Journal of Shellfish Research, Vol. 22, No. 3, 621–631, 2003.

STRATEGIES TO MITIGATE THE IMPACT OF *CIONA INTESTINALIS* (L.) BIOFOULING ON SHELLFISH PRODUCTION

C. E. CARVER, A. CHISHOLM, AND A. L. MALLET*
Mallet Research Services Ltd., 4 Columbo Drive, Dartmouth, Nova Scotia, Canada B2X 3H3

ABSTRACT A sudden increase in the population of the solitary ascidian *Ciona intestinalis* (L.) is causing serious biofouling problems for shellfish growers on the Atlantic coast of Nova Scotia, Canada. The objective of the present study was to document the growth, spawning, and recruitment patterns of this species, and to develop strategies to minimize its impact on the culture of European oysters at two locations in Lunenburg Bay, Nova Scotia. Profiles of condition index, which may be indicative of spawning activity, suggested that the *C. intestinalis* population at the Bayport site spawned from mid-May through June, whereas the population at Mason's Beach spawned from mid-July to mid-August. Histological assessment of reproductive status indicated a period of gametogenesis in March-April (>3°C) followed by spawning from mid-May to mid-August (>8°C). Although mature eggs were observed in the ovary in July-August, spawning trials suggested a decline in the fecundity of the Bayport population during this period. Two main recruitment events were observed at Mason's Beach (June and August), but only one at Bayport (June). From the data on fecundity and settlement rates, it was estimated that a 100-mm long *C. intestinalis* (0.6 g dry weight) may produce 12,000 eggs in a season and that recruitment intensity may reach 3,000 individuals m^{-2}. Laboratory predation trials indicated that rock crabs (*Cancer irroratus*) consumed significantly more *C. intestinalis* than did green crabs (*Carcinus maenas*). A maximum predation rate of 11 individuals per day per rock crab (80 mm carapace width) was recorded at peak water temperatures of 18°C. In a series of chemical width eradication trials, exposure to 5% acetic acid was found to be a more effective strategy for eliminating *C. intestinalis* than hydrated lime, saturated brine, or hypochlorite solution. Total mortality was observed following exposure to 5% acetic acid for 15 to 30 s, with no corresponding mortality in the control mussels or oysters. Initial field trials indicated that spraying with acetic acid might prove to be an effective means of eliminating *C. intestinalis* under commercial conditions.

KEY WORDS: *Ciona intestinalis,* tunicates, biofouling, shellfish production, predation

INTRODUCTION

Ciona intestinalis is a solitary phleobranchiate ascidian, or tunicate, which occurs on natural substrates such as rocky bottoms and eelgrass beds, or on artificial structures such as aquaculture gear, marker buoys, dock pilings, and boat hulls (Petersen & Riisgard 1992, Connell 2000, Mazouni et al. 2001). Although native to the northern Atlantic Ocean (Van Name 1945, Plough 1978), this species is now distributed worldwide, most likely as a result of dispersion by shipping activities (Monniot & Monniot 1994, Lambert & Lambert 1998). Published accounts indicate that *C. intestinalis* has recently become a serious biofouling problem for many shellfish culture operations including those in Scotland (Karayucel 1997), South Africa (Hecht & Heasman 1999), and Chili (Uribe & Etchepare 2002). In eastern Canada, the severe impact of *C. intestinalis* biofouling was first documented in 1997 at a mussel farm in Lunenburg Bay, Nova Scotia (Cayer et al. 1999). In an unprecedented recruitment event, this tunicate species heavily colonized the mussel sleeves, causing a substantial reduction in growth and the eventual loss of the crop. Subsequent reports of significant *C. intestinalis* recruitment at several other shellfish growing sites in Nova Scotia suggest that this species has become a widespread biofouling problem. In a similar scenario, the nonindigenous club tunicate *Styela clava* has recently infested several mussel farms on the eastern coast of Prince Edward Island and is now recognized as a serious threat to the viability of the mussel industry (Boothroyd et al. 2002).

Information on the basic life-history traits of *C. intestinalis* originates primarily from natural populations in northern European waters (Gulliksen 1972, Svane 1983, Petersen et al. 1995, Petersen et al. 1997). Under these conditions, the life cycle of *C. intestinalis* is reportedly 12 to 18 mo, with growth and longevity varying in response to temperature and food levels (Millar 1952, Petersen et al. 1995). Growth rates in terms of length are estimated at 1 to 3% day^{-1} or 10 to 20 mm mo^{-1} (Dybern 1965, Petersen et al. 1995). In contrast, reports from Japan indicate that *C. intestinalis* has a life span of 3 mo in the summer at temperatures of 20 to 26°C, and 6 mo in the winter at 14°C (Yamaguchi 1975). The timing of reproductive activity also varies depending on temperature. In more northerly regions, such as in Sweden, reproductive activity peaks in May and June, whereas in warmer zones, such as Britain, gamete release may occur throughout the year (Dybern 1965, Gulliksen 1972). Given the various life-history strategies of this species, it is important to document this basic information for *C. intestinalis* populations in Atlantic Canada.

The primary objective of this study was to develop a strategy to mitigate the impact of *C. intestinalis* on an oyster culture operation in Lunenburg, Nova Scotia. In contrast to mussel culture, oysters are contained in a cage from which the tunicates can be removed without losing the inventory. Heavy infestations, however, have the potential to depress shellfish growth, and to increase mortality due to competition for food (Lesser et al. 1992) and obstruction of water flow (Uribe & Etchepare 2002). The removal of these tunicates from the grow-out structures and oyster inventory is labor intensive, and, in some cases, disposal of the waste biomass can be costly. A series of field and laboratory experimental trials were undertaken from November 1999 to November 2000 for the following purposes: (1) to document the local distribution of *C. intestinalis*; (2) to investigate the growth, spawning, and recruitment patterns of this species; and (3) to evaluate possible biological and chemical strategies for eliminating this species from the culture equipment and the oyster inventory.

*Corresponding author. E-mail: amallet@ns.sympatico.ca

MATERIALS AND METHODS

Field Ecology

Distribution, Growth, and Condition

Several exploratory dives aimed at documenting the local distribution of *C. intestinalis* were carried out at the two field sites in Lunenburg Bay, Bayport, and Mason's Beach, in the fall of 1999 and the fall of 2000 (Fig. 1).

Two experimental oyster tables with oyster bags containing adult *C. intestinalis* (year 1999 class) were set up at each of the two grow-out sites (i.e., Mason's Beach and Bayport) on October 30, 1999 (Fig. 2). Temperature recorders were attached to the tables at each site. The two experimental groups were sampled monthly from November 1999 to May 2000 and then every 3 wk until September 2000. On each occasion, a random sample of 10 individuals was collected from each site to evaluate their condition index. Each individual was measured and dissected to obtain estimates of wet tunic and wet body weight, and then they were dried overnight at 60°C for 24 h and reweighed. The condition index was calculated as dry body weight divided by total dry weight.

In early June 2000, oyster bags with recently recruited individuals were transferred to the experimental tables. Growth in terms of length, whole wet weight, and whole dry weight were estimated for the newly settled year 2000 cohort. Ten individuals from each site were measured, weighed, and then dried. Due to the difficulty in obtaining measurements from individuals in a fully extended position, a relationship was derived between body diameter when contracted and body length when alive and fully extended. This was used to estimate the mean body length of the cohort over time. A final sample was collected in November 2000 to document the development of the year 2000 class.

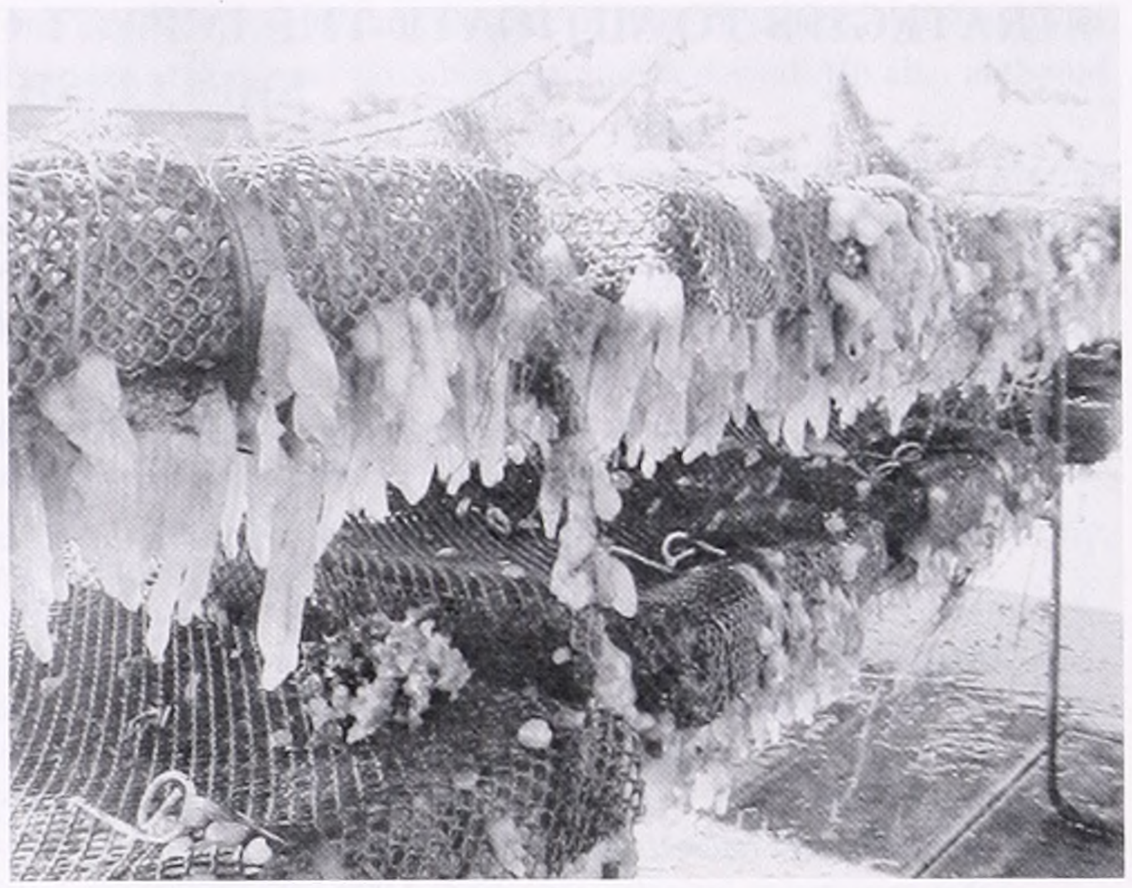

Figure 2. Photograph of an oyster table with oyster bags. Tunicates are apparent on the lower side of the oyster bags.

Reproductive Status

Five individuals from each year class at each site were dissected and weighed, and the body was fixed in 1% glutaraldehyde and 4% formaldehyde. The samples were then sent to the Diagnostics Laboratory at the Atlantic Veterinary School (Prince Edward Island) for histological processing. The tissues were embed-

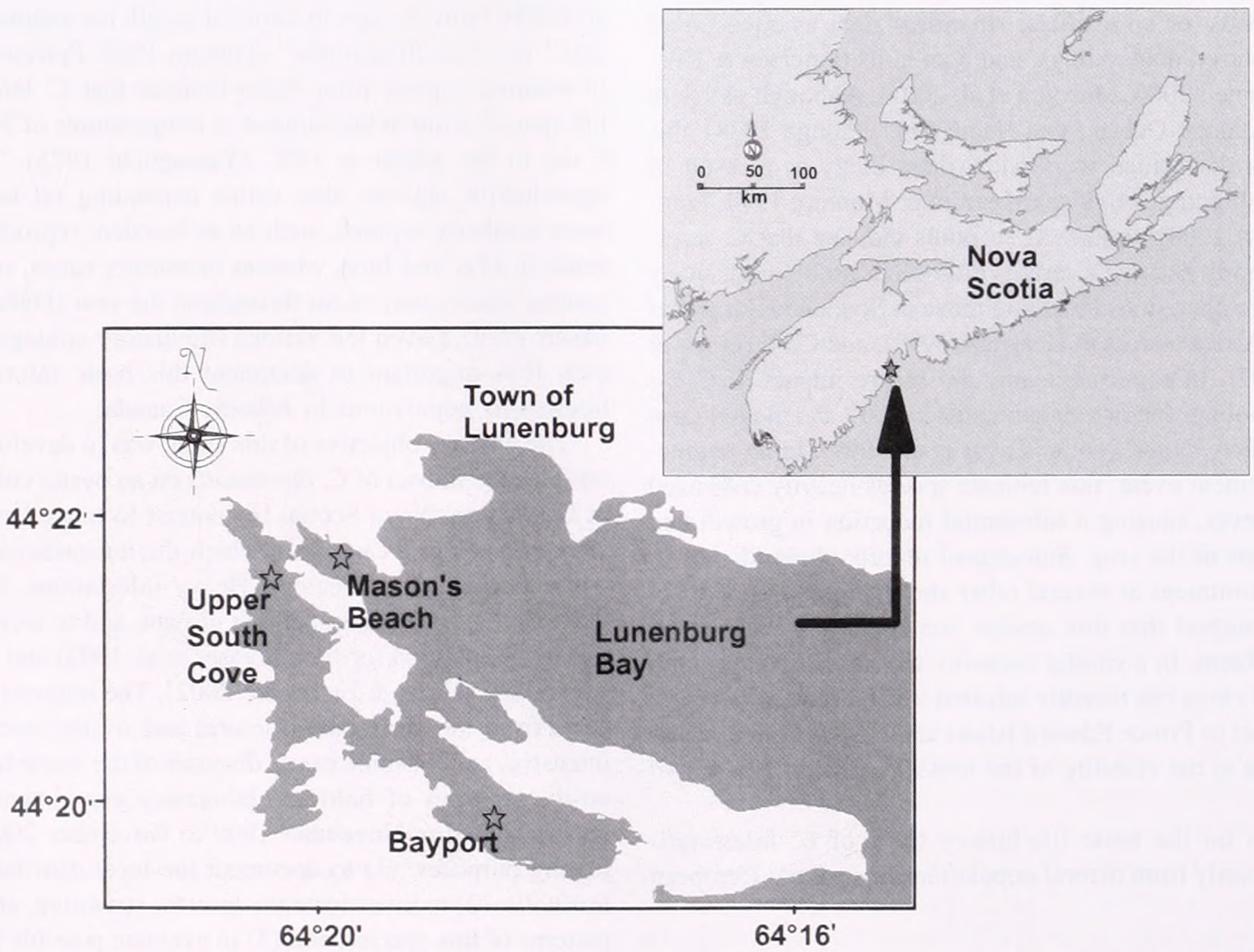

Figure 1. Map of Lunenburg Bay showing the location of the mussel farm in Upper South Cove, the site of the initial *C. intestinalis* infestation, and the two experimental sites, Bayport and Mason's Beach.

ded in paraffin and sectioned (6-μm thick), and the sections were stained with hematoxylin and eosin. The histology sections were assessed for reproductive status using a Weibel graticule (two fields per slide). The contents of each field were assigned to five categories: empty of follicle tissue; eggs in early development stage; eggs in late development stage; mature eggs; and regressing eggs. Mature eggs are surrounded by a thick layer called the vitelline coat, which clearly distinguishes them from immature or developing eggs. These data were used to estimate the proportion of the ovary that contained follicle tissue and the proportion of that area occupied by eggs in various stages of development. The cross-sectional area of the ovary was also measured using an image analyzer system.

Recruitment

Four recruitment plates (~200 cm^2) cut from clean but used oyster bags (4-mm mesh) were attached to the lower side of each oyster table on each sampling occasion. Plates deployed on the previous sampling trip were retrieved and were placed in separate plastic containers filled with seawater for transfer to the laboratory. The plates were examined with a stereomicroscope to detect the presence of newly recruited juvenile *C. intestinalis*. The plates were then placed in flowing filtered seawater (50 μm) for 2 to 3 wk to allow for the development of very small individuals that may not have been counted initially. The plates were then reassessed, and the maximum of the two counts was retained. The final counts for both sides of each plate were tallied and divided by the available solid area to estimate the intensity of settlement over the previous sampling period. The data were plotted such that any settlement that was observed at the end of a particular interval was assigned to the midpoint of that interval.

Laboratory Trials

Larval Development

The objective of the first series of trials (January–May 2000) was to induce natural spawning in the laboratory, to document the various phases of larval development, and to devise a protocol for rearing juveniles. Adult *C. intestinalis* from the 1999 cohort were collected from the field populations at each sampling event and were transferred to a flow-through system running at ambient temperature with unfiltered water. Spawning trials were undertaken on January 19, February 16, February 28, March 13, March 30, April 18, and May 3. To determine whether the adults possessed competent gametes, attempts were made to trigger spontaneous spawning by exposing individuals to a natural-light regimen for 24 h. When this proved unsuccessful, adults were strip-spawned and cross-fertilized to determine whether the eggs were competent. Fertilization trials were conducted at ambient water temperatures (0–6°C).

Fecundity

A series of five spawning trials were conducted in the quarantine unit at the Bedford Institute of Oceanography from May 15 to August 25, 2000, to estimate the fecundity of individuals obtained from the 1999 *C. intestinalis* cohort at both sites. Several individuals from the newly recruited 2000 cohort were included in July and August in an attempt to determine the minimum size at which spawning was initiated. The first four trials each lasted from 14 to18 d (May 15–June 2, June 8–26, July 4–21, and July 25–August 10), but the fifth trial (August 14–25) was discontinued after 10 days because of technical problems with the water supply system.

Five individuals of various sizes from each site were placed in separate 500-mL Mason jars in a tank of ambient flowing seawater. The water was prefiltered through a 40-μm mesh to remove any risk of contamination from eggs originating outside the system. The water level in the main reservoir was adjusted such that the flowing water just cleared the top of each jar; the objective was to allow sufficient flow for gas exchange and particle renewal but not enough to entrain the eggs. Control jars were placed downstream in the tank to estimate whether eggs were being lost. No eggs were retrieved from the control jars, and observations of fecal deposition suggested that negatively buoyant particles, including eggs, were retained inside their respective jars.

The experimental tank was set up approximately 3 m from an east-facing window such that the dawning light each morning would induce normal spawning behavior (Lambert & Brandt 1967). Every second or third day, the individual tunicates were transferred to new jars, and the contents of each old jar were screened through a 60-μm mesh to retain any eggs (150 μm size) produced over the previous 48 to 72 h. The jar and the screen were well rinsed with filtered seawater to remove any eggs stuck to the surface and were then flushed with hot freshwater to avoid contamination between samples. The eggs from each jar were collected in a petri dish and were counted using a stereomicroscope. Fecundity was estimated in terms of eggs produced per individual per day over the duration of the trial. At the end of each trial, the surviving individuals were dissected for assessment of dry body weight.

Methods of Control

Natural Predation

A series of predation experiments were set up in flowing seawater tanks in the quarantine unit at the Bedford Institute of Oceanography, Nova Scotia. Various sizes of *C. intestinalis* attached to weighted pieces of oyster bag were offered to a range of potential predators including starfish (*Asterias vulgaris*), green crabs (*Carcinus maenas*), rock crabs (*Cancer irroratus*), and hermit crabs (*Pagurus acadianus*). The first three trials were conducted in late January 2000 at water temperatures of 2 to 4°C. The second series of five trials focused on assessing the predation activity of rock crabs versus green crabs at a range of temperatures. Trials were undertaken on February 4 to 14 (2°C), April 13 to May 3 (5°C), July 27 to 31 (15°C), August 8 to 10 (18°C), and August 14 to 15 (18°C). The crabs ranged in carapace width (CW) from 40 to 100 mm, and the tunicate prey ranged in length from 15 to 125 mm. The duration of the experiments had to be reduced in the later trials to ensure that the supply of prey was not exhausted prior to the end of the trial. Predation rates were calculated in terms of individual tunicates consumed per crab per day.

Chemical Treatment

A series of physical/chemical eradication trials were undertaken in the laboratory from February to August 2000. The chemicals tested included sodium hypochlorite (10–60 parts per million), hydrated lime (1–4%), saturated brine, freshwater, and acetic acid (1–5%). The effectiveness of heated freshwater (40°C and 60°C) for eradicating *C. intestinalis* was also investigated. Various sizes of tunicates were used in each trial to determine whether younger

stages might be eliminated more easily than older stages. Mussels and oysters were also included in the trials to ascertain whether the treatment could potentially be used to remove tunicates from shell surfaces or from gear containing shellfish.

RESULTS

Local Distribution and Conditions

Diving surveys carried out at both sites in the fall of 1999 and the fall of 2000 did not identify any *C. intestinalis* attached to natural substrates, including rocks or eelgrass. None were observed on local wharf pilings at Bayport, but there was a substantial population attached to the bottom of a floating dock at Mason's Beach. Otherwise, *C. intestinalis* was only observed attached to oyster tables or suspended culture gear such as mussel sleeves and longlines. Both experimental sites typically have a lower incidence of *C. intestinalis* than the more sheltered Upper South Cove, the site of the original 1997 infestation, where the conditions tend to be warmer and more productive (Mallet & Carver 1993). Temperature profiles for the two experimental sites were virtually identical (Fig. 3).

C. intestinalis: *1999 Year Class*

Growth and Condition Index

Estimates of body length and total wet weight per individual for the 1999 year class showed low variation over time or location (mean values: November 1999 69 mm and 5.9 g, respectively; September 2000 76 mm and 6.7 g, respectively). The mean dry weight per individual remained at 0.3 to 0.4 g (range 0.1–0.9 g) for the duration of the study, and the overall relationship between whole dry weight (g) and body length (mm) was estimated as $y = 0.0000106x^{2.38}$ ($r^2 = 0.91$). Note that both primary tissues, the outer tunic and the body, are composed of approximately 95% water. Although the smaller individuals did grow from April to September 2000, the mortality of the larger individuals during the summer obscured any population growth trend.

During the colder months, the condition index (dry body weight/total dry weight) declined slightly from 44% in November 1999 (6°C) to 40% in late February 2000 (0°C) (Fig. 4). The condition index then increased sharply at both sites to a maximum of 60% at Bayport in late April, and 55% at Mason's Beach in

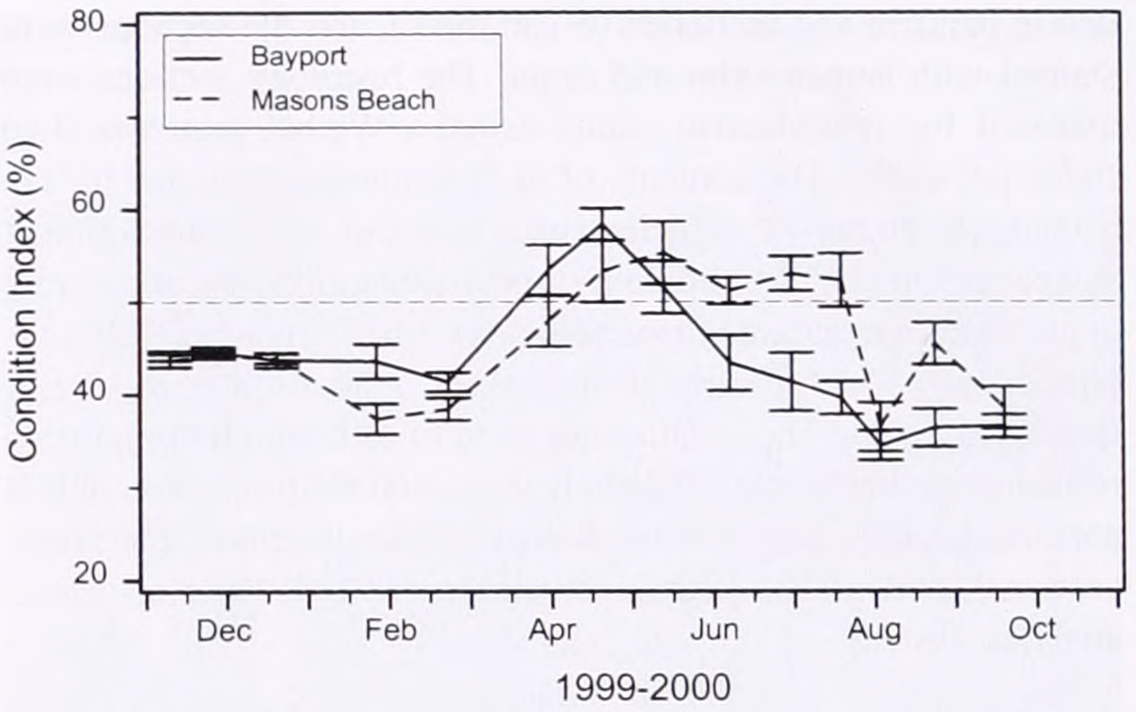

Figure 4. Condition index (dry body weight/total dry weight) for the year 1999 class of *C. intestinalis* at Bayport and Mason's Beach.

mid-May. At that time, the ambient water temperature at both sites was in the 6 to 9°C range (Fig. 3). The condition index of *C. intestinalis* at the Bayport site declined steadily from late April to early August, stabilizing at 35%. This profile would suggest that spawning started between April 20 and May 13, and continued through June and July. In contrast, the 1999 cohort at Mason's Beach exhibited a slight drop in condition in May but then maintained a condition index of >50% until mid-July, at which time values declined sharply. If the condition index is related to reproductive status, this profile suggests that the major spawning event at Mason's Beach occurred after mid-July or later than at Bayport.

Reproductive Status

Data on the reproductive status of *C. intestinalis* were pooled over the two sampling sites. The mean cross-sectional area of the adult ovary increased from 10 mm^2 in November 1999 to 25 mm^2 in late January 2000, declined slightly in February-March, and then rebounded in April-May to 24 mm^2. Between May 13 and June 7, the mean size of the ovary fell to approximately 10 mm^2, where it remained until September. Estimates of the proportion of the ovary occupied by follicle tissue ranged from 55 to 70% from November 1999 to March 2000, increased to 90% in April-May, and then declined to 70% in July (Fig. 5). The follicle area occu-

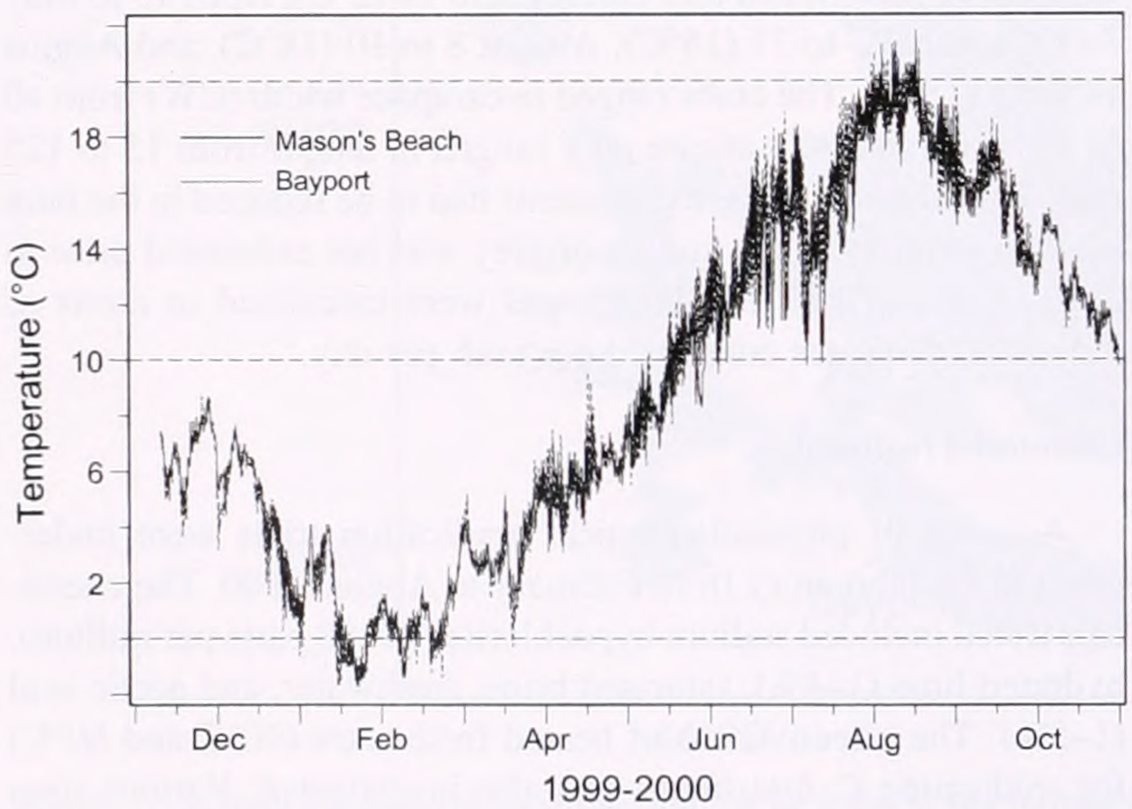

Figure 3. Temperature profiles for Bayport and Mason's Beach from November 1999 to November 2000.

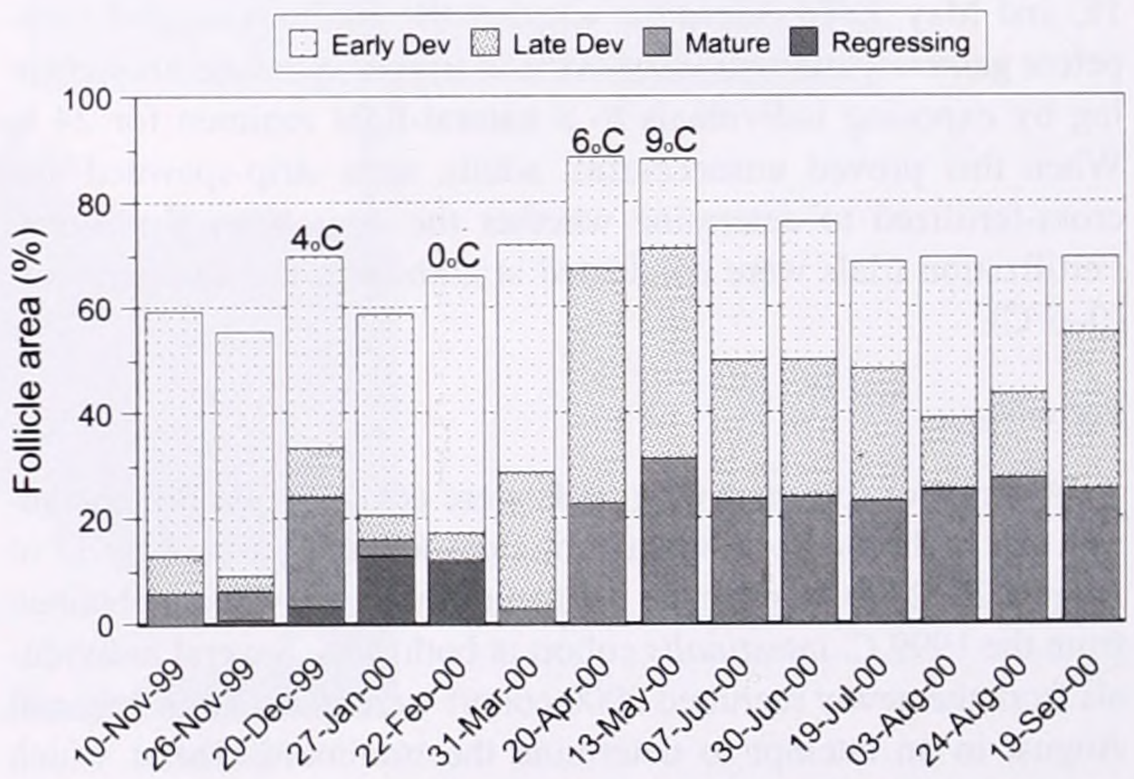

Figure 5. Reproductive status of the year 1999 class of *C. intestinalis*: proportion of the ovary that contained follicle tissue with early development, late development, mature, or regressing eggs.

pied by mature eggs increased from 10% in November to 35% in December 1999, but then declined from January to late March 2000. By mid-May, the incidence of mature eggs had returned to 30% and remained relatively stable until mid-September.

In summary, it would appear that egg development proceeded during the late fall 1999 when water temperatures exceeded 2°C but ceased when temperatures fell below 2°C in January-February 2000. During this latter period, there was an abundance of hemocytes in the ovary, which suggested that resorption or regression may have been occurring. By late March (4°C), the follicle area was starting to increase, as was the incidence of late development and mature egg stages. The major period of gametogenesis occurred in April through to mid-May (4–9°C), followed by the initiation of spawning in mid-to-late May. This event was coincident with a significant decline in the size of the ovary as well as the proportion of follicle tissue. From early June onward, the production of mature eggs continued, but the size of the ovary remained smaller than in April–May.

Fecundity

A total of five 2-wk spawning trials (May–July 2000) were carried out at ambient temperature in the laboratory using individuals collected from the two field sites. The number of eggs produced per individual varied widely from day to day, but there was no consistent decline in the rate of egg production over time within a trial. The maximum daily production estimated for a single individual was 1998 eggs day^{-1}, or a total of 5994 eggs over 3 days (May 19–22). (Table 1). The maximum fecundity for a single individual averaged over one trial period was 533 eggs d^{-1}.

Fecundity was positively correlated with whole dry weight (Fig. 6). The results indicated that individuals with dry weights as low as 0.1 g (40-mm long) could produce up to 200 eggs day^{-1}, whereas individuals with dry weights of 0.9 g (120-mm long) could produce as many as 500 eggs day^{-1} (averaged over 10–18 days). In general, fecundity was higher for the individuals from Mason's Beach than for those from Bayport. Estimates of mean fecundity for Bayport individuals (Table 2) showed a steady decline in egg production from May 15 onward. This was consistent with the profile of condition index (Fig. 4). The data for Mason's Beach suggest that the 1999 year class was producing >250 eggs ind^{-1} day^{-1} in May-June. However, unlike the Bayport population, the individuals at Mason's Beach continued to produce >100 eggs day^{-1} through July-August. This was consistent with the higher condition index for this population.

TABLE 2.

Egg production rates for year 1999 class *C. intestinalis* (eggs ind^{-1} day^{-1}) from the two experimental sites over time.

Trial Duration	Bayport (eggs ind^{-1} d^{-1})	Mason's Beach (eggs ind^{-1} d^{-1})
May 15–June 2	183 ± 80	221 ± 98
June 8–June 26	172 ± 71	257 ± 70
July 4–July 21	97 ± 34	160 ± 40
July 25–August 10	35 ± 15	150 ± 30

Values given as mean ± SE.

Larval/Juvenile Development

From January 19 to March 30 2000, eggs were obtained by dissection because of failures to trigger spontaneous spawnings. Very few mature eggs were obtained from January through March, and the sperm rapidly lost motility. In the few instances in which mature eggs were obtained, fertilization was generally poor (<10%), and development did not proceed to the larval stage. In the April 18 and May 3 trials, however, larvae were successfully produced both by spontaneous spawning and dissection. As in the earlier trials, the eggs were fertilized at ambient temperature (6–9°C in April-May) and then were allowed to gradually warm up to 15°C in the dark.

The development of *C. intestinalis* eggs at 15°C typically took 24 to 36 h, hatching and growth of the tadpole larvae lasted 24 h, followed by settlement and metamorphosis over another 12 h for an approximate total of 3 days to the juvenile stage (see also Berrill 1947). Larvae were successfully settled on plastic petri dishes, where they metamorphosed into juveniles. The dishes were submerged in a 10-L tank, and the water was changed every 2 to 3 days. The juveniles proved to be remarkably resilient and survived for weeks with minimal handling/feeding. A series of photos were taken to document the development of *C. intestinalis* from the egg to the juvenile phase (Fig. 7a, b, c, d, e, and f). It should be noted that the species identity of *C. intestinalis* was confirmed by the presence of single refringent bodies in the halo of follicle cells that surround the egg (Byrd & Lambert 2000).

TABLE 1.

Results of first spawning trial (May 15–June 2) indicating the individual variability in daily egg production rate over time. *C. intestinalis* individuals were brought in from the two field sites on May 13 and were held in flowing seawater until Jun 2.

Ind No.	May 15–17	May 17–19	May 19–22	May 22–24	May 24–26	May 26–29	May 29–31	May 31–Jun 2	Mean Eggs day^{-1}	Length (mm)	Whole Dry Weight (g)
M1	7	292	91	378	615	206	717	378	317	89	0.41
M2	2	1032	66	120	165	178	72	224	223	65	0.47
M3	0	0	0	0	0	0	54	0	6	56	0.27
M4	0	798	1998	363	0	398	0	44	533	89	0.91
M5	0	228	0	6	0	6	5	0	28	79	0.37
B1	0	0	0	105	74	126	60	102	59	65	0.16
B2	0	339	18	1158	453	416	140	1674	491	89	0.53
B3	117	344	45	135	281	164	71	135	155	74	0.36
B4	42	77	43	0	174	78	80	0	62	61	0.27
B5	225	180	111	0	392	0	371	0	148	70	0.29
Mean	44	329	237	226	215	157	157	256	202	74	0.40

Abbreviations: M = Mason's Beach; B = Bayport.

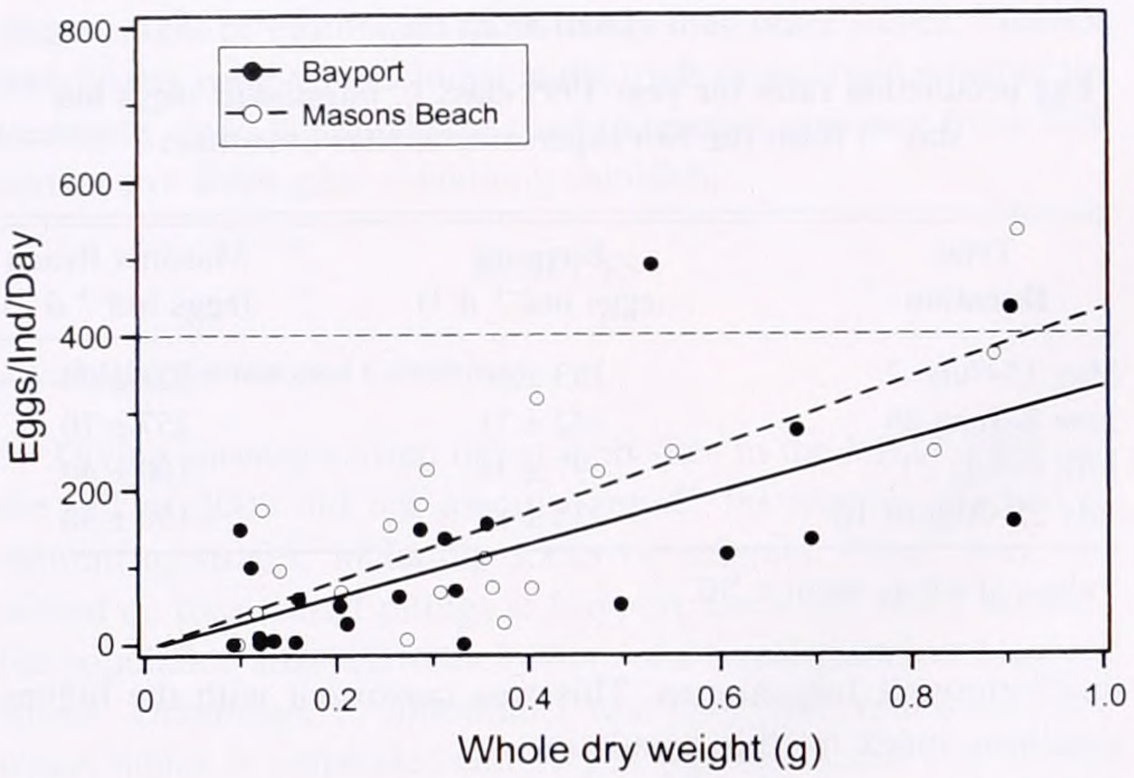

Figure 6. Fecundity (eggs ind^{-1} day^{-1}) versus whole dry weight of the 1999 *C. intestinalis* from Mason's Beach and Bayport from May through August 2000. Data from individuals that died during the trials were not included.

C. intestinalis: 2000 Year Class

Recruitment Patterns

The observed recruitment profiles suggest one settlement event at Bayport and two at Mason's Beach (Fig. 8). Estimates of settlement intensity ranged as high as 47 per 100 cm^2 of solid collector area, but levels often varied substantially among replicate plates. The timing of the settlement peak at Bayport (May 13–June 29) is consistent with the condition index/spawning profile for the year 1999 class (Fig. 4). In the case of Mason's Beach, the timing of the second recruitment peak (Aug 3–24) closely followed the decline in condition observed at that site (Fig. 4). The absence of a decline in condition in May-June may indicate that the first recruitment event at Mason's Beach was related to an influx of larvae from other areas such as Bayport or Upper South Cove. However, the fecundity trials confirmed that Mason's Beach adults were capable of producing eggs from mid-May onward. Recruitment plates deployed from August 24 to September 19 exhibited some new settlement at Mason's Beach, but no juveniles were observed on the plates deployed from September 19 to November 29, 2000.

Growth Rate

The growth rate of the first year 2000 cohort in terms of body length was relatively steady from mid-July through to mid-September and then decreased, possibly due to declining water temperature or the onset of maturity (Fig. 9). Continued growth in terms of whole dry weight through October-November was apparently related to an increase in body weight as opposed to length (Fig. 10). Whereas profiles of mean body length were similar for the two sites, estimates of whole dry weight were consistently higher at Mason's Beach than at Bayport. At Mason's Beach in November 2000, the mean body length was 96 mm, the whole wet weight was 11 g, and the whole dry weight was 0.7 g. These values were consistently higher than those for the year 1999 class the previous November. Individuals from the second year 2000 cohort at Mason's Beach had a body length of 36 mm, a mean wet weight of 0.5 g, and a mean dry weight of 0.05 g on November 29, 2000. The overall relationship between whole dry weight (g) and body length (mm) for the year 2000 class was estimated as $y = 0.00000801x^{2.4}$ ($r^2 = 0.93$).

Reproductive Status

Profiles of the percentage of follicle area as well as the proportion of the follicle area occupied by mature eggs increased rapidly between July 19 and August 3, 2000 (Fig. 11). At that time, the first year 2000 cohort had a mean length of 47 mm and a mean whole dry weight of approximately 0.1 g. Although dry weight continued to increase through the fall, gonad area increased only slightly to approximately 10 mm^2, and follicle area remained at 70%. Over the same period, the proportion of mature eggs declined from 40 to 20% in late November. Final values for all three reproductive indices were slightly higher than those for the 1999 year class recorded 1 y previously.

Fecundity

A few individuals from the year 2000 class did produce eggs in the July-August fecundity assessment trials. Estimates were typically <10 eggs day^{-1} in the July 27 trial but increased to as high as 460 eggs day^{-1} for the largest individual in the August 14 trial. The mean daily egg production was higher for the Mason's Beach recruits (245 eggs day^{-1}) than for the Bayport recruits (25 eggs day^{-1}), which was consistent with the greater dry weight of the former group (Fig. 10). The presence of mature eggs in the ovary from early August onward (Fig. 11) suggested that individuals from the first year 2000 cohort were likely spawning during this late summer period. However, given that there were 1999 individuals still spawning in August, the relative contribution of the first 2000 cohort to the second year 2000 recruitment peak cannot be ascertained.

Methods of Control

Natural Predators

A series of predation experiments were set up in flow-through seawater tanks at the Bedford Institute of Oceanography. Various sizes of *C. intestinalis* attached to weighted pieces of oyster bag were offered to a range of potential predators including starfish (*A. vulgaris*), green crabs (*C. maenas*),* rock crabs (*C. irroratus*), and hermit crabs (*P. acadianus*). In the first three trials, conducted in late January 2000 at water temperatures of 2 to 4°C, only the green crabs and, in particular, the rock crabs showed any feeding activity. The rock crabs were observed to use two different feeding strategies, depending on the size of the prey. Small *C. intestinalis* individuals (15–35 mm CW) were generally consumed whole, although after extracting the body tissues the tunic was rejected. Larger individuals (35–125 mm CW) were cut open with the

TABLE 3.

Effectiveness of various chemicals for the elimination of *C. intestinalis* (% mortality) under laboratory conditions.

Chemical Treatment	Duration (min)	Mortality (%)
Sodium hypochlorite 60 ppm	20	0
Salt brine (saturated)	8	25
Hydrated lime (4%)	6	50
Fresh water (15°C)	1	10
Fresh water (40°C)	1	66
Acetic acid (5%)	0.5	95

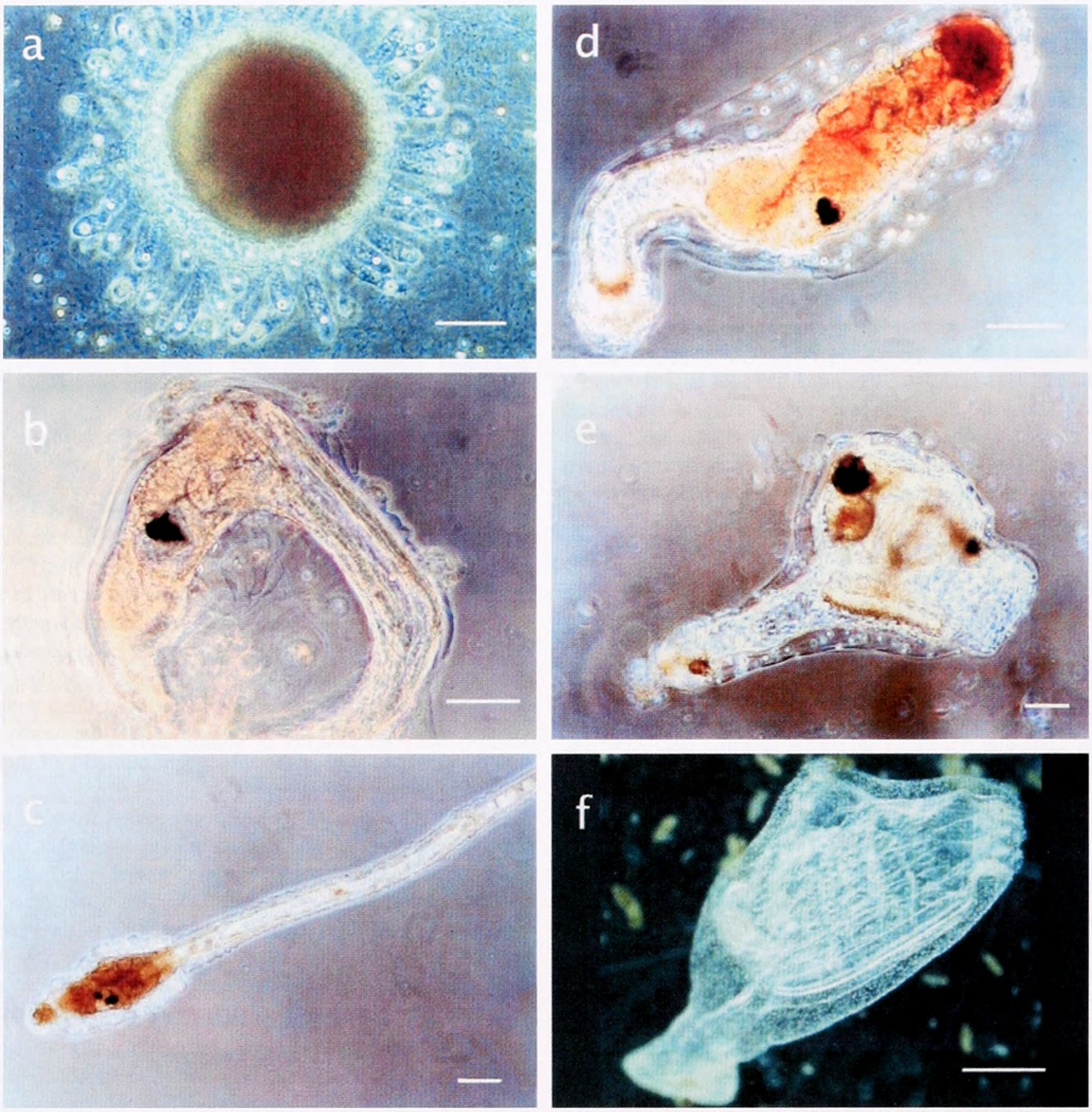

Figure 7. Photographs of the various stages of development of *C. intestinalis* reared under laboratory conditions: (a) egg (150 µm) with vitelline coat and follicle cells (protrusions) with distinctive refringent bodies (bright spots); (b) tadpole larvae with otolith (dark spot) and notochord hatching from egg (250 µm); (c) tadpole larvae with notochord and adhesive papillae on head (800 µm long); (d) metamorphosing larvae (360 × 120 µm) developing peduncle for attachment and resorbing tail; (e) juvenile (525 µm) with developing siphons; and (f) juvenile (1.3 mm) with stigmata or slits evident in the branchial chamber (photo (f) courtesy of Dr. Dan Jackson, (Department of Fisheries and Oceans). Scale bars for (a) to (e) are 50 µm; scale bar for (f) is 500 µm.

claws, and the body tissues were dragged out and consumed, leaving the empty tunic attached to the original substrate.

The predation trials undertaken at a range of temperatures indicated that rock crabs (50–90 mm CW) may consume as many as 11 *C. intestinalis* ind day^{-1} (35–80 mm long) at 18°C (Fig. 12). Predation rates were substantially lower at <6°C, but activity was steady. The trials also suggested that the small to medium rock crabs (<80 mm CW) tended to consume greater numbers of the <35-mm tunicates than did the larger crabs (Fig. 13). In general, the green crabs showed less interest than the rock crabs in preying on tunicates. There was also a tendency among the smaller green crabs (50 mm CW) to consume the <80-mm tunicates and ignore the larger individuals.

Physical/Chemical Eradication Trials

The results of the various eradication trials indicated that exposure to 5% acetic acid was by far the most effective strategy (Table 3). After the first trial, which indicated that a 1-min exposure to 5% acetic acid was sufficient to cause 100% mortality, further trials were carried out using shorter intervals. Exposure times of 5 to 10 sec were found to be insufficient, but 30 sec was generally 95% effective. The application of this chemical treatment by spraying or by immersing the tunicates proved equally useful. Rinsing post-treatment was included in the protocol to mimic conditions in the field where the acetic acid would be rapidly diluted by seawater. Oysters and mussels >20 mm in shell

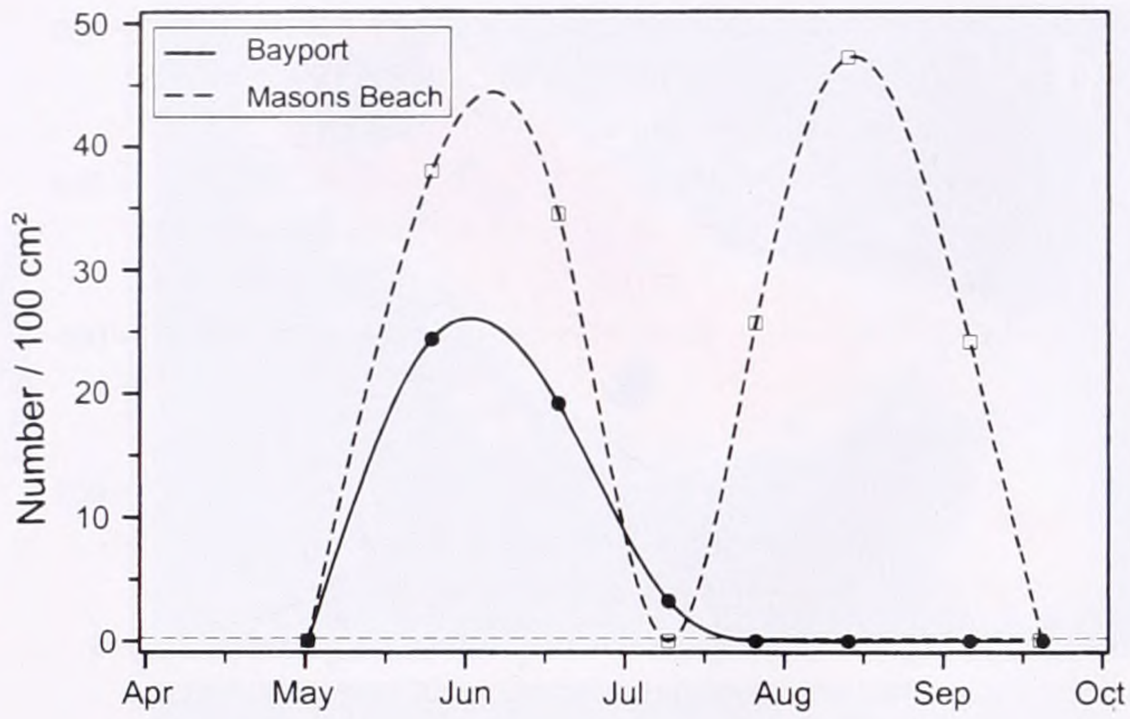

Figure 8. Projected recruitment profiles for the two year 2000 cohorts of *C. intestinalis* at Bayport and Mason's Beach.

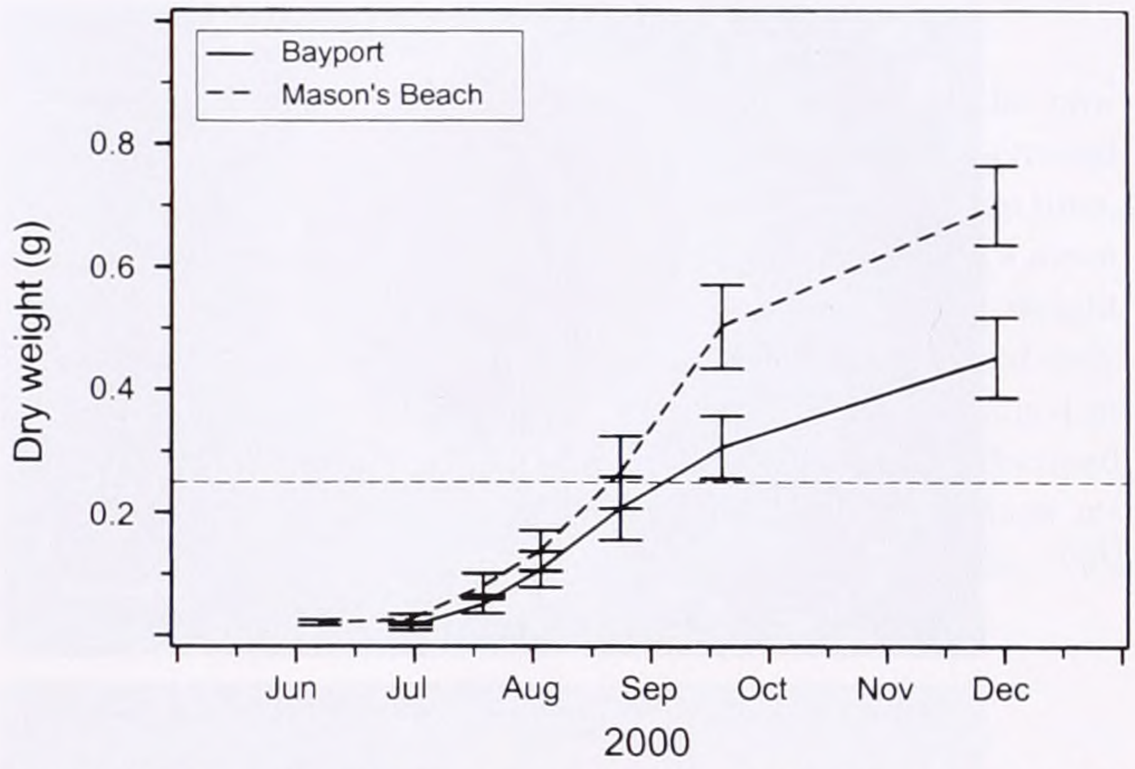

Figure 10. Increase in whole dry weight (g) over time for the year 2000 cohort of *C. intestinalis* at Bayport and Mason's Beach.

length (SL) were typically unaffected by the acetic acid spray/dip, but control mussels <10 mm SL died in one comparative trial.

Other chemical methods were consistently less effective at eradicating tunicates. Exposure to hydrated lime for 8 min was 70% effective, whereas saturated brine was only 20% effective over the same exposure time. Solutions of sodium hypochlorite at concentrations up to 60 parts per million for as long as 20 min had no impact on tunicate survival. Exposure to freshwater for 1 min resulted in only 10% mortality. Longer exposure times may be more effective, but under field conditions this may not be practical. A 1-min exposure to 40°C freshwater was 100% effective at eradicating *C. intestinalis*, but the European oyster (40 mm SL) and one of the two mussels (50 mm SL) also died.

The second phase of the eradication trials was to test the effectiveness of acetic acid treatment on *C. intestinalis* attached to oyster grow-out bags in the field. It should be noted that these trials were preliminary and were only assessed at a qualitative level. To administer the treatment, the acetic acid solution was placed in a garden-spraying unit. Goggles, gloves, and appropriate clothing were used, and care was taken to ensure that the bag being sprayed was located downwind. Although there was a slight smell, the fumes were rapidly dispersed in the open air. The treatment protocol followed was similar to that developed in the laboratory trials: 5% acetic acid spray for 30 s followed by air exposure for 30 s. Note that the *C. intestinalis* individuals had ample time to fully contract before being exposed to the treatment.

The preliminary trials were carried out on August 24 and September 19, 2000. The oyster bags were covered with a heavy settlement of 70 to 80-mm-long year 2000 individuals. In each case, individuals on one third of the bay were covered to act as controls. It should be noted that the health of these control individuals was apparently not affected by either the nearby application of the acetic acid or the subsequent mortality of their immediate neighbors.

The effectiveness of the treatment varied from 60 to 100%, depending largely on the density of the settlement (qualitative assessment). In the second field trial (September 19), 10 European oysters (40 mm SL) were placed in the bags while the acetic acid treatment was administered. On average, 80% of the oysters were alive when the bag was examined on November 29. Although these small-scale experiments were in no way conclusive, the results were sufficiently promising to warrant further trials.

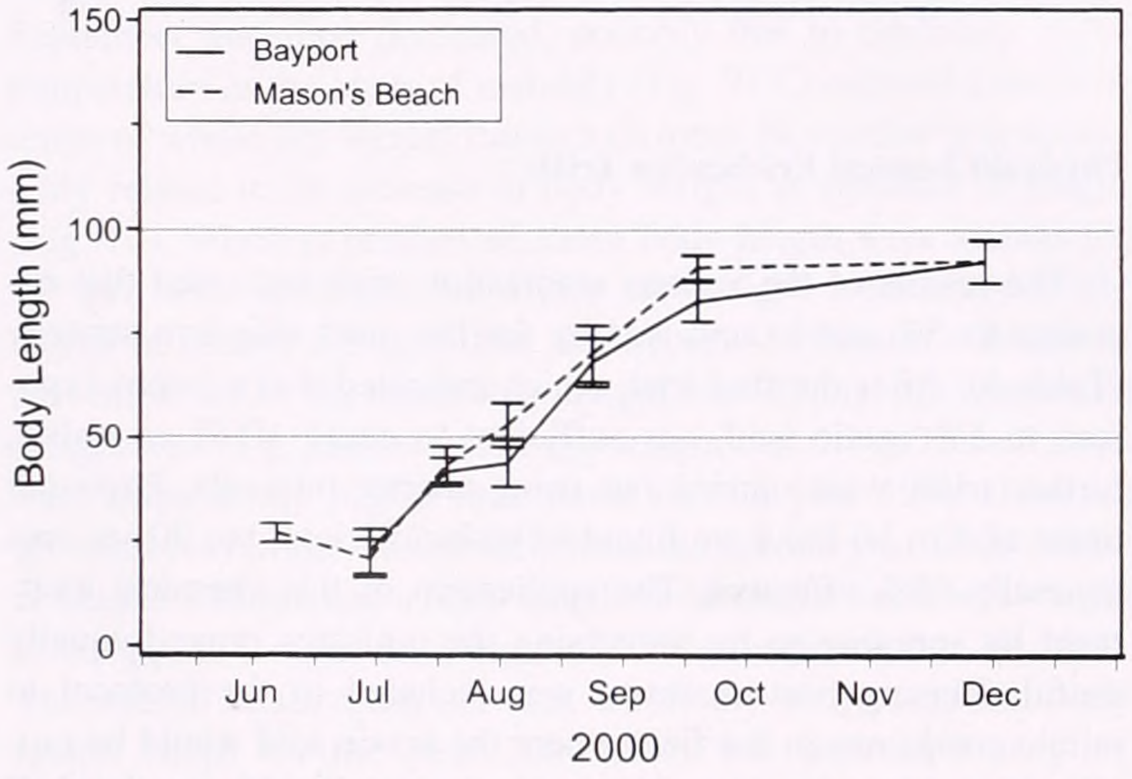

Figure 9. Increase in body length (mm) over time for the year 2000 cohort of *C. intestinalis* at Bayport and Mason's Beach.

DISCUSSION

Natural Distribution

In northern Europe, substantial natural populations of *C. intestinalis* are found in eelgrass beds or attached to rocky substrates (Dybern 1965, Gulliksen 1973, Petersen & Riisgard 1992). Diving surveys in 1999-2000 aimed at documenting the local distribution of *C. intestinalis* at the two study sites in Lunenburg found no individuals attached to natural substrates. Two independent surveys of the Bayport area carried out in August 2000 and August 2001 also failed to locate any *C. intestinalis* on eelgrass or rocky bottom areas (Barry MacDonald, Department of Fisheries and Oceans, pers. comm.). It would appear that the distribution of this species is generally restricted to man-made structures, such as floating docks and aquaculture gear.

The sudden proliferation of the *C. intestinalis* population in Lunenburg is a classic example of the potential impact of man-made structures on the settlement and survival of sessile invertebrate species (Connell & Glasby 1999). Various Australian studies comparing the species assemblages found on pier pilings and pontoons versus adjacent natural substrates have suggested that the introduction of artificial structures may effectively increase local species abundance and diversity (Butler & Connolly 1996, Glasby

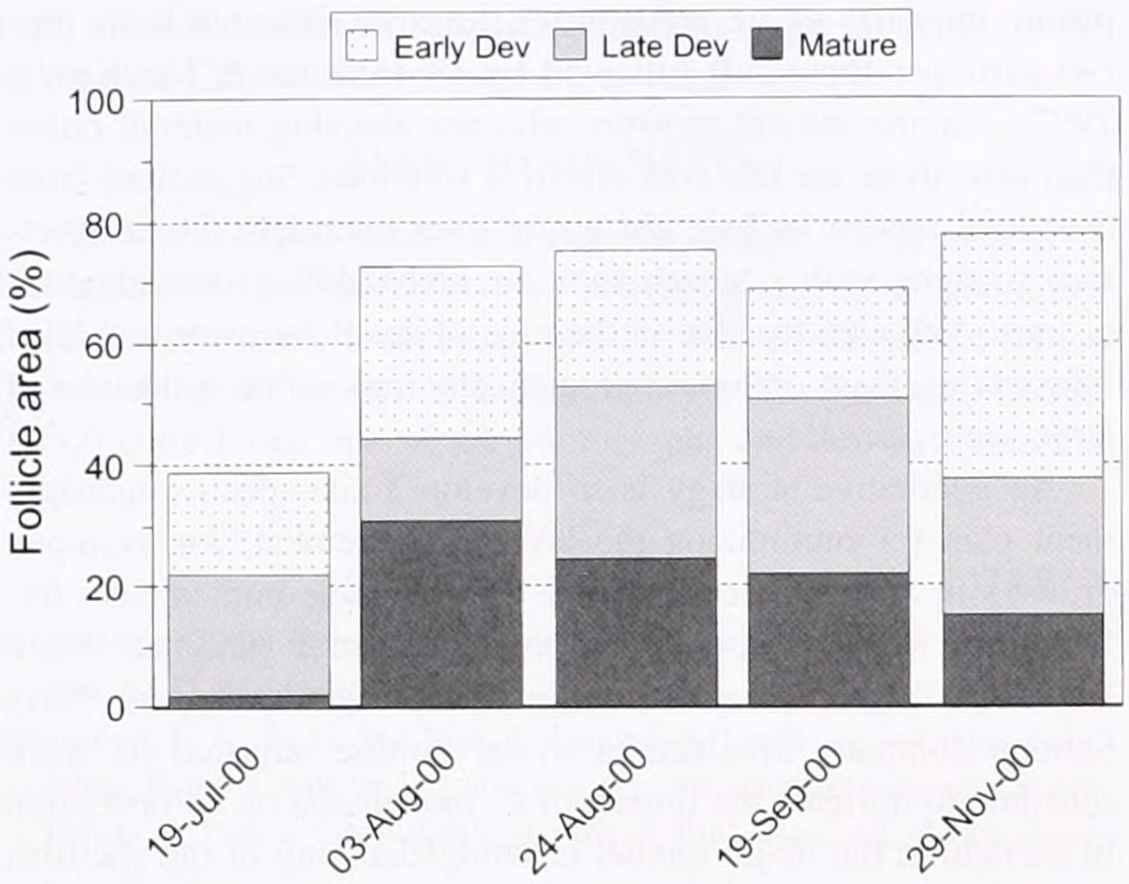

Figure 11. Reproductive status of the first year 2000 cohort of *C. intestinalis:* proportion of the ovary that contained follicle tissue with early development, late development, or mature eggs.

1999, Connell & Glasby 1999, Connell 2000). In particular, solitary ascidians such as *C. intestinalis* were typically more abundant at marinas than at reference locations (Glasby 1997). Ascidians in general have been recognized as the dominant biofouling organism on oysters grown in rope culture in L'Etang de Thau (France) (Mazouni et al. 2001).

The conspicuous absence of *C. intestinalis* from natural substrates suggests that manmade structures may function as a refuge from predation. Field studies in Denmark and Norway have reported that variations in spatial abundance of this species are linked to predation by sea stars (*Asterias rubens*), plaice (*Pleuronectes platessa*), and cod (*Gadus morhua)* (Gulliksen 1972, Gulliksen & Skjaeveland 1973). Natural predators include jellyfish, sea stars, rock crabs, hermit crabs, dog whelks, and surface-feeding fish (Gulliksen 1972, Yamaguchi 1975, Svane 1983, Olesen et al.1994). Recently settled juvenile stages may also be susceptible to dislodgment by surface grazers such as gastropods and sea urchins (Svane 1983). Predation trials conducted in the present study demonstrated that the rock crab, *C. irroratus*, can rapidly excise the body tissues of *C. intestinalis* from the heavy tunic and may consume as many as 11 ind day^{-1} during the summer months.

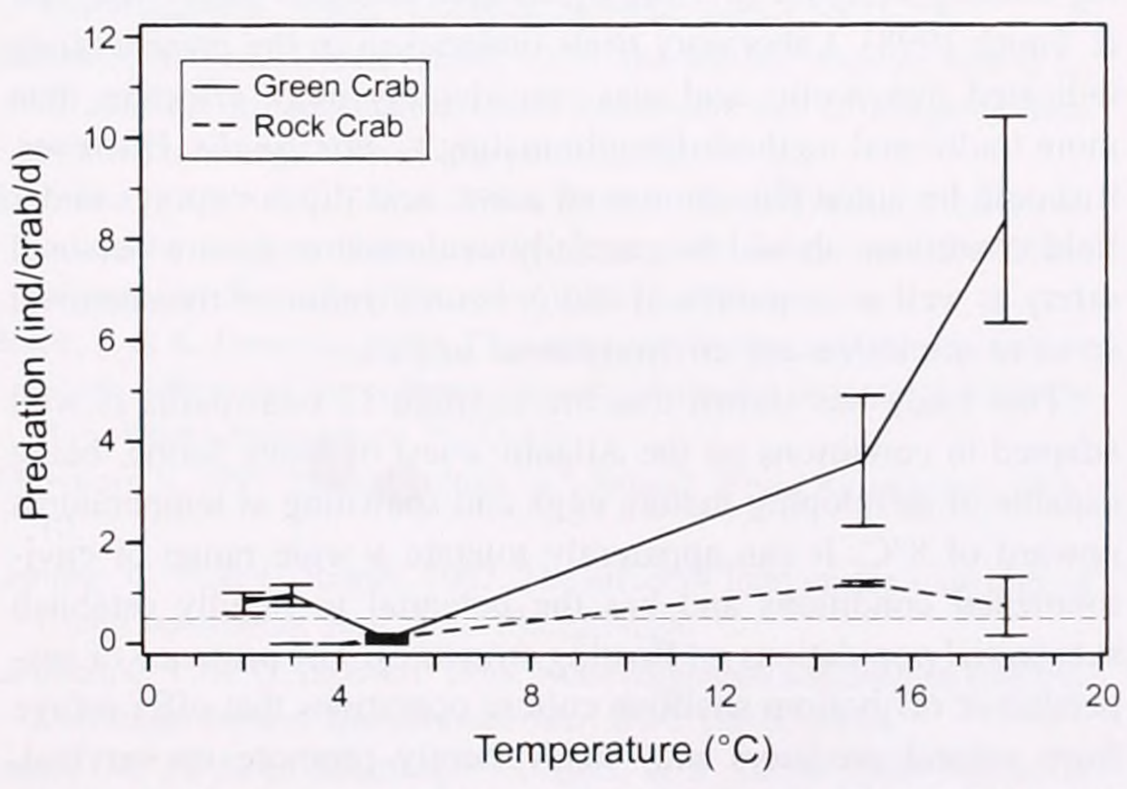

Figure 12. Predation rates of two crab species (ind crab^{-1} day^{-1}) on *C. intestinalis* (35–80 mm long) for a range of temperatures.

Field observations also suggested that predators, in particular crabs, were actively reducing the abundance of *C. intestinalis* on the upper surface of the oyster bags but were apparently unable to access individuals attached to the underside of the bags (Fig. 2). Other surface-feeding predators such as sea stars may also play a role in controlling the distribution of small individuals, but, apart from the crab activity, there was no indication of any significant predation pressure on the larger tunicates.

Life History Traits

Field observations suggested that most of the individuals from the 1999 year class died prior to November 2000. This pattern of mortality, apparently as a result of natural senescence, was consistent with life span estimates of 12 to 18 mo for *C. intestinalis* in Scandinavian waters (Petersen et al. 1995). Similar to Lunenburg, reports from Sweden indicate that *C. intestinalis*, which settles in the summer, spawns the following spring and dies during the winter (Dybern 1965). Reports from Japan suggest the life span of this species is apparently determined by cumulative environmental temperature (Nomaguchi 1974). Thus, individuals that settle early in the summer, such as those in the first year 2000 cohort at Lunenburg, may die at a younger age than those that settle in late summer.

Estimates of growth rate in terms of body length for the year 2000 cohort were approximately 20 mm mo^{-1} from July through September, which is similar to estimates from Swedish (Petersen et al. 1995) and Chilean waters at 12 to 21 mm mo^{-1} (Uribe & Etchepare 2002). Observations on maximum size in terms of body length (100–140 mm) were higher than the 60 mm reported for Japanese waters (Yamaguchi 1975). Perhaps this species can attain a larger body size under colder conditions.

The results of the histological assessment and the spawning trials indicated that individuals that settled in May-June were capable of initiating egg production and spawning by August of the same year. This was consistent with observations from Sweden where two breeding generations of *C. intestinalis* have been found to co-occur in populations living close to the surface (Dybern 1965).

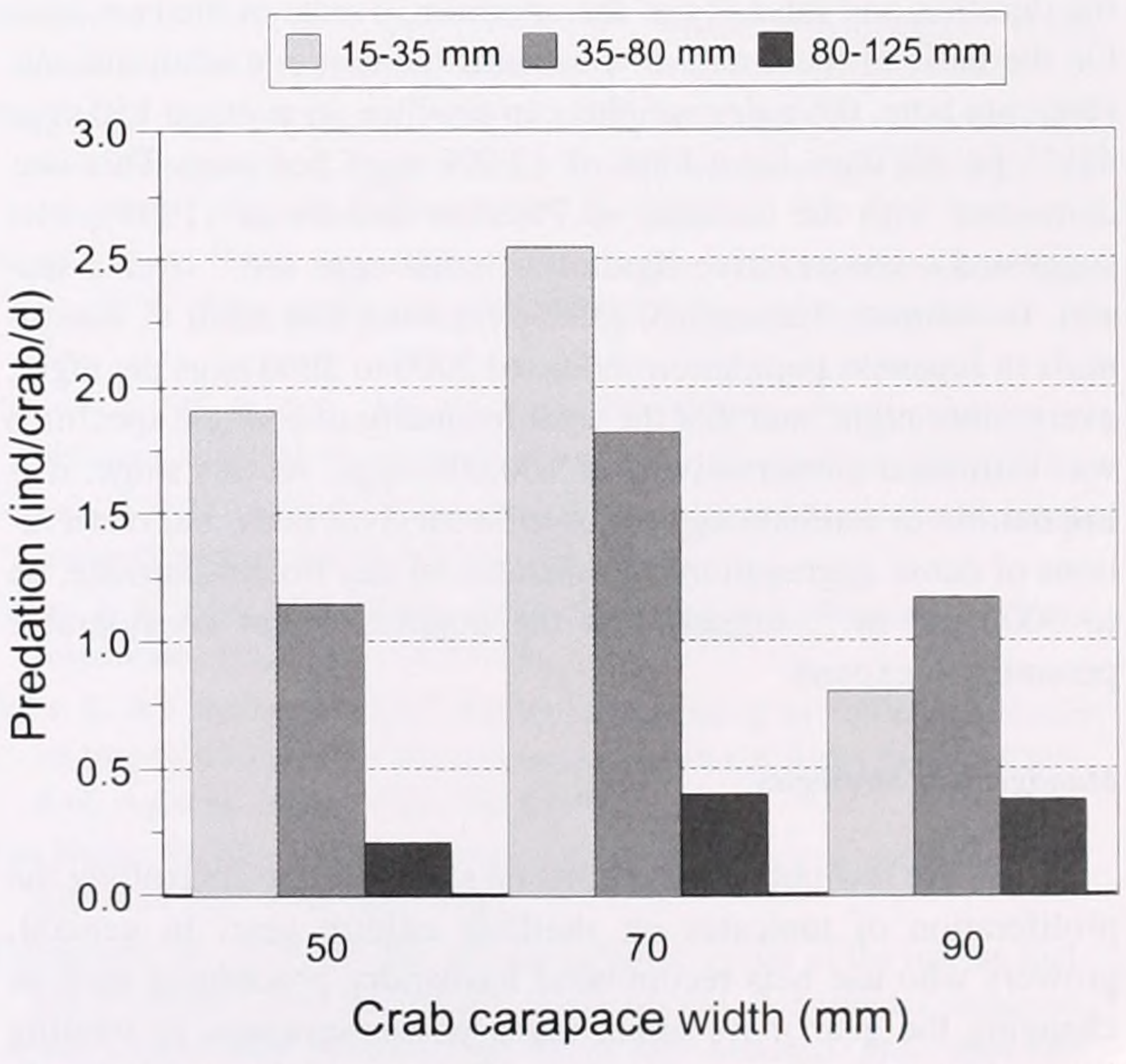

Figure 13. Predation rates of various sizes of rock crabs (ind crab^{-1} day^{-1}) on a range of sizes of *C. intestinalis*.

Yamaguchi (1975) also reported that *C. intestinalis* reached sexual maturity within 2 mo of settlement in winter, and within 1 mo at higher summer temperatures. This variability confirms that reproductive capability is size-dependent rather than age-dependent (Petersen et al. 1995).

Gulliksen (1972) concluded that the lowest temperature for the production of cionid larvae in Norwegian populations was in the range of 6 to 8°C, or comparable to their deep water winter temperatures. This was generally consistent with observations from the present study, which indicated possible gonad regression in January-February at <3°C, gametogenesis in March-April-May at 4 to 8°C, and production of competent gametes from mid-May onward when ambient temperatures exceeded 8°C.

The juvenile settlement data indicated that *C. intestinalis* populations in adjacent inlets may differ in their spawning and recruitment patterns. In Bayport, the recruitment peak was observed in May-June, whereas at Mason's Beach recruitment peaks were recorded in May-June and again in early August. The timing of the first peak was consistent with the histological data, indicating the presence of mature eggs in both populations in early May, and the spawning trials, suggesting that these two populations were capable of releasing eggs. However, unlike the Bayport population, the condition index for the Mason's Beach population remained relatively high until mid-July, suggesting that they did not spawn in May-June. It should be noted that the use of the condition index (body dry weight/total dry weight) as an index of spawning may be misleading. Petersen et al. (1995) found that this index reflected the level of growth but did not link it to spawning activity. It is possible that the relatively high condition index values for the Mason's Beach population in June-July were related to higher food levels at that site rather than to a delay in the onset of spawning activity. It remains unclear whether the first recruitment event at Mason's Beach originated from larvae produced by the local population or from other spawning populations such as those in Bayport and Upper South Cove.

Unlike many shellfish species that spawn over an interval of weeks, *C. intestinalis* can apparently spawn continuously over a 3-mo period (mid-May through mid-August). Information on reproductive status combined with estimates of fecundity illustrated the duration and intensity of the spawning events at the two sites. On the basis of these data it was estimated that one adult tunicate (100 mm long, 0.6 g dry weight) can produce on average 150 eggs day^{-1} for 60 days for a total of 12,000 eggs per year. This was consistent with the estimate of Petersen and Svane (1995), who suggested a conservative figure of 10,000 eggs ind^{-1} over a season. In contrast, Yamaguchi (1975) reported that adult *C. intestinalis* in Japanese populations released 2000 to 3000 eggs per night, every other night, and that the total fecundity of a single specimen was estimated conservatively at 100,000 eggs. At this stage, it is impossible to estimate egg to juvenile survival rates, but observations of dense aggregations of tunicates on any floating surface, up to 3000 ind m^{-2}, suggest that the population has considerable potential to expand.

Management Strategies

There are few published reports on strategies for controlling the proliferation of tunicates on shellfish culture gear. In general, growers who use nets recommend husbandry procedures such as changing the gear more often, using power sprayers, or treating bags with antifouling compounds. Other suggestions include exposing tunicates to air, fresh water, lime, or saturated brine dips (90 parts per thousand) followed by air (Shearer & MacKenzie 1997). Among mussel growers who use sleeving material rather than nets, there are few cost-effective solutions. Suggestions from anecdotal reports include air-drying lines overnight, *in situ* pressure washing with a bleach solution, and stabbing individual tunicates. Although feasible in the case of small farms or low-level infestations, these options are logistically impossible in the case of large-scale operations.

An alternative strategy is to develop a site-specific management plan for minimizing the level of settlement; for example, growers in South Africa re-sleeve their mussels immediately following the recruitment of *C. intestinalis* (Hecht & Heasman 1999). Based on the recommendations of the present study, the Nova Scotian company involved in oyster culture adjusted its work schedule to mitigate the impact of *C. intestinalis* on its operation. In particular, the major annual cleaning/changing of the shellfish and the culture gear was postponed from May to September when the heaviest settlement had passed; this strategy has since proved to be a reasonably cost-effective option for the company. Because *C. intestinalis* tends to occur in highly aggregated distributional patterns (Havenhand & Svane 1991, Svane & Havenhand 1993, Petersen & Svane 1995), the annual eradication of the broodstock population from the culture gear may reduce future recruitment levels.

Encouraging natural predation is always a preferred strategy for pest management in aquaculture (e.g., Enright et al. 1983) but may only have a limited application in this instance. At an estimated recruitment level of 25 ind 100 cm^{-2}, 3000 tunicates may settle on one oyster bag; even at a consumption rate of 11 tunicates day^{-1} at peak water temperatures, it would take one crab 273 days to clean one bag. Moreover, tunicates that have settled directly on the shellfish inventory are not accessible. Field observations suggest, however, that natural predation, possibly by rock crabs, may play an important role in reducing the abundance of tunicates during the winter. Another potential control method that has yet to be investigated is based on the hypothesis that recently recruited *C. intestinalis* may be vulnerable to dislodgement by surface grazers such as periwinkles (*Littorina littorea*). Enright et al. (1983) reported that the addition of periwinkles to lantern nets containing European oysters resulted in a significant reduction in biofouling levels.

Chemical treatment protocols including lime and brine immersion have been developed for the purpose of eliminating the fouling tunicate *Molgula* sp. from oyster spat collector units (MacNair & Smith 1998). Laboratory trials undertaken in the present study indicated that acetic acid was considerably more effective than more traditional methods for eliminating *C. intestinalis*. However, it should be noted that the use of acetic acid dips or sprays under field conditions should be carefully evaluated to ensure personal safety as well as containment and/or neutralization of the chemical so as to minimize any environmental impact.

This study has shown that the ascidian *C. intestinalis* is well adapted to conditions on the Atlantic coast of Nova Scotia, being capable of developing mature eggs and spawning at temperatures upward of 8°C. It can apparently tolerate a wide range of environmental conditions and has the potential to rapidly establish substantial populations on floating structures. The presence of suspended or off-bottom shellfish culture operations that offer refuge from natural predators may inadvertently promote its survival. Given the tendency of *C. intestinalis* to attach to the hulls of ships,

local maritime traffic will likely facilitate its dispersal to other sites in Atlantic Canada over the next few years

ACKNOWLEDGMENTS

We would like to thank Dr. Ellen Kenchington [Department of Fisheries and Oceans (DFO)] for providing access to laboratory facilities for sample processing, and Dr. Ken Freeman (DFO) for his advice on the design of the laboratory trials. Dr. Dan Jackson (DFO) contributed one of the photographs and demonstrated the use of the image analyzer system. Dr. Peter Strain (DFO) was very helpful in assessing the potential environmental impact of certain chemicals. Staff from Lunenburg Shellfish Inc. provided field support in sampling the experimental tables and conducting the preliminary eradication trials. Special thanks are extended to Dr. Donald Douglas, Industrial Technical Advisor, for his contribution to the experimental design of this project, and to the National Research Council Industrial Research Assistance Program for partial funding.

LITERATURE CITED

Berrill, N. J. 1947. The development and growth of *Ciona*. *J. Mar. Biol. Ass. U. K.* 26:616–625.

Boothroyd, F. A., N. G. MacNair, T. Landry, A. Locke, & T. J. Davidson. 2002. Dealing with an aquatic invader: the clubbed tunicate (*Styela clava*) in Prince Edward Island waters. Bull. Aquacul. Assoc. Can. 102:98–99.

Butler, A. J. & R. M. Connolly. 1996. Development and long term dynamics of a fouling assemblage of sessile marine invertebrates. *Biofouling* 9:187–209.

Byrd, J. & C. Lambert. 2000. Mechanism of the block to hybridization and selfing between the sympatric ascidians *Ciona intestinalis* and *Ciona savignyi*. *Mol. Reprod. Dev.* 55:109–116.

Cayer, D., M. MacNeil & A. G. Bagnall. 1999. Tunicate fouling in Nova Scotia aquaculture: a new development. *J. Shellfish Res.* (abstract) 18:327.

Connell, S. D. 2000. Floating pontoons create novel habitats for subtidal epibiota. *J. Exp. Mar. Biol. Ecol.* 247:183–194.

Connell, S. D. & T. M. Glasby. 1999. Do urban structures influence local abundance and diversity of subtidal epibiota? A case study from Sydney Harbour, Australia. *Mar. Environ. Res.* 47:373–387.

Dybern, B. I. 1965. The life cycle of *Ciona intestinalis* (L.) *f. typica* in relation to the environmental temperature. *Oikos* 16:109–131.

Enright, C., D. Krailo, L. Staples, M. Smith, C. Vaughan, D. Ward, P. Gaul, & E. Borghese. 1983. Biological control of fouling in oyster aquaculture. *J. Shellfish Res.* 3:41–44.

Glasby, T. M. 1997. Analysing data from post-impact studies using asymmetrical analyses of variance: a case study of epibiota on marinas. *Aust. J. Ecol.* 22:448–459.

Glasby, T. M. 1999. Differences between subtidal epibiota on pier pilings and rocky reefs at marinas in Sydney, Australia. *Est. Coast. Shelf Sci.* 48:281–290.

Gulliksen, B. 1972. Spawning, larval settlement, growth, biomass, and distribution of *Ciona intestinalis* L. (Tunicata) in Borgenfjorden, North-Trondelag, Norway. *Sarsia* 51:83–96.

Gulliksen, B. 1973. The vertical distribution and habitat of the ascidians in Bjorgenfjorden, North-Trondelag, Norway. *Sarsia* 52:21–28.

Gulliksen, B. & S. H. Skaeveland. 1973. The sea-star *Asterias rubens* L., as predator on the ascidian *Ciona intestinalis* (L.) In Borgenfjorden, North-Trondelag, Norway. *Sarsia* 52:15–20.

Havenhand, J. N. & I. Svane. 1991. Roles of hydrodynamics and larval behaviour in determining spatial aggregation in the tunicate *Ciona intestinalis*. *Mar. Ecol. Prog. Ser.* 68:271–276.

Hecht, T. & K. Heasman. 1999. The culture of *Mytilus galloprovincialis* in South Africa and the carrying capacity of mussel farming in Saldanha Bay. *World Aquaculture* 30:50–55.

Karayucel, S. 1997. Mussel culture in Scotland. *World Aquaculture* 28:4–10.

Lambert, C. C. & L. Brandt. 1967. The effect of light on the spawning of *Ciona intestinalis*. *Biol. Bull.* 132:222–228.

Lambert, C. C. & G. Lambert. 1998. Non-indigenous ascidians in southern California harbors and marinas. *Mar. Biol.* 130:675–688.

Lesser, M. P., S. E. Shumway, T. Cucci, & J. Smith. 1992. Impact of fouling organisms on mussel rope culture: interspecific competition for food among suspension-feeding invertebrates. *J. Exp. Mar. Biol. Ecol.* 165:91–102.

MacNair, N. & M. Smith. 1998. An investigation into the effects of lime and brine immersion treatments on *Molgula* sp. (sea grape) fouling on oyster collectors on Prince Edward Island. *P.E.I. Tech. Rep.* 219:13 pp.

Mallet, A. L. & C. E. Carver. 1993. Temporal production patterns in various size groups of the blue mussel in eastern Canada: spatial, temporal, stock and age variation. *Mar. Ecol. Prog. Ser.* 67:35–41.

Mazouni, N., J.-C. Gaertner, & J.-M. Deslou-Paoli. 2001. Composition of biofouling communities on suspended oyster cultures: an in situ study of their interactions with the water column. *Mar. Ecol. Prog. Ser.* 214:93–102.

Millar, R. H. 1952. The annual growth and reproductive cycle in four ascidians. *J. Mar. Biol. Assoc. U.K.* 31:41–61.

Monniot, C. & F. Monniot. 1994. Additions to the inventory of eastern tropical Atlantic ascidians: arrival of cosmopolitan species. *Bull. Mar. Sci.* 54:71–93.

Nomaguchi, T. A. 1974. Seasonal variations in life span of the ascidian *Ciona intestinalis*. *Exp. Geront.* 9:231–234.

Olesen, N. J., K. Frandsen, & H. U. Riisgard. 1994. Population dynamics, growth and energetics of jellyfish (*Aurelia aurita*) in a shallow fjord. *Mar. Ecol. Prog. Ser.* 105:9–18.

Petersen, J. K. & H. U. Riisgard. 1992. Filtration capacity of the ascidian *Ciona intestinalis* and its grazing impact in a shallow fjord. *Mar. Ecol. Prog. Ser.* 88:9–17.

Petersen, J. K. & I. Svane. 1995. Larval dispersal in the ascidian *Ciona intestinalis* (L.): evidence for a closed population. *J. Exp. Mar. Biol. Ecol.* 186:89–102.

Petersen, J. K., O. Schou, & P. Thor. 1995. Growth and energetics in the ascidian *Ciona intestinalis*. *Mar. Ecol. Prog. Ser.* 120:175–184.

Petersen, J. K., O. Schou, & P. Thor. 1997. *In situ* growth of the ascidian *Ciona intestinalis* (L.) and the blue mussel *Mytilus edulis* in an eelgrass meadow. J. *Exp. Mar. Biol. Ecol.* 218:1–11.

Plough, H. H. 1978. Sea squirts of the Atlantic continental shelf from Maine to Texas. Baltimore, MD: Johns Hopkins University Press, 118 pp.

Shearer, L. W. & C. L. MacKenzie. 1997. Effects of salt solutions of different strengths on oyster enemies. *Fish. Aquaculture* 15:97–103.

Svane, I. 1983. Ascidian reproductive patterns related to long-term population dynamics. *Sarsia* 68:249–255.

Svane, I. & J. N. Havenhand. 1993. Spawning and dispersal in *Ciona intestinalis*. *Mar. Ecol.* 14:53–66.

Uribe, E. & I. Etchepare. 2002. Effects of biofouling by *Ciona intestinalis* on suspended culture of *Argopecten purpuratus* in Bahia Inglesa, Chile. *Bull. Aquacul. Assoc. Can.* 102:93–95.

Van Name, W. G. 1945. The North and South American ascidians. *Bull. Am. Mus. Nat. Hist.* 84:1–476.

Yamaguchi, M. 1975. Growth and reproductive cycles of the marine fouling ascidians *Ciona intestinalis, Styela plicata, Botrylloides violaceus*, and *Leptoclinum mitsukurii* at Aburatsubo-Moroiso. *Mar. Biol.* 29: 253–259.