 12 |
| August 7 | 38509 | 3 | 5.00 (1.73) | 4 | 7 |
| | 38510 | 5 | 3.80 (1.30) | 2 | 5 |
| | 38511 | 6 | 4.83 (1.47) | 3 | 6 |
| August 11 | 38515 | 0 | N/A | N/A | N/A |
| | 38514 | 7 | 3.14 (2.12) | 1 | 7 |
| | 38516 | 7 | 4.57 (1.90) | 3 | 8 |
| August 21 | 38517 | 2 | 3.50 (2.12) | 2 | 5 |
| | 38519 | 0 | N/A | N/A | N/A |
| | 38512 | 3 | 2.67 (0.58) | 2 | 3 |
| August 27 | 38520 | 2 | 6.50 (4.95) | 3 | 10 |
| | 38521 | 2 | 2.50 (0.71) | 2 | 3 |
| | 38522 | 4 | 2.50 (0.58) | 2 | 3 |
| September 2 | 38580 | 2 | 5.50 (4.95) | 2 | 9 |
| | 38581 | 2 | 3.50 (0.71) | 3 | 4 |
| | 38582 | 3 | 2.67 (0.58) | 2 | 3 |

**Appendix H.** Sampling protocol for seed collection trials, 1999. Standard deviation is in parentheses.

| **Deployment** Date | Seed Bag ID # | Total # Clams | Mean Shell Length (mm) | Min. Shell Length (mm) | Max. Shell Length (mm) | Monofilament Weight (g) | Block Weight (g) |
|---|---|---|---|---|---|---|---|
| June 22 | 20501 | 345 | 12.32 (3.44) | 3.66 | 21.81 | 502.99 | 478.49 |
| | 20502 | 729 | 12.56 (3.48) | 3.67 | 26.59 | 479.21 | 475.22 |
| | 20503 | 753 | 12.16 (3.29) | 3.36 | 23.63 | 798.39 | 927.36 |
| | 20504 | 695 | 11.28 (3.54) | 2.94 | 22.27 | 765.92 | 567.37 |
| | 20505 | 731 | 12.82 (2.96) | 3.46 | 20.04 | 761.79 | 497.11 |
| | 20506 | 658 | 13.28 (3.52) | 3.79 | 22.62 | 499.20 | 416.36 |
| | 20507 | 630 | 12.97 (3.26) | 4.02 | 22.00 | 369.43 | 606.45 |
| | 20508 | 517 | 12.26 (3.44) | 4.18 | 23.85 | 531.60 | 988.37 |
| June 29 | 20509 | 103 | 10.95 (5.95) | 2.74 | 22.65 | 262.49 | 397.32 |
| | 20510 | 59 | 10.89 (5.20) | 2.83 | 24.02 | 318.39 | 491.02 |
| | 20511 | 105 | 9.91 (5.98) | 1.88 | 23.23 | 284.73 | 1186.98 |
| | 20512 | 88 | 9.16 (5.00) | 2.07 | 19.96 | 297.60 | 889.92 |
| July 6 | 20513 | 119 | 5.35 (3.08) | 1.90 | 17.69 | 306.40 | 841.57 |
| | 20514 | 93 | 6.62 (3.18) | 2.45 | 15.00 | 275.33 | 389.27 |
| | 20515 | 147 | 5.56 (2.70) | 2.37 | 19.11 | 329.55 | 463.35 |
| | 20516 | 59 | 6.15 (2.96) | 2.80 | 14.84 | 279.87 | 683.19 |
| July 13 | 20517 | 50 | 5.97 (2.60) | 2.98 | 13.31 | 385.71 | 497.40 |
| | 20518 | 72 | 9.32 (4.44) | 3.34 | 20.13 | 424.98 | 676.11 |
| | 20519 | 85 | 8.55 (4.52) | 2.48 | 20.68 | 530.56 | 512.56 |
| | 20520 | 85 | 8.52 (4.00) | 3.14 | 18.10 | 330.82 | 689.25 |
| July 20 | 20521 | 39 | 7.38 (3.62) | 3.16 | 17.13 | 423.90 | 379.95 |
| | 20522 | 80 | 7.20 (3.35) | 3.30 | 18.43 | 278.94 | 432.67 |
| | 20523 | 64 | 7.58 (3.61) | 2.52 | 16.61 | 389.82 | 274.21 |
| | 20524 | 118 | 5.89 (3.13) | 1.71 | 16.35 | 417.63 | 322.57 |

| Deployment Date | Seed Bag ID # | Total # Clams | Mean Shell Length (mm) | Min. Shell Length (mm) | Max. Shell Length (mm) | Monofilament Weight (g) | Block Weight (g) |
|---|---|---|---|---|---|---|---|
| July 27 | 20525 | 299 | 5.61 (2.00) | 1.93 | 18.60 | 496.36 | 415.92 |
| | 20526 | 259 | 5.55 (2.42) | 2.23 | 18.86 | 374.46 | 370.02 |
| | 20527 | 203 | 5.97 (2.50) | 2.57 | 19.99 | 346.59 | 417.60 |
| | 20528 | 368 | 5.97 (2.00) | 2.20 | 16.51 | 344.16 | 479.35 |
| August 3 | 20529 | 305 | 6.59 (2.19) | 2.09 | 13.44 | 486.08 | 476.81 |
| | 20530 | 481 | 5.95 (2.09) | 1.88 | 13.80 | 301.96 | 402.89 |
| | 20531 | 287 | 6.87 (2.40) | 2.14 | 14.77 | 383.63 | 201.06 |
| | 20532 | 99 | 5.93 (1.71) | 2.64 | 10.18 | 430.00 | 0.00 |
| August 10 | 20533 | 587 | 5.87 (1.75) | 2.04 | 11.83 | 225.16 | 361.30 |
| | 20534 | 263 | 5.16 (1.78) | 1.42 | 11.00 | 243.58 | 337.72 |
| | 20535 | 129 | 6.08 (1.62) | 3.08 | 10.08 | 400.26 | 359.03 |
| | 20536 | 1565 | 5.62 (1.70) | 1.57 | 12.00 | 461.47 | 424.40 |
| August 17 | 20537 | 295 | 5.62 (1.25) | 2.69 | 11.09 | 192.16 | 340.22 |
| | 20538 | 73 | 5.34 (1.38) | 2.03 | 8.06 | 170.30 | 360.47 |
| | 20539 | 937 | 5.56 (1.37) | 1.97 | 9.60 | 267.36 | 335.26 |
| | 20540 | 1227 | 6.16 (1.36) | 1.61 | 12.97 | 257.14 | 407.58 |
| August 24 | 20541 | 163 | 5.10 (1.37) | 1.54 | 8.00 | 169.15 | 329.43 |
| | 20542 | 7 | 6.49 (1.14) | 5.33 | 8.23 | 191.83 | 330.81 |
| | 20543 | 114 | 4.99 (1.75) | 1.57 | 9.05 | 219.36 | 625.91 |
| | 20544 | 134 | 5.91 (1.28) | 2.48 | 8.92 | 221.23 | 564.02 |
| August 31 | 20545 | 36 | 3.17 (1.32) | 1.49 | 7.03 | 437.56 | 375.98 |
| | 20546 | 17 | 5.15 (1.17) | 3.61 | 7.21 | 386.59 | 349.41 |
| | 20547 | 24 | 4.29 (1.57) | 1.63 | 7.39 | 466.97 | 238.51 |
| | 20548 | 10 | 5.86 (1.32) | 4.62 | 7.99 | 239.90 | 278.67 |