POPULATION DYNAMICS OF A NON-INDIGENOUS COLONIAL ASCIDIAN TUNICATE IN A SUBARCTIC HARBOUR

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by

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A thesis submitted to the
School of Graduate Studies
in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

DEPARTMENT OF BIOLOGY, FACULTY OF SCIENCE
MEMORIAL UNIVERSITY OF NEWFOUNDLAND

December 2012

ST. JOHN'S NEWFOUNDLAND AND LABRADOR CANADA

ABSTRACT

Botryllus schlosseri (Subphylum Tunicata: Class Ascidiacea) is a non-indigenous ascidian species of global and national interest, which has extensive populations along the south coast of insular Newfoundland. Economically, this species has been of concern to industry, management, and policymakers because non-indigenous ascidian species have been a severe and costly nuisance for bivalve aquaculture. Ecologically, the presence of this temperate-adapted species in Newfoundland represents an expansion of its global range into subarctic waters. Thus, I aimed to describe the population dynamics of *B. schlosseri* in Arnold's Cove, Placentia Bay, Newfoundland and Labrador, Canada, by determining the temporal and spatial patterns of recruitment and the seasonal cycle of colony abundance. In addition, I aimed to compile a checklist of extant indigenous and non-indigenous ascidian species of eastern Canada with an emphasis on species from Newfoundland and Labrador.

Artificial plates were used to determine recruitment rates among three sites, depths (1.0, 2.5, and 4.0 m from the water surface), and substrate types (aluminum, PVC, and wood in 2010; only PVC in 2011), in Arnold's Cove. Concurrently, density and cover of colonies were determined from the analysis of high-definition video surveys of a belt transect of wharf pilings. Seasonal biomass production was estimated from carbon to nitrogen (C:N) ratios and dry weight per unit area of dissected tissue subsamples.

The seasonal window for recruitment was from early August to mid-October. Recruitment rates were greater near the water surface than at other depths, and on PVC in comparison to aluminum and wood substrates. Maximum recruitment rates on PVC at 1.0 m were 29.3 and 43.5 $m^{-2}d^{-1}$ in September of 2010 and 2011, respectively, coincident with maximum seasonal seawater temperatures of 16-17°C. Colonies were present year-round on pilings. In the upper subtidal zone, monthly mean cover ranged from an annual minimum of 0.6% in May to a maximum of 2.8% in October. Colony size and biomass, though not C:N ratios, had a significant seasonal signal.

These findings suggest that recruitment was predominantly constrained by seawater temperature within the short productive season, and that the population was sustained from one year to the next because of high cover of overwintering colonies. The efficacy of utilising PVC to track recruitment of *Botryllus schlosseri*, and perhaps other closely related ascidian species is supported by my data. Future management of *B. schlosseri* should target mitigation efforts before the annual onset of sexual reproduction and recruitment in July and within the upper 3-4 m of the water column.

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ACKNOWLEDGMENTS

My time on this thesis project introduced me to the strange world of ascidian biology. For this, I am tremendously grateful to have Dr. Don Deibel, Professor (Research), and Dr. Cynthia H. McKenzie, Adjunct Professor, as my co-supervisors. I thank Dr. Deibel for his sound advice, academic support, and financial assistance, and Dr. McKenzie for her enthusiasm, guidance, and expertise. I am indebted to the generous input and constructive criticisms from Drs. Deibel, McKenzie, Raymond J. Thompson, and Patrick Gagnon who were members of my Supervisory Committee. I benefited from many knowledgeable mentors over the past several years. In particular, I value the time and insights from Drs. Maria L. Palomares, J. Ben Lowen, and Matthew L. Rise. Also, I am very thankful to the Internal and External Examiners for examining my thesis.

This research would not be possible without technical help in the field and in the laboratory. I acknowledge the invaluable assistance from Mai Aoki, Gavin Applin, Danny Au, Susan Barney, Laura Chomyshyn, James Devereaux, Jerry Ennis, Ainsley Latour, Kenneth Law, Kyle Matheson, Robert O'Donnell, Mark O'Flaherty, Andrew Perry, Matthew Pittman, Dave Poitras, Philip Sargent, Meredith Schofield, Jeanette Wells, Terri Wells, and Damian Whitten.

Funding for this research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Supplemental Strategic Grant to Drs. Deibel, McKenzie, Rise, and Thompson. I am thankful to the Arnold's Cove

Harbour Authority for the use of the wharves and floating docks. Partnerships with Fisheries and Oceans Canada (Newfoundland Region), the Newfoundland and Labrador Department of Fisheries and Aquaculture, the Newfoundland Aquaculture Industry Association, and other industry partners were instrumental to the successful completion of this research.

During the course of my studies in St. John's, I was motivated by the unconditional love from family and the moral support from friends. In particular, I was very fortunate to have met and befriended Junjie Hu, Hanbyal Kim, Chai W. Phua, and Qingheng Xu.

CO-AUTHORSHIP STATEMENT

I am the principal author responsible for all aspects of the research described in this thesis, including formulating research questions, literature review, research design, data collection, data analysis, and preparation of manuscripts. Drs. Don Deibel and Cynthia H. McKenzie contributed to the identification and design of the research. Chapter 2—including any resulting publications unless requested otherwise—is co-authored with Mai Aoki, Kenneth Law, and Drs. Maria L. Palomares, Don Deibel, and Cynthia H. McKenzie. Aoki and Law assisted with data collection and Dr. Palomares contributed to the design of Chapter 2. Chapters 3 and 4—including any resulting publications unless requested otherwise—are co-authored with Drs. J. Ben Lowen, Don Deibel, and Cynthia H. McKenzie. Dr. Lowen assisted with data collection and provided statistical advice.

ABBREVIATIONS

AIS	aquatic invasive species
AIS-AZMP	Aquatic Invasive Species-Atlantic Zone Monitoring Program
ANOVA	analysis of variance
CCWP	colony cover on wharf pilings
CHN	carbon, hydrogen, nitrogen
C:N	carbon to nitrogen
COI	cytochrome *c* oxidase subunit I
CV	coefficient of variation
DD	degree day
DCWP	densities of colonies on wharf pilings
DFO	Fisheries and Oceans Canada
GBIF	Global Biodiversity Information Facility
GLM	generalised linear model
GP	general Poisson
HD	high-definition
NAIA	Newfoundland Aquaculture Industry Association
NB	negative binomial
NIA	non-indigenous ascidian
NMNH	National Museum of Natural History
NTU	nephelometric turbidity units
OBIS	Ocean Biogeographic Information System
psu	practical salinity units
PVC	polyvinyl chloride
QS	quotient of similarity
RAS	rapid assessment survey

RDST	recruitment on different substrate types
RPVC	recruitment on PVC
SCUBA	self contained underwater breathing apparatus
SD	standard deviation
ZIGP	zero-inflated general Poisson
ZINB	zero-inflated negative binomial
ZIP	zero-inflated Poisson

CHAPTER 1 – INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction and objectives

Newfoundland and Labrador is increasingly susceptible to biological invasions by ascidian species in the advent of human-mediated global environmental change. To date, *Botryllus schlosseri* (Pallas, 1766), *Botrylloides violaceus* Oka, 1927, and *Ciona intestinalis* (Linnaeus, 1767) are the only non-indigenous ascidian (NIA) species that have been identified in the waters of insular Newfoundland (United States Navy 1951, Hooper 1975, Callahan 2009, Callahan et al. 2010, Sargent et al., in preparation) despite the island being an international maritime destination for over 500 years. Chapter 1 introduces the economic and ecological threats associated with NIA species and, subsequently, describes the biology of *B. schlosseri*, which is currently the more widely distributed NIA species in Newfoundland.

The potential adverse impacts of *Botryllus schlosseri* (commonly known as the golden star tunicate), *Botrylloides violaceus* (the violet tunicate), and *Ciona intestinalis* (the vase tunicate) have been of immediate concern to the region's bivalve aquaculture since these species were reported on the south coast of Newfoundland between 2006 and 2007 (Callahan 2009, Callahan et al. 2010), and 2012 (Sargent et al., in preparation). The need for science-based approaches to the management of NIA species is recognised and supported by industry, managers, and

policymakers in Newfoundland and Labrador. Therefore, the objectives of this thesis were threefold. Firstly, I aimed to better understand the relative regional diversity of this taxonomic group and to facilitate the identification of current and prospective NIA species. Checklists of extant indigenous and non-indigenous ascidian species of eastern Canada were compiled with an emphasis on species from Newfoundland and Labrador. Secondly, I aimed to describe the patterns of recruitment of *B. schlosseri* in Newfoundland waters by determining the seasonal window for recruitment, the temporal and spatial patterns in recruitment within a harbour, and the affinity of larvae for various substrate types. Thirdly, I aimed to determine the seasonal timing of minimum and maximum abundance of *B. schlosseri* in Newfoundland by documenting the seasonal cycle of colony abundance and biomass production.

With these objectives in mind, Chapter 2 reviews the history and distribution of NIA species in eastern Canada and presents an account of extant indigenous and non-indigenous ascidian species that have been reported from the coasts of the Arctic, Labrador, insular Newfoundland, the Gulf of St. Lawrence, and the Maritimes. *Botryllus schlosseri* was studied in Chapters 3 and 4 under the general prediction that its population dynamics differs in subarctic waters, which includes the waters around Newfoundland (Dunbar 1953), from its habitual temperate waters. Chapter 3 presents the temporal and spatial patterns of recruitment on artificial plates, which have implications for the seasonal timing of future mitigation efforts and the efficacy of using PVC plates to monitor for NIA species. Chapter 4 presents the

seasonal cycle of colony abundance on wharf pilings, which offers an insight into the present status of biological invasion of this NIA species in Newfoundland. Lastly, Chapter 5 summarises the results from these studies, and provides a discussion of the current understanding of the population dynamics of *B. schlosseri* in a subarctic harbour.

1.2 Non-indigenous ascidian species

Economically, high abundances of NIA species can damage the real and or perceived value of marine resources, can incur high costs to process aquaculture products, and can increase the risk of regional spread. Vectors for the spread of NIA species include: (1) the movement of fouled ship hulls and aquaculture equipment and products and (2) berthing ships near floating docks and wharf structures (Ramsay et al. 2008, Davis and Davis 2009). Some NIA species, such as the colonial ascidian, *Didemnum vexillum*, are considered to be an aquatic invasive species (AIS) because they have negative economic and ecological impacts. Fouling ascidian species grow on a variety of commercially important species such as crabs, lobsters, mussels, oysters, quahogs, and scallops (Bernier et al. 2009, Morris et al. 2009, Carman and Grunden 2010, Fisheries and Oceans Canada 2011a). In particular, these invasive ascidian species are a threat to bivalve aquaculture sustainability because of the costs associated with removing them from fouled equipment and products (Lambert 2002, Davis and Davis 2009, 2010, Locke and Carman 2009). For instance, the estimated cost of one non-indigenous solitary ascidian, *Styela clava* (the clubbed tunicate), to bivalve aquaculture is approximately CAD $26,000,000

year^{-1} (after correcting for inflated values; Andrea Locke, personal communication), which represents ca. 15% of the value of the industry (Colautti et al. 2006). Mitigation can be costly and counter-productive. For example, in Prince Edward Island, high pressure water treatments to remove colonies of *Botryllus schlosseri* from mussel lines can release viable fragments into the water column (Paetzold and Davidson 2010, Arens et al. 2011).

Ecologically, the introduction of NIA species can alter the biological diversity (number of species, relative abundance of different species, etc.) of benthic communities, compete with indigenous biota for space, and carry exotic diseases (Harms and Anger 1983, Blum et al. 2007, Dijkstra et al. 2007, Lutz-Collins et al. 2009). For example, Dijkstra et al. (2007) reported a long-term change in the community composition of NIA species in the Gulf of Maine, which initially consisted of only *Botryllus schlosseri* in 1979-1980, and later three additional NIA species in 2003-2005. This change may represent a scenario of invasional meltdown, which postulates that early biological invaders can modify the ecosystem, and in turn facilitates secondary biological invasions (Green et al. 2011). In addition to an increase in species numbers, Dijkstra et al. (2007) indicated that this succession represented an increase in total space occupied by NIA species. On Georges Bank, *Didemnum vexillum* exhibited invasive levels of abundance of ca. 50-90% cover, which considerably modified the marine landscape (Bullard et al. 2007, Osman and Whitlatch 2007). In British Columbia, direct competition for space with NIA species is cited as one of the causes for designating the oyster, *Ostrea lurida,* of "special

concern" by the Committee on the Status of Endangered Wildlife in Canada (Trimble et al. 2009, Committee on the Status of Endangered Wildlife in Canada 2011).

Maritime traffic is an important vector for the historical and contemporary introduction of NIA species, primarily via attachment of organism to boat hulls (Clarke Murray et al. 2011) and movement of fouled aquaculture equipment and products (Locke et al. 2007, Izquierdo-Muñoz et al. 2009, Carman et al. 2010, Paetzold and Davidson 2010). Within a harbour, high abundances of colonies on the hulls of moored ships and wharf structures further increases the risk of recruitment of new individuals onto the hulls of neighbouring ships. Ballast water is not a likely vector for the introduction of ascidian species (Lambert and Lambert 1998, Lambert 2002). Van Name (1945) and Berrill (1950) suggested that *Botryllus schlosseri* was introduced from Europe to the east coast of North America on ship hulls. As early as the 1830s, transport of *B. schlosseri* via hull fouling is supported by observations of colonies on a ship moored to a wharf in Boston, Massachusetts, for several months (Couthouy 1838). Moreover, Visscher (1928) examined over 250 metal-hulled ships and found that 10% were fouled with ascidian species. This list included *B. schlosseri*, which was reported on one of 100 ships surveyed. Similarly, Yan and Huang (1993) reported that *B. schlosseri* fouled ship hulls at a frequency of 12.5% in Daya Bay, China. By the mid-twentieth century, this species had become a common fouling organism in harbours and naval bases (Berrill 1950, United States Navy 1951, Woods Hole Oceanographic Institute 1952). However, it is also probable that *B. schlosseri* and other ascidians (e.g., the cryptogenic solitary ascidian, *Ciona*

intestinalis) were dispersed in the past by wooden sailing ships (Van Name 1945, Carlton and Hodder 1995). Carlton and Hodder (1995) demonstrated that *B. schlosseri* could foul the wooden hull of a sixteenth century replica sailing ship. An analysis of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) in *B. schlosseri* implicated transoceanic gene flow (López-Legentil et al. 2006). The analysis of the COI sequence suggested that the northwestern part of the Mediterranean Sea is the most probable origin of the Newfoundland clade of *B. schlosseri* (Callahan et al. 2010). On the west coast of North America, one of the multiple introductions of *B. schlosseri* may have originated from a shipment of fouled oysters (*Crassostrea* sp.) because this ascidian species was conspicuously established at an oyster aquaculture site (Lambert and Lambert 1998).

Factors that can influence the successful establishment of a biological invader in a novel system include the intra-specific characteristics of the biological invader (invasiveness of a species), the abiotic and biotic characteristics of an invaded community (invasibility of an ecosystem), or a combination of both. Multiple NIA species exhibit qualities associated with invasiveness, such as rapid growth, high fecundity, physiological tolerance to a broad range of environmental conditions (including pollution), prior invasion success, and wide distribution. The invasibility of benthic communities is attributed to the availability of nutrients and resources, to the existence of vacant or under-utilised niches, and to the disturbance regime of the local environment. For example, new spaces created by man-made structures are susceptible to fouling by NIA species. Also, ecosystems with high biodiversity tend to

be less susceptible to biological invasions, which may be a function of niche availability (Stachowicz et al. 1999, 2002a).

NIA species, including the chromatically diverse *Botryllus schlosseri*, are sometimes unidentified or misidentified. For example, in North America, *Didemnum vexillum* was initially referred to as *Didemnum* sp. A. The identity of *B. schlosseri* from Monterey Bay, California, was unknown and assumed to be *Botryllus* sp., until morphological and molecular evidence indicated similarity to *B. schlosseri* from Woods Hole, Massachusetts (Boyd et al. 1990). Accordingly, the identity of *B. schlosseri* from insular Newfoundland was confirmed (Gretchen Lambert, personal communication) from preserved specimens from our laboratory (Table A-1 in the Appendix). In addition, the COI sequence of samples from insular Newfoundland was consistent with sequences for *B. schlosseri* published in GenBank (Callahan et al. 2010).

At the national level, the Aquatic Invasive Species Task Group was formed in 2002 by the Canadian Council of Fisheries and Aquaculture Ministers to address the threat of AIS in Canada (Canadian Council of Fisheries and Aquaculture Ministers-Aquatic Invasive Species Task Group 2004). A national strategy was formalised by 2004, which resulted in collaborative research, monitoring programmes, rapid response planning, risk analyses, and a regulatory framework through Fisheries and Oceans Canada (DFO). For instance, risk analysis showed the geographic likelihood of invasion by the colonial ascidians, *Botryllus schlosseri*, *Botrylloides violaceus*, and *Ciona intestinalis* on the Pacific and Atlantic coasts of Canada (Therriault and

Herborg 2008a). Risk analysis maps suggested that large regions, including waters of insular Newfoundland, were within the temperature and salinity tolerance ranges of these three species.

At the provincial level, the management of AIS in Newfoundland and Labrador adheres to the precautionary principle. In collaboration with Memorial University of Newfoundland, DFO (Newfoundland Region) received funding in 2006 to conduct a Rapid Assessment Survey (RAS) of four high risk coastal harbours around the island to survey for indigenous and non-indigenous ascidians (Callahan et al. 2010). The four harbours that were chosen for this RAS were determined by a study funded by DFO and the Newfoundland Aquaculture Industry Association (NAIA; Baines 2007) to assess vessel traffic and high risk harbours. In addition to RAS, DFO has been involved with the Aquatic Invasive Species-Atlantic Zone Monitoring Program (AIS-AZMP) to track the recruitment of AIS using grey PVC plates. Since 2007, monitoring surveys targeting AIS throughout the province are ongoing to provide the most up-to-date maps, which are published and updated periodically by DFO. Fortunately, NIA species are not yet a nuisance for bivalve aquaculture in Newfoundland and Labrador. However, immediate concerns have resulted in some shipment closures of aquaculture products. In 2007, the NAIA and DFO Newfoundland Region formed the Newfoundland and Labrador Aquatic Invasive Species Advisory Committee. NAIA, DFO, and the provincial Department of Fisheries and Aquaculture collaboratively published field guides on AIS, including *Botryllus schlosseri* and *Botrylloides violaceus*, to raise public and industry

awareness. In collaboration with others, I wrote two fact sheets on *B. schlosseri* and *B. violaceus* in Newfoundland, which were published by DFO (Fisheries and Oceans Canada 2011b, 2011c). Finally, interim progress reports of research on *B. schlosseri* and *B. violaceus* at Memorial University of Newfoundland were published to communicate progress on AIS research to the aquaculture industry and the general public (Applin 2011, Lowen 2011, Ma 2011).

1.3 The biology of *Botryllus schlosseri*

Botryllus schlosseri is an ascidian species within a polyphyletic group of ca. 3,000 extant species that diverged from the vertebrate lineage ca. 500 million years ago. Around 350 BCE, Aristotle was the first to recognise ascidians as animals (Lambert 2005, Voultsiadou and Vafidis 2007, Shenkar and Swalla 2011), but they were later erroneously grouped with gorgonians, sea pens, and sponges (Linnæus 1758, Pallas 1766) and molluscs (Gould 1870). Schlosser and Ellis (1756) are credited with giving the earliest detailed account of an ascidian species: *B. schlosseri* (Shenkar and Swalla 2011). *B. schlosseri* was originally described as *Alcyonium schlosseri* by Pallas (1766) and then re-assigned to the genus *Botryllus* by Gaertner (1774). In North America, it was re-described from New York as *Botryllus gouldii* by Verrill (1871). *B. gouldii* was probably incorrectly considered as an indigenous species until Van Name (1945) implicated it as *B. schlosseri* and as a non-indigenous species in North America (Carlton 2009).

There are two distinct stages in the life cycle of *Botryllus schlosseri*—the zooid and the larva. In the zooid stage, a group of genetically identical zooids

embedded in a common tunic comprises a colony. The zooid is ovate or tear-shaped, whereas the colony is asymmetrical and flat, such that zooids are arranged two dimensionally into stellate systems (Figure 1.4.1, B). However, the colony may form irregular three-dimensional lobes when space for colonisation becomes scarce. Systems of zooids are encased in the tunic with a shared common vascular system and associated ampullae. The tunic is composed of an animal cellulose-like compound known as tunicin and is translucent, gelatinous, and smooth (Berrill 1950, Boyd et al. 1990, Saito and Okuyama 2003). The maximum colony size is ca. 100 mm in diameter and 1-1.5 mm in thickness when not lobate (Boyd et al. 1990, Saito and Okuyama 2003). The system consists of 3-12 (Berrill 1950) or 5-18 (Milkman 1967) zooids surrounding a common cloaca (Figure 1.4.1, C). Systems range from 5-10 mm in diameter. Each zooid is 2-2.8 mm long and consists of an inhalent siphon, which is distal to the cloaca at the anterior end, and an exhalent siphon, which is proximate to the cloaca at the posterior end (Berrill 1950, Boyd et al. 1990, Saito and Okuyama 2003). If present, palleal buds (ca. 1 mm), ova (230-250 μm), and embryos (280-300 μm) are visible through the tunic (Boyd et al. 1990, Saito and Okuyama 2003, Voskoboynik et al. 2007; Figure 1.4.1, D). The tadpole larva is 1.6 mm long and consists of a 0.4 mm ovate head (or trunk) and a tail (Boyd et al. 1990, Saito and Okuyama 2003; Figure 1.4.1, A). The larva lacks a functional mouth (Berrill 1950).

Colour polymorphism in colonies of *Botryllus schlosseri* has been briefly treated in the literature. Colour ranges from bright orange to gold to dark brown and

purplish-black (Berrill 1950, Lambert and Lambert 1998, Boyd et a. 1990). Upon close examination, orange, yellow, or white patterns on zooids can be distinguished from the brown, grey, or black margins around the system of zooids (Carver et al. 2006a). In the eastern Mediterranean Sea, where *B. schlosseri* has been extensively investigated, the dominant colour morph is brown, with a natural frequency of 80-93% (Rinkevich et al. 1998). Pigmented patterns are sometimes present, such as peristomatic rings around the cloaca or intersiphonal bands situated between the inhalent and exhalent siphons. In eastern North America, the zooids are commonly dark purple to purplish-brown (Verrill 1871, Verrill and Smith 1874, Van Name 1945). Verrill and Smith (1874) described nine and named seven morphotypes of *B. schlosseri* from eastern United States of America (*B. schlosseri* varieties *albida*, *annulata*, *atrox*, *bicolor*, *farinacea*, *stella*, and *variegata*). I have recognized 10 morphotypes of *B. schlosseri* from Newfoundland based on six discrete, two-state character traits (see Appendix D). I have named these morphotypes uniform orange, orange peristomatic-ringed, inhalent- and peristomatic-ringed orange, inhalent-ringed orange, white translucent, uniform, intersiphonal-banded, inhalent-ringed, inhalent- and peristomatic-ringed, and peristomatic-ringed.

The *Botryllus schlosseri* zooid exhibits several forms of asexual reproduction. The oozooid and the blastozooid are distinguished based on their developmental origins. The oozooid is ca. 0.5 mm in length and is the founding individual of the colony that arises upon settlement and metamorphosis of the larva (Saito and Okuyama 2003). The oozooid is resorbed when the first blastozooid(s) replace(s) it.

The blastozooids arise via blastogenesis (or palleal budding), a process in which zooids undergo a synchronised colony-wide blastogenic cycle. The cycle begins at the moment when palleal buds open their siphons to feed, thereby becoming a functional blastozooid, and ends with the resorption of the same blastozooid and takeover by the next generation of blastozooids (Rinkevich et al. 1992, Ballarin et al. 2008). Takeover lasts ca. 30 h and often results in an increase in the size and total number of blastozooids in a colony (Lauzon et al. 1993). In addition to blastogenesis, asexual reproduction of zooids can be induced by vascular budding from intact vascular tissues when a colony is stripped of all its blastozooids (Satoh 1994). Propagation by fragmentation is another form of asexual reproduction at the colonial level, which can be facilitated by natural and anthropogenic disturbances (Paetzold and Davidson 2010). However, natural occurrences of fragmentation may be rare (Brunetti 1974). For instance, Grosberg (1988) observed two cases of viable fragmentation in ca. 3,000 colonies. Natural mechanisms of dispersal of fragments include (a) drifting in the water column, (b) attaching to carapaces of rock crab (*Cancer irroratus*) and American lobster (*Homarus americanus*), and (c) rafting on eelgrasses and seaweeds (Worcester 1994, Bernier et al. 2009).

Botryllus schlosseri is hermaphroditic and reproduces sexually by ovoviviparous embryogenesis. Testes and egg follicles develop on young palleal buds (Manni et al. 1994, Satoh 1994). However, oocytes arise from germ cells (gametes), which originate from undifferentiated coelomic cells known as hemoblasts (Manni et al. 1993, 1994, Satoh 1994, Kawamura and Sunanaga 2010).

Young oocytes differentiate in the gonadal blastemas of young palleal buds (Mukai 1977), and migrate from older to younger palleal buds via the vascular system until they are developmentally mature (Manni et al. 1994, Satoh 1994). Finally, the mature oocyte is discharged into the atrial cavity (ovulation) and recruited by the egg follicle (Mukai 1977), which holds a single ovum and is connected by the follicle stalk to the brood pouch (Satoh 1994). Each zooid produces 1-3 ova (Sabbadin 1971, Boyd et al. 1990, Manni et al. 1994). Sperm mature after ovulation (Mukai 1977) and are released ca. 2 d after the start of the blastogenic cycle (Milkman 1967). In the field, colonies reach sexual maturity at a minimum age of ca. 49 d (seven blastogenic cycles; Chadwick-Furman and Weissman 1995). Self-fertilisation is uncommon (Sabbadin 1971, Yund et al. 1997). Spawning occurs when sperm are liberated through the cloaca and into the water column in a synchronised, colony-wide manner (Boyd et al. 1990, Saito and Okuyama 2003). The ovum is fertilised by the sperm at the beginning of the blastogenic cycle and develops into an embryo (Manni et al. 1993, Manni et al. 1994). The mature embryo is hatched at the end of the blastogenic cycle and the free-swimming larva is expelled through the cloaca and into the water column in a synchronised, colony-wide manner (Stewart-Savage et al. 2001). Thus, the length of the brooding period (from fertilisation to hatching) is the same as the length of the concurrent blastogenic cycle (Grosberg 1982, Westerman et al. 2009).

An allorecognition system in *Botryllus schlosseri* enables colonies to distinguish between kin and non-kin upon physical contact (Sabbadin and Astorri

1988, Ben-Shlomo et al. 2001, Rinkevich 2005). Colonies in contact that share one or both histocompatibility alleles are recognised as kin. This results in the complete or partial integration of tissues (by resorption) of partner colonies forming a natural chimera. Otherwise, rejection results in inflammation at the site of contact between non-kin tissues. Chimeric colonies are genetically non-homogeneous and consist of tissues from two or more partners. In a chimera, the proportion of partner tissue can change after each successive blastogenic cycle. This has led some authors to believe that chimeras may exhibit short-term plasticity to environmental changes that may facilitate long-term evolutionary adaptation (Rinkevich and Yankelevich 2004, Rinkevich 2005). However, parasitism among partner tissues has been documented, which may have counter-adaptive significance associated with the cost of somatic maintenance (Pancer et al. 1995).

The states of colonial regression and degeneration are distinguishable in *Botryllus schlosseri*, and are generally linked to abiotic and biotic controls, such as seawater temperature and genetically-programmed colony lifespan (Milkman 1967, Millar 1971, Brunetti et al. 1980, Rinkevich et al. 1992). Blastogenesis continues uninterrupted during regression; however, it is likely that siphons remain closed (Milkman 1967, Brunetti et al. 1980). Regression is a reversible process when environmental conditions become favourable (Brunetti et al. 1980). Degeneration is an irreversible process, which is characterised by indiscernible zooids. A colony can remain in this state for a protracted period of time prior to death.

Divergent life-history strategies were documented within a population of *Botryllus schlosseri* in Woods Hole, Massachusetts (Grosberg 1988). Colonies of that population were semelparous and iteroparous. Semelparous morphs produced only one clutch of larvae before death (Grosberg 1988), whereas iteroparous morphs produced three or more clutches (maximum 7-10; Grosberg 1988). Clutch size per zooid tends to be larger in semelparous morphs than in iteroparous morphs (Grosberg 1988). However, not all populations contain both morphs. For example, colonies from a population in Monterey Bay, California, were all iteroparous (Chadwick-Furman and Weissman 1995). In the field, the lifespan of colonies varied from three months for spring-born colonies to eight months for autumn-born colonies (Chadwick-Furman and Weissman 1995).

Botryllus schlosseri has evolved a sedentary lifestyle that features active suspension feeding. Zooids filter ambient seawater through the inhalent siphon collecting phytoplankton, zooplankton, and perhaps, suspended organic particles (Millar 1971). In the laboratory, healthy colonies can be maintained on a mixed diet of algae (Boyd et al. 1986), although colonies can live without food for months (Milkman 1967).

Predation on *Botryllus schlosseri* and competition for space among colonies of this species and other benthic species are predominant inter- and intra-specific interactions. Natural predators include shell-less gastropods (e.g., nudibranchs), shelled gastropods (*Anachi lafresnay, Erato volute,* and *Mitrella lunata*), and winter flounder (*Pseudopleuronectes americanus*; Milkman 1967, Millar 1971, Dijkstra et al.

2007, Epelbaum et al. 2009a, Whitlatch and Osman 2009). Larvae and newly settled colonies are susceptible to predation (Osman and Whitlatch 1998, 2004, Whitlatch and Osman 2009). Laboratory experimental studies have demonstrated that if no other food source is available, *Strongylocentrotus droebachiensis* (the green sea urchin), *Dermasterias imbricate* (the leather sea star), and *Hermissenda crassicornis* (the opalescent sea slug) will graze on adult colonies. However, only *S. droebachiensis* grazes on juvenile colonies (Epelbaum et al. 2009a).

Generally, *Botryllus schlosseri* is a dominant competitor for space in benthic communities, and can out-compete, or co-dominate, with other ascidian species, barnacles, bryozoans, and sponges (Harms and Anger 1983, Schmidt and Warner 1986, Boyd et al. 1990, Nandakumar 1996). Yet, some colonial ascidians, such as *Botrylloides violaceus* and *Diplosoma listerianum*, can out-compete *B. schlosseri* for space on experimental artificial substrates (Schmidt and Warner 1986, Gittenberger and Moons 2011). Colonial ascidians overgrowing bryozoans are not always lethal, and both can persist for months (Todd and Turner 1988). Skeleton shrimps (*Caprella* spp.) are often attached to the tunic of *B. schlosseri*, but this inter-specific relationship is not well understood (Epelbaum et al. 2009a). Skeleton shrimps also occur on *B. schlosseri* in Newfoundland (personal observation).

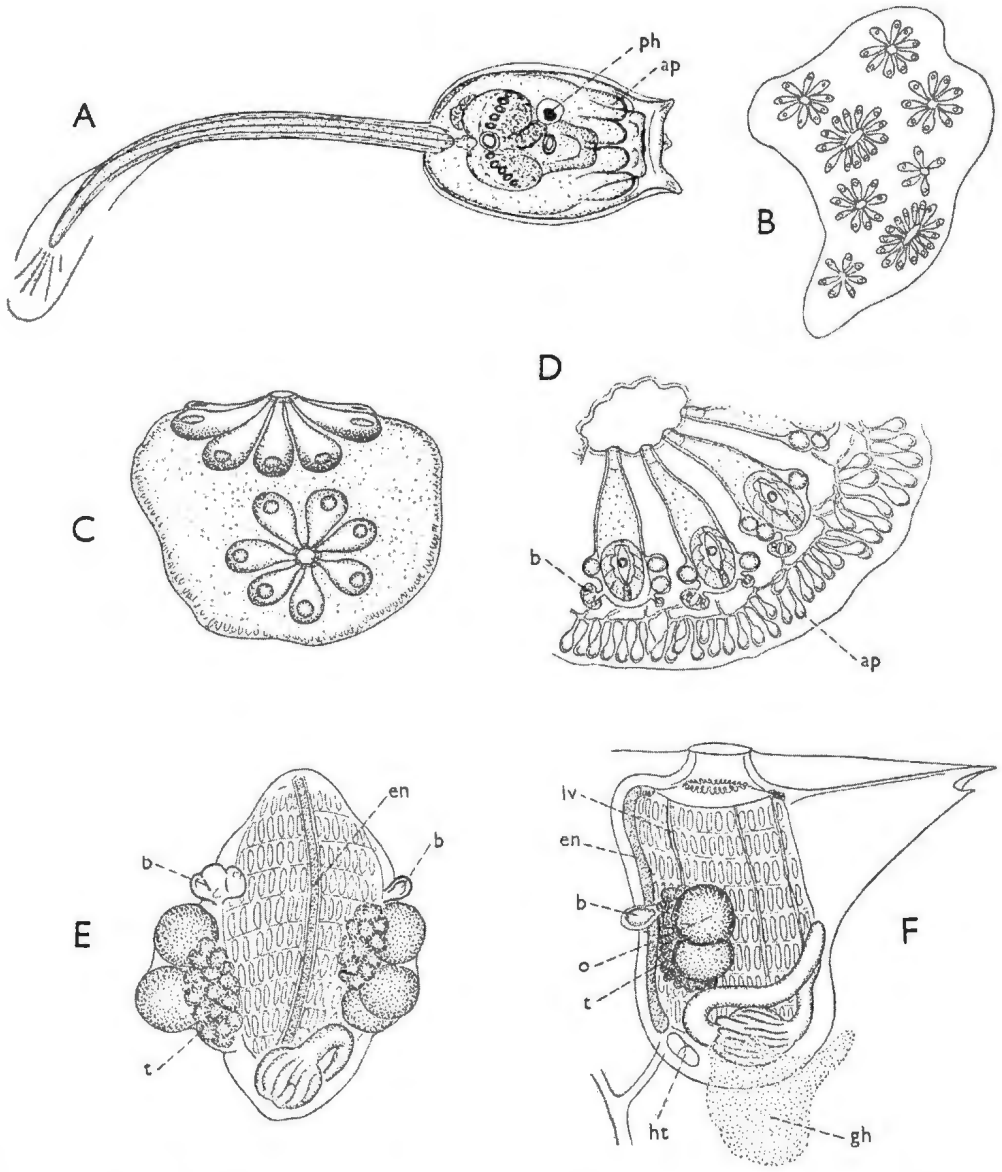

Figure 1.4.1 *Botryllus schlosseri.* (A) Larva; (B) colony with stellate systems; (C) two systems showing zooids around a common cloaca; (D) three zooids of a system with exhalent siphons opening into the common cloaca and ampullae at the margin of the tunic; (E) ventral view of zooid; (F) zooid viewed from the left with a 'ghost' of the preceding zooid; ap = ampulla, b = bud, en = endostyle, gh = 'ghost', ht = heart, lv = longitudinal vessel, o = ovum, ph = photolith, and t = testis. Figure adapted from Berrill (1950).

CHAPTER 2 – AN ACCOUNT OF EXTANT ASCIDIANS OF EASTERN CANADA

2.1 Introduction

The understanding of the biological diversity of the ascidian fauna is relatively coarse given that, at the continental scale, 82 species of ascidians inhabit the waters of western Canada and the United States of America (Pellegrin et al. 2007), and 88 are reported from the eastern United States of America (Plough 1978). At smaller spatial scales, 37 ascidian species occur in the Gulf of St. Lawrence (Brunel et al. 1998), and 26 species in coastal Massachusetts (Shenkar and Swalla 2011). However, based on the distributional data found in the monograph of Van Name (1945), there are 12 ascidian species in the Bay of Fundy (Shenkar and Swalla 2011). For the Bay of Fundy, this low number of species compared to neighbouring regions suggests that this water body is not biologically diverse with ascidian species, or that the ascidian faunal composition is poorly understood and the number of species underestimated. There may be a general knowledge gap in the systematics of ascidians due to a lack of studies and, perhaps, taxonomic expertise in a given region (Schander and Willassen 2005).

Marine ecosystems are presently threatened by the invasion of non-indigenous ascidian (NIA) species, which have become a Canadian (LeGresley et al. 2008, Ramsay et al. 2008, Callahan et al. 2010, Sephton et al. 2011) and global problem (Lambert 2007, 2009, Locke and Carman 2009). In response, a growing number of investigators have aimed to understand the process of NIA introductions,

to minimise their detrimental ecological and economic impacts and to control their potential spread. In southern California, as many as 14 NIA species have been identified (Lambert and Lambert 1998, 2003). In contrast, five NIA species have been reported from British Columbia (*Botrylloides violaceus*, *Botryllus schlosseri*, *Ciona intestinalis*, *Didemnum vexillum*, and *Styela clava*). To date, five NIA species (*Ascidiella aspersa*, *B. violaceus*, *B. schlosseri*, *D. listerianum*, *S. clava*) and one cryptogenic ascidian (*C. intestinalis*) have been reported from eastern Canada. Presently, eastern Canada is susceptible to introduction of *D. vexillum* because it is already established in northern Maine (Martin et al. 2010, 2011). This susceptibility underscores the value of up-to-date knowledge on the distribution of NIA species at both continental and regional spatial scales.

Although the identification, invasion history, and geographic distribution of NIA species are important to industry, management, and policymakers, it is also valuable to have an informed description of indigenous ascidians. Unfortunately, a good account of the local diversity and distribution of indigenous and non-indigenous ascidians is often inaccessible, incomplete, or missing. For instance, the indigenous ascidian fauna of Newfoundland might not be fully known because many specimens held at the provincial museum were not identified to species (personal observation) and might be not be verifiable because older collections described in the literature may be lost.

The main purpose of this investigation was to compile checklists of extant indigenous and non-indigenous ascidian species of eastern Canada, with emphasis

on species from Newfoundland and Labrador, and to compare the diversity of the ascidian fauna among the coasts of the Arctic, Labrador, insular Newfoundland, the Gulf of St. Lawrence, and the Maritimes. This information can improve the understanding of the ascidian fauna at regional spatial scales. I tested the hypothesis that there are significant differences in the composition of the ascidian fauna among regions, such that neighbouring regions are more similar than between distantly separated regions.

2.2 Materials and methods

2.2.1 Occurrence records

Ascidian records from 1852 to the present were collected from 49 published sources and two online databases: the Ocean Biogeographic Information System (OBIS; Vanden Berghe 2007) and the Global Biodiversity Information Facility (GBIF; GBIF 2012), accessed on January 15, 2012, at http://www.iobis.org/ and http://www.gbif.org/ respectively (see Table A-2 in Appendix A for a list of sources organised by region). I searched all ascidian records that were geographically referenced to Canada and excluded records from western Canada *post hoc*. Records that were not identified to species were excluded. OBIS contained 558 ascidian occurrence records from four databases: the Bay of Fundy Species List (Pohle et al. 2004), the DFO Maritime Research Vessel Trawl Surveys Fish Observations (Clark and Branton 2007), the National Museum of Natural History (NMNH) Invertebrate Zoology Collections (Department of Invertebrate Zoology 2007), and the North American Sessile Marine Invertebrate Survey (Ruiz et al. 2005). GBIF contained 961

records from four databases: the Atlantic Reference Centre Museum of Canadian Atlantic Organisms: Invertebrates and Fishes Data (Atlantic Reference Centre 2006), the NMNH Invertebrate Zoology Collections (Department of Invertebrate Zoology 2007), the North American Sessile Marine Invertebrate Survey (Ruiz et al. 2005), and the Peabody Invertebrate Zoology DiGIR Service (Yale Peabody Museum 2009). Two of these databases (the NMNH Invertebrate Zoology Collections and the North American Sessile Marine Invertebrate Survey) are housed in both OBIS and GBIF.

All scientific names were updated to be consistent with the World Register of Marine Species (Appeltans et al. 2012) and the Integrated Taxonomic Information System (Integrated Taxonomic Information System 2012), accessed on January 15, 2012, at http://www.marinespecies.org/ and http://www.itis.gov/ respectively. All records from eastern Canada were categorised by region as follows: the Arctic (from the Beaufort Sea to Baffin Bay; including Hudson Bay), Labrador, insular Newfoundland, the Atlantic coast of Nova Scotia, the Gulf of St. Lawrence coasts of Quebec, Prince Edward Island, New Brunswick, and Nova Scotia, and the Bay of Fundy coasts of New Brunswick and Nova Scotia.

Native species with two or fewer records in the literature were evaluated for inclusion in the checklist. *Didemnum roseum* is an arctic species that is restricted to the Old World and is a close ally of the relatively common *Didemnum albidum* in the New World (Van Name 1945). *Halocynthia aurantium* is restricted to the Pacific coast and is very similar to the widely distributed *Halocynthia pyriformis* on the Atlantic coast (Van Name 1945, Millar 1966, Haydar 2010). Thus, to conform to the

conventional treatment of their taxonomy based on their distributions, I considered records of *D. roseum* in eastern Canada to be *D. albidum* and records of *H. aurantium* in eastern Canada to be *H. pyriformis*. I am uncertain about the identification of *Aplidium translucidum* (record from Kongulaksiarvik, Labrador), *Didemnum granulatum* (record from Hopedale, Labrador), *Molgula pugetiensis* (record from Port de Grave, Newfoundland), and *Styela squamosa* (record between Halifax and La Have Bank, Nova Scotia) listed in OBIS and GBIF. These four species were not included in the checklist because of a lack of corroborating published literature to support their inclusion. Although *Clavelina concrescens*, *Lissoclinum wandeli*, and *Styela gelatinosa* have been reported from the Davis Strait, off the west coast of Greenland, these species were not included because there are no other records from the coasts of Canada, and I believe that these are primarily Old World species (Van Name 1945, Millar 1966). *Molgula occidentalis* (record from northern Gulf of St. Lawrence coast of Quebec) was excluded because it is distributed from North Carolina to the West Indies (Van Name 1945). *Polycarpa albatrossi* (record from the Bay of Fundy) was excluded because it is strictly an abyssal species (Van Name 1945). The checklist included *Ascidia dijmphniana* (record from Labrador) because it is a rare arctic species that has been documented in Baffin Bay (Van Name 1945) and *Hartmeyeria arctica* because it was originally described in Mason Bay, Northwest Territories (Korczynski 1989). The checklist includes *Molgula arenata* (a questionable record from the Bay of Fundy) because it is distributed from

Massachusetts to New Jersey (Haydar 2010). A total of eight ascidian species were excluded from the checklist (Table 2.3.1).

Ascidian records from published sources and online databases were supplemented by our dive surveys and with some observations from the AIS-AZMP. SCUBA divers collected specimens from Newfoundland during Rapid Assessment Surveys (RAS; September 2006 to October 2008; Callahan et al. 2010) and additional field surveys (September 2009 to March 2010). Specimens from the RAS were preserved in ethanol. Indigenous specimens collected in September 2009 and February 2010 were transported to the laboratory, where they were maintained alive in flowing unfiltered seawater and identified and photographed within a few days. In January 2010, samples of non-indigenous *Botrylloides violaceus* (n = 2) and *Botryllus schlosseri* (n = 5), along with several indigenous ascidian specimens, were sent to Gretchen Lambert for taxonomic verification.

In 2012, observations made as part of and in collaboration with the AIS-AZMP included the first reports of *Ascidiella aspersa* on the Atlantic coast of Nova Scotia and on the Bay of Fundy coast of New Brunswick (Dawn Sephton, personal communication), *Ciona intestinalis* on the south coast of insular Newfoundland (Sargent et al., in preparation), and *Diplosoma listerianum* and *Styela clava* on the Atlantic coast of Nova Scotia (Andrea M. Moore and Kevin C. K. Ma, unpublished data).

Table 2.3.1 List of ascidian species that were excluded from the checklist.

Species	Authority	Record from eastern Canada	References*
Aplidium translucidum	(Ritter, 1901)	Labrador	[1], [2]
Clavelina concrescens	Hartmeyer, 1924	Arctic	[3]
Didemnum granulatum	Tokioka, 1954	Labrador	[1]
Lissoclinum wandeli	Hartmeyer, 1924	Arctic	[3]
Molgula occidentalis	Traustedt, 1883	Gulf of St. Lawrence coast of Quebec	[4]
Molgula pugetiensis	Herdman, 1898	Insular Newfoundland	[1], [2]
Polycarpa albatrossi	(Van Name, 1912)	Bay of Fundy coast of Nova Scotia	[1]
Styela gelatinosa	(Traustedt, 1886)	Arctic	[3], [5]
Styela squamosa	Herdman, 1881	Atlantic coast of Nova Scotia	[1], [2]

* References for Table 2.3.1: [1] GBIF; [2] OBIS; [3] Van Name (1945); [4] Brunel et al. (1998); [5] Millar (1966).

2.2.2 Indigenous, cryptogenic, and non-indigenous criteria

Each ascidian species in this study was assigned to a status of indigenous, cryptogenic, or non-indigenous based on their historical, biogeographic range. *Ascidiella aspersa*, *Botrylloides violaceus*, *Botryllus schlosseri*, *Diplosoma listerianum*, and *Styela clava* are considered non-indigenous in eastern Canada (Carver et al. 2006a, 2006b, Clarke and Therriault 2007, Locke 2009, Mackenzie 2011). *Ciona intestinalis* is considered cryptogenic to eastern Canada except in the Arctic, Prince Edward Island, and insular Newfoundland (see below). I assumed all other ascidian species to be naturally distributed and indigenous.

Botrylloides violaceus and *Styela clava* are indigenous to the north-western Pacific Ocean (Carlton 1979, Carver et al. 2006a, Clarke and Therriault 2007, Goldstien et al. 2011, Lejeusne et al. 2011). *Botryllus schlosseri* is indigenous to the Mediterranean Sea (Carver et al. 2006a, Lejeusne et al. 2011) and *Ascidiella aspersa* to Europe (Stachowicz et al. 2002b, Carlton 2009). *B. schlosseri* and *Diplosoma listerianum* are cryptogenic to the North Sea of Europe (Haydar 2010). *B. violaceus* was likely introduced to eastern North America via transport of aquaculture equipment (Dijkstra et al. 2007), whereas *A. aspersa*, *B. schlosseri*, *D. listerianum*, and *S. clava* were probably introduced via transport on fouled ship hulls (Van Name 1945, Dijkstra et al. 2007, Therriault and Herborg 2008a, Haydar 2010, Mackenzie 2011).

Ciona intestinalis is generally believed to be native in European waters (Carver et al. 2006b, Therriault and Herborg 2008a, Zhan et al. 2010) and in arctic

and subarctic waters (Van Name 1945, Berrill 1950, Millar 1966). However, there are several potential varieties of *C. intestinalis* described in the literature, including formae *typica*, *gelatinosa*, *longissima*, and *tenella* (Van Name 1945, Millar 1966, Therriault and Herborg 2008b). Formae *gelatinosa* and *longissima* were reported from arctic and subarctic waters of northern Europe (Van Name 1945, Millar 1966). I considered *C. intestinalis* from the arctic waters of northern Canada (records from Foxe Basin and northeastern Baffin Island; Atkinson and Wacasey 1989, Aitken and Fournier 1993) to be a cold water variety, either forma *gelatinosa* or *longissima*. Therefore, *C. intestinalis* can be considered as an indigenous species in the arctic. Forma *tenella* is now no longer used, but it was reported from northern New England and the Bay of Fundy (Verrill 1871, Van Name 1945, Millar 1966). In the present study, I treated *C. intestinalis* from eastern Canada as cryptogenic. At regional spatial scales, *C. intestinalis* from the Atlantic waters of Canada, the Gulf of St. Lawrence, and the Bay of Fundy can be considered as a cryptogenic species, except in Prince Edward Island and insular Newfoundland. On these two islands, the absence of historical records suggests that *C. intestinalis* can be considered as a non-indigenous species.

As a result of empirical and genetic evidence (Haydar 2010), *Molgula manhattensis*, previously considered cryptogenic in eastern Canada, is believed to be indigenous. In the present study, I treat *M. manhattensis* as indigenous in eastern Canada despite its controversial origins.

2.2.3 Analysis

The similarity between any two regions was evaluated with the Sørensen similarity index by calculating the quotient of similarity (QS).

$$QS = \frac{2C}{A + B}$$

where A and B represent the number of ascidian species in regions A and B, respectively, and C the number of species shared between these two regions. Accordingly, QS values range from 0 (dissimilar) to 1 (similar). I chose the Sørensen similarity index because the regions are distinct areas and the data consists of the presence and absence of ascidian species.

In the laboratory, living ascidian specimens from the field surveys were photographed with a 7.1 megapixel Canon PowerShot SD750 digital camera. Each specimen was placed in a glass beaker and immersed in ambient seawater. A measuring tape was positioned under the glass beaker in each frame to provide scale to the nearest 1 mm. The specimen was left undisturbed for ≥ 1 min prior to photography. The maximum chord length of the individual for solitary ascidians or of the colony for colonial ascidians was determined using ImageJ (version 1.44).

2.3 Results

2.3.1 Checklist

A total of 56 ascidian species from 10 families and 29 genera was reported from eastern Canada (Table 2.3.2). Using the criteria described in Section 2.2.2, I

determined that 50 of these species are indigenous, five are non-indigenous, and one is cryptogenic. Fourteen species (25%) belong to the order Aplousobranchia, eight (14%) to the Phlebobranchia, and 34 (61%) to the Stolidobranchia. Forty species were geographically referenced to the Arctic, 25 to Labrador, 37 to insular Newfoundland, and 34 to the Atlantic coast of Nova Scotia (Table 2.3.2). In the Gulf of St. Lawrence, 34 species were referenced to Quebec, 17 to Prince Edward Island, five to New Brunswick, and 11 to Nova Scotia (Table 2.3.2). In the Bay of Fundy, 35 species were referenced to New Brunswick, and 32 to Nova Scotia (Table 2.3.2). Few species were reported from the Gulf coasts of Prince Edward Island, New Brunswick, and Nova Scotia. Of the 56 indigenous ascidian species, the distribution of 55 were reviewed and categorised by Haydar (2010). Based on the categories established by Haydar (2010), four species (7%) were restricted to the northeast coast of North America, 16 (29%) exhibited a continuous amphi-Atlantic distribution, 10 (18%) exhibited a disjunct amphi-Atlantic distribution, and 25 (46%) were limited to arctic and or subarctic waters (Table A-3 in Appendix A).

The ascidian fauna of eastern Canada (excluding the southern Gulf of St. Lawrence due to the lower number of sources) exhibited compositional differences along a north-south latitudinal gradient. Not surprisingly, the faunas of the Bay of Fundy coasts of New Brunswick and Nova Scotia were the most similar (QS = 0.96; Table 2.3.3). The northern faunas of the Arctic and Labrador were the least similar to the southern faunas of the Bay of Fundy coasts of New Brunswick and Nova Scotia (QS ranged from 0.53-0.58; Table 2.3.3). The faunas were relatively similar among

insular Newfoundland, the Atlantic coast of Nova Scotia, and the Gulf of St. Lawrence coast of Quebec (*QS* ranged from 0.76-0.82; Table 2.3.3), but these regions also have many shared species with northern and southern regions.

Table 2.3.2 Checklist of 56 extant ascidian species of eastern Canada. QC = Quebec; PEI = Prince Edward Island; NB = New Brunswick; NL = Newfoundland; NS = Nova Scotia. References are given in Table A-2 in Appendix A. The authority name and year, distribution, and status for each species are listed in Table A-3 in Appendix A.

	Region									
					Gulf of St. Lawrence				Bay of Fundy	
Species	Arctic	Labrador	Insular NL	Atlantic NS	QC	PEI	NB	NS	NB	NS
Aplidium glabrum	×	×	×	×	×				×	×
Aplidium mutabile	×									
Aplidium pallidum	×		×	×	×				×	×
Aplidium spitzbergense									×	×
Aplidium stellatum									×	×
Ascidia callosa	×	×	×	×	×				×	×
Ascidia obliqua	×	×	×	×	×		×		×	×
Ascidia prunum	×	×	×	×	×	×		×	×	×
Ascidia dijmphniana	×	×								
Ascidiella aspersa				×					×	
Bostrichobranchus pilularis		×	×	×	×	×	×		×	×
Boltenia echinata	×	×	×	×	×				×	×
Boltenia ovifera	×	×	×	×	×				×	×
Botrylloides aureum	×	×	×		×					
Botrylloides violaceus			×	×		×		×	×	
Botryllus schlosseri			×	×	×	×		×	×	×
Chelyosoma macleayanum	×	×		×	×				×	×
Ciona intestinalis	×		×	×	×	×		×	×	×
Cnemidocarpa finmarkiensis	×		×							

Table 2.3.2 Continued.

Species	Region									
	Arctic	Labrador	Insular NL	Atlantic NS	Gulf of St. Lawrence				Bay of Fundy	
					QC	PEI	NB	NS	NB	NS
Cnemidocarpa mollis	×			×	×				×	×
Cnemidocarpa mortenseni	×			×						
Cnemidocarpa rhizopus	×				×					
Corella borealis	×									
Dendrodoa aggregata	×	×	×	×	×					
Dendrodoa carnea			×		×	×			×	×
Dendrodoa grossularia	×	×	×	×	×		×		×	×
Dendrodoa pulchella	×	×	×	×	×					
Didemnum albidum	×	×	×	×	×	×		×	×	×
Didemnum candidum			×		×				×	×
Diplosoma listerianum				×	×					
Distaplia clavata	×		×	×	×				×	×
Eudistoma vitreum	×		×							
Eugyra glutinans		×	×		×					
Halocynthia pyriformis	×	×	×	×	×	×		×	×	×
Hartmeyeria arctica	×									
Kukenthalia borealis	×									
Leptoclinides faeroensis	×		×	×						
Lissoclinum aureum	×		×						×	×
Microcosmus glacialis	×		×	×						
Molgula arenata									×	×

Table 2.3.2 Continued.

Species	Region									
	Arctic	Labrador	Insular NL	Atlantic NS	Gulf of St. Lawrence				Bay of Fundy	
					QC	PEI	NB	NS	NB	NS
Molgula citrina	×	×	×	×	×	×	×	×	×	×
Molgula complanata	×	×	×		×	×			×	×
Molgula griffithsii	×	×	×	×	×	×			×	×
Molgula manhattensis					×	×			×	×
Molgula provisionalis									×	×
Molgula retortiformis	×	×	×	×	×				×	×
Molgula siphonalis	×		×		×				×	×
Pelonaia corrugata	×	×	×	×	×	×		×		
Polycarpa fibrosa	×		×	×	×	×		×	×	×
Rhizomolgula globularis	×	×								
Styela canopus				×					×	×
Styela clava				×		×				
Styela coriacea	×	×	×	×	×	×		×	×	
Styela rustica	×	×	×	×	×	×	×	×		
Synoicum pulmonaria	×	×	×							
Tridemnum tenerum			×	×					×	×
Number of species	40	25	37	34	34	17	5	11	35	32

Table 2.3.3 Quotient of similarity (*QS*) matrix. QC = Quebec; PEI = Prince Edward Island; NB = New Brunswick; NL = Newfoundland; NS = Nova Scotia.

		Arctic	Labrador	Insular NL	Atlantic NS	Gulf of St. Lawrence				Bay of Fundy	
						QC	PEI	NB	NS	NB	NS
Arctic		1.00	0.71	0.78	0.70	0.73	0.39	0.18	0.35	0.59	0.58
Labrador			1.00	0.71	0.64	0.75	0.48	0.33	0.39	0.53	0.53
Insular NL				1.00	0.76	0.82	0.56	0.24	0.46	0.72	0.70
Atlantic NS					1.00	0.76	0.55	0.26	0.49	0.72	0.67
Gulf of St. Lawrence	QC					1.00	0.59	0.26	0.44	0.75	0.76
	PEI						1.00	0.27	0.79	0.54	0.49
	NB							1.00	0.25	0.20	0.22
	NS								1.00	0.39	0.33
Bay of Fundy	NB									1.00	0.96
	NS										1.00

2.3.2 Specimens from insular Newfoundland

I collected and identified eight indigenous ascidian species and two NIA species from Newfoundland (Table A-1 in Appendix A). Measurements were made of a subsample of specimens. In September, *Dendrodoa carnea* (mean maximum chord length ± one SD = 0.5 ± 0.2 cm, n = 3) was collected in Salmonier Arm at a depth of 6-11 m. In February, *Aplidium glabrum* (2.2 ± 1.4 cm, n = 4), *Ascidia callosa* (4.9 ± 2.9 cm, n = 8), *Boltenia echinata* (2.1 ± 0.5 cm, n = 3), *Didemnum albidum* (2.0 cm, n = 1), and *Halocynthia pyriformis* (5.1 ± 2.4 cm, n = 4) were collected in Logy Bay at a depth of 11 m. I collected overwintering colonies of *Botryllus schlosseri* (n = 4, depth of 1-2 m) in February in Arnold's Cove and *Botrylloides violaceus* (n = 9, depth 2-8 m) in March in Belleoram. Figure 2.3.1 shows photographs of live ascidian species collected in the present study.

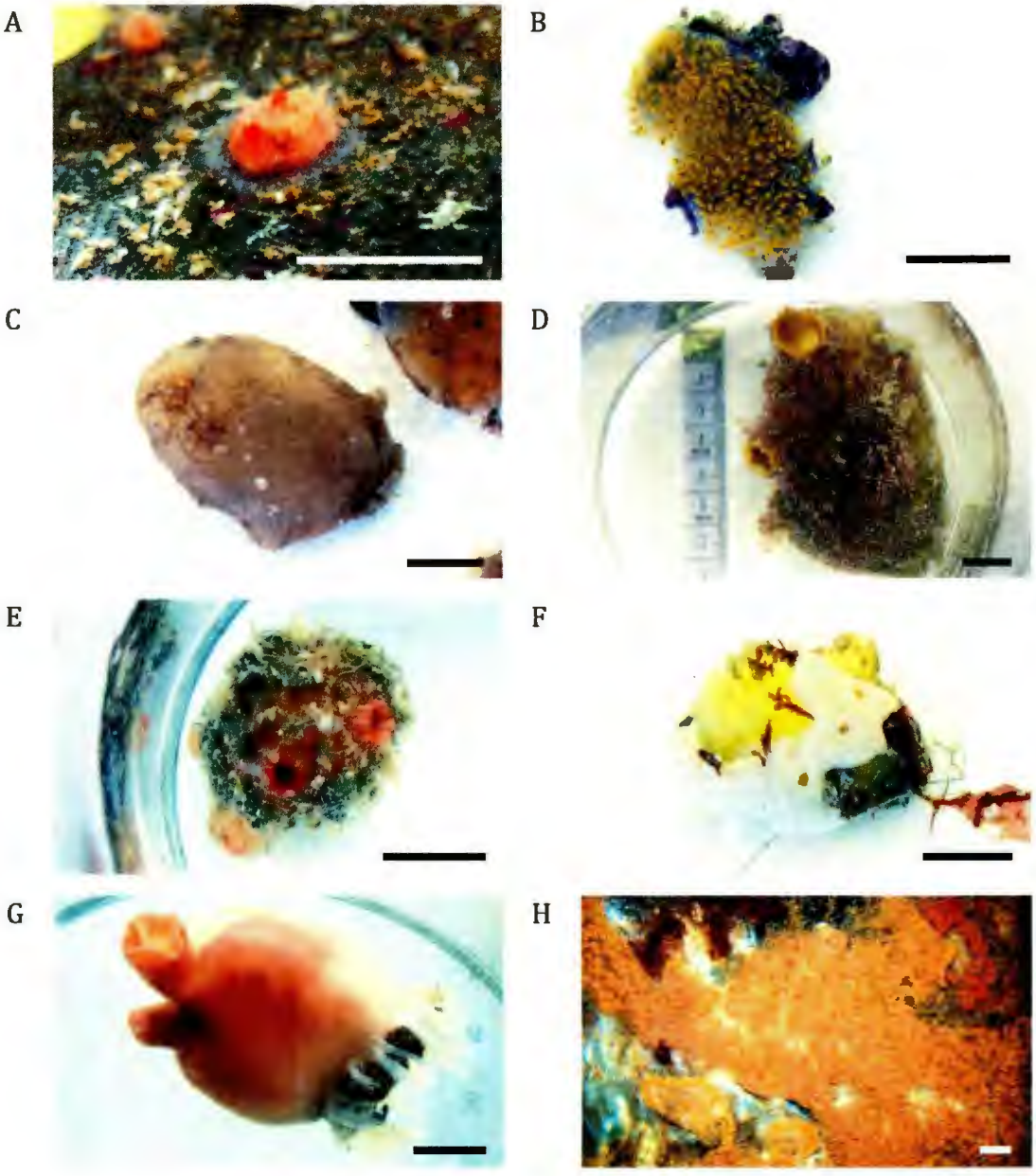

Figure 2.3.1 Photographs of living specimens of ascidian species from Newfoundland. (A) *Dendrodoa carnea*; (B) *Aplidium glabrum*; (C) and (D) *Ascidia callosa*; (E) *Boltenia echinata*; (F) *Didemnum albidum* encrusting *Molgula* sp.; (G) *Halocynthia pyriformis*; (H) several colonies of *Botrylloides violaceus* on wharf piling. Scale bars = 1 cm.

2.4 Discussion

2.4.1 Invasion history of *Botryllus schlosseri* in North America: A case study

Historically, the earliest records of *Botryllus schlosseri* in North America are from Massachusetts—near Boston in ca. 1838 and 1870 (Couthouy 1838, Gould 1870), and south of Cape Cod in 1904 and 1905 (Sumner et al. 1913). The first report of *B. schlosseri* on the west coast of North America was from San Francisco, California, in 1944-1947 (United States Navy 1951, Cohen and Carlton 1995). In western Canada, this species was first observed in harbours on southern Vancouver Islands, British Columbia, ca. 1998 (Lambert and Lambert 1998). In eastern Canada, *B. schlosseri* was first reported at a United States naval base in Argentia, south coast of Newfoundland, in 1944-1947 (United States Navy 1951). Subsequently, *B. schlosseri* was reported on the Bay of Fundy coast of New Brunswick (Linkletter et al. 1977), the Atlantic, Bay of Fundy, and Bras d'Or Lake coasts of Nova Scotia (Gosner 1971, Carver et al. 2006a), and on the west coast of Newfoundland (Bonne Bay and St. Paul's Inlet; Hooper 1975). It was more recently observed in Prince Edward Island (Carver et al. 2006a, Locke et al. 2007), on the Bay of Fundy coast of New Brunswick (LeGresley et al. 2008, Martin et al. 2011), the Atlantic, Bay of Fundy, Bras d'Or Lake, and southern Gulf of St. Lawrence coasts of Nova Scotia (Clancey and Hinton 2003), and the east and south coasts of Newfoundland (present study, Callahan et al. 2010). Today, this NIA species has a cosmopolitan distribution mainly in temperate waters on all continents except Antarctica, although some populations persist in the boreal waters of Alaska (Lambert 2001, Stoner et al.

2002), northern Japan (Rinkevich and Saito 1992), and Scandinavia (Ben-Shlomo et al. 2010).

2.4.2 Distribution of non-indigenous ascidian species in eastern Canada

In eastern Canada, NIA species have been reported from the Bay of Fundy, the Gulf of St. Lawrence, and insular Newfoundland, but not from Labrador and the Arctic. The melting of the arctic sea ice linked to global climate change may cause the Northwest Passage to become a viable commercial shipping route during the short ice-free periods in the summer, which may inadvertently introduce prospective NIA species to the Arctic on fouled ship hulls (Lambert et al. 2010). Furthermore, ships navigating over long distances through icy waters are often equipped with heated sea chests. Hypothetically, this heating may increase survival of the fouling NIA species (Lambert et al. 2010).

Presently, *Botryllus schlosseri*, *Botrylloides violaceus*, and *Ciona intestinalis* are the only NIA species known to be established in Newfoundland (Figure A-1 in Appendix A). On the south coast, Callahan et al. (2010) reported *B. schlosseri* attached to the hull of a small boat moored in Argentia on December 7, 2006, after nearly 60 years had elapsed since the first report by the United States Navy (1951). On the east coast, it was reported in Foxtrap in late October, 2011, by Dave Poitras, and subsequently confirmed during a field survey on December 5, 2011 (Table A-1 in Appendix A). For the first time in Newfoundland, *B. violaceus* was reported on wharf structures in Belleoram in September, 2007 (Callahan et al. 2010) and *C. intestinalis* in Burin, Little Bay, and Marystown in 2012 (Sargent et al., in

preparation). At present, extensive populations of *B. schlosseri* have been detected in at least 15 coastal sites—namely Argentia, Arnold's Cove, Baine Harbour, Fox Harbour, Foxtrap, Garden Cove, Harbour Breton, Hermitage, the vicinity of Kingwell, Lamaline, Little Bay, Long Harbour-Mount Arlington Heights, North Harbour, the vicinity of North Tilt Island, and Northeast Arm (Figure A-1 in Appendix A). *B. schlosseri* was not found during a recent search in Bonne Bay where it was originally reported by Hooper (1975). In Newfoundland, *B. violaceus* has only been recorded at Belleoram, where it still occurs. For the temperate species *B. schlosseri* and *B. violaceus*, this represents an expansion of their global range into subarctic waters. Moreover, the multi-year, island-wide surveys suggest both are at the northern boundary of their ranges, particularly since they are absent from the northeast coast of insular Newfoundland (Fisheries and Oceans Canada 2011b, 2011c).

2.4.3 Diversity of the ascidian fauna

Accurate checklists of indigenous species are important for protecting biodiversity, identifying knowledge gaps, and monitoring the status of non-indigenous species. A checklist of indigenous and non-indigenous ascidian species provides essential information needed for managing biological invaders, which includes (a) correctly identifying established and prospective non-indigenous species, (b) determining future changes to local species richness, and (c) determining the distribution and extent of biological invasions. Descriptions of ascidian species indigenous to Newfoundland and Labrador were difficult to access in the literature. In particular, distributional information compiled by Van Name

(1945), in one of the most comprehensive and authoritative monographs of the ascidian fauna of North and South America, was organised by species rather than by region.

Neighbouring regions tend to be similar in their ascidian faunal composition, except for regions along the southern Gulf of St. Lawrence. However, the notable regional dissimilarity among the Gulf coasts of Prince Edward Island, New Brunswick, and Nova Scotia, coupled with the few species that were reported and the low number of sources from those regions (Table A-2 in Appendix A), suggests that the ascidian fauna of the southern Gulf of St. Lawrence is poorly understood. Although the northern and southern region have some species in common (e.g., the widely distributed *Didemnum albidum*, *Halocynthia pyriformis*, and *Molgula citrina*), there is a general north-south latitudinal gradient in the composition of the ascidian fauna. Notably, the northern faunas of the Arctic and Labrador are markedly dissimilar to the southern faunas of the Bay of Fundy coasts of New Brunswick and Nova Scotia (Table 2.3.3).

In conclusion, the checklist provides a faunal baseline to monitor potential ascidian invaders. The reports of established populations of NIA species are of immediate concern to industry, management, and policymakers in eastern Canada. With the advent of global climate change, there are concerns about the potential biological invasion of NIA species such as *Didemnum vexillum* into eastern Canada, and about potential range expansions of *Ascidiella aspersa*, *Diplosoma listerianum*, and *Styela clava* to Newfoundland.

CHAPTER 3 – PATTERNS OF RECRUITMENT IN A POPULATION OF *BOTRYLLUS SCHLOSSERI*

3.1 Introduction

Ongoing global environmental change may increase the rate of introductions of aquatic invasive species (AIS; Stachowicz et al. 2002b). In particular, arctic and subarctic waters are becoming vulnerable to biological invasions due to favourable climatic changes that create favourable conditions (e.g., rise in seawater temperatures; Stachowicz et al. 2002b), and to higher volumes of maritime traffic as the ice becomes thinner (Lambert et al. 2010). AIS pose real and potential ecological and economic threats, which have stimulated industry, management, and policymakers to monitor for new biological invaders and to minimise damage caused by current invaders. Additionally, many anthropogenic vectors that facilitate the introduction of AIS to novel systems have been identified, i.e., ballast water exchange, ship hull fouling, and transport of fouled commercial seafood products (Lambert and Lambert 1998, Locke et al. 2007, Carman et al. 2010, Paetzold and Davidson 2010, Clarke Murray et al. 2011).

The impact of non-indigenous ascidian (NIA) species has become problematic for the sustainability of bivalve aquaculture. Presently, five NIA species (*Ascidiella aspersa*, *Botrylloides violaceus*, *Botryllus schlosseri*, *Diplosoma listerianum*, and *Styela clava*) and one cryptogenic ascidian (*Ciona intestinalis*) are known to be established in eastern Canada (see Chapter 2). Only *B. schlosseri*, *B. violaceus*, and *C. intestinalis*

have been reported in the subarctic waters of Newfoundland (United States Navy 1951, Hooper 1975, Callahan et al. 2010, Sargent et al., in preparation). Shortly after the recent reports of NIA species on the south coast of Newfoundland in 2006-2007 (Callahan et al. 2010), federal authorities attempted to eradicate *B. violaceus* using limited existing ecological data on the species (Fisheries and Oceans Canada 2012). In Newfoundland, the distribution of NIA species is currently known only from coastal harbours and, fortunately, these species are not yet a nuisance for bivalve aquaculture.

Botryllus schlosseri is more widely distributed in Newfoundland, which suggests that this temperate NIA species may have made genetic, physiological, and or phenological changes to become established in the subarctic. In particular, field and laboratory observations indicate that the lowest seawater temperature for sexual reproduction in *B. schlosseri* is 10-13°C (presence of eggs; Brunetti 1974, Brunetti et al. 1980, 1984, Westerman 2007, Westerman et al. 2009) and for recruitment is 10-15°C (presence of juvenile colonies; Brunetti 1974, Yund and Stires 2002, Westerman et al. 2009). However, these observations, including the preference of this species to colonise aluminum substrates rather than wood and PVC (Tyrrell and Byers 2007), were made on colonies from temperate waters. Therefore, the main objective of the present study was to determine the inter-annual and seasonal patterns of recruitment in a population of *B. schlosseri,* with the prediction that these processes are limited by subarctic environmental conditions. In addition, I aimed to understand spatial patterns of recruitment at a scale of tens of

metres and recruitment patterns on different substrate types, which provide initial ecological information of this NIA species in Newfoundland that can be useful to the region's industry, managers, and policymakers. Therefore, I tested the hypotheses that (1) the seasonal onset of recruitment is constrained by the temperature threshold of 13°C, which is the cited temperature threshold for sexual reproduction based on field observations in the literature; (2) vertical and geographical patterns of recruitment within a harbour are limited by larval dispersal; and (3) recruitment is greater on aluminum or on wood plates than on PVC.

3.2 Materials and methods

3.2.1 Study area and study population

The study was carried out in Arnold's Cove, NL, Canada, which is situated at the northern end of Placentia Bay at 47°45' N, 54°00' W (Figure A-1 in Appendix A). The harbour is naturally sheltered such that ice formation during the winter is atypical (Catto et al. 1999). The harbour is characterised by intra-provincial, inter-provincial, and international vessel traffic by local industries and recreational users. Tides in Placentia Bay are semi-diurnal with maximum spring tides of 2.1 m and neap tides up to 1.5 m. A population of *Botryllus schlosseri* that is fouling boat hulls, buoys, floating docks, and fixed wharf structures most likely provided the source of larvae. Three sites were selected under the ramp to each of three floating docks of the government wharf (Fig. 3.2.1). The sites were shaded from direct sunlight and spaced approximately 15-20 m apart. Bottom depth was ca. 6 m. All three floating docks were attached to a permanent wharf.

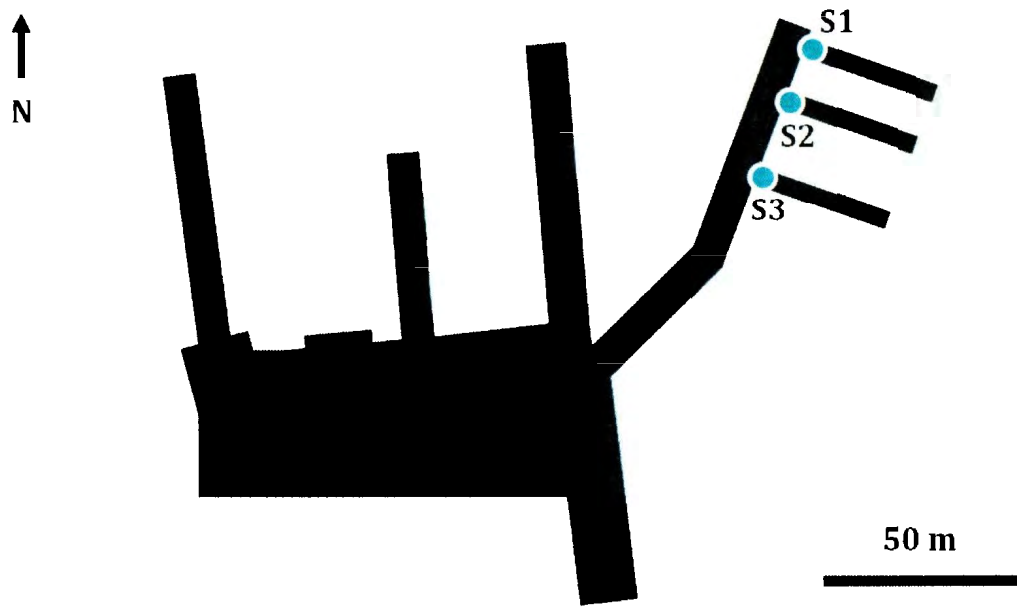

Figure 3.2.1 Schematic map of the government wharf in Arnold's Cove, Placentia Bay, NL, Canada. Cross hatched area indicates land. Solid black area indicates permanent wharf structure and floating docks. S1 = site #1; S2 = site #2; S3 = site #3. Scale bar = 50 m.

3.2.2 Sampling design

The study spanned 21 months that consisted of two recruitment seasons, i.e., one season per annum. For the first season, artificial plates of different substrate types (aluminum, PVC, and wood; see Section 3.2.3 on the preparation of artificial plates) were deployed from March 17 to December 8, 2010. The deployment of aluminum and wood plates was terminated after the first season. For the second season, only PVC plates were deployed from December 8, 2010, to November 15, 2011, to determine inter-annual variability in recruitment. Concurrently, supplementary PVC plates (n = 15) were deployed adjacent to site #1 on July 20, 2010, prior to the seasonal onset of recruitment, to monitor for the first release of recruits.

For the 2010 sampling season, there were 11 contiguous sampling dates when new artificial plates were deployed and existing plates were retrieved (Table A-4 in Appendix A). The sampling effort intensified in September and October to improve temporal resolution during the high recruitment period. A sampling array of 18 horizontal artificial plates (10 × 10 cm each) was moored at each of the three sites for a deployment period of four weeks (mean of 28.0 d per deployment). Sites were spaced ca. 15-20 m apart. An array consisted of equal numbers of aluminum, PVC, and wooden plates that were randomly distributed at 1.0, 2.5, and 4.0 m depths below the water surface. Mooring lines were spaced 20 cm apart within an array.

The 2011 sampling season was intended to continue monitoring of recruitment and address the question of inter-annual variability in recruitment.

There were seven contiguous sampling dates when artificial plates were deployed and existing plates were retrieved (Table A-4 in Appendix A). An array of six PVC plates was moored at each of the three sites for a deployment period of 4-11 weeks (mean 48.6 d per deployment). Sampling depths were the same as in 2010 (see above).

Seawater temperature, salinity, *in situ* fluorescence, and turbidity were monitored with a YSI Multiparameter Sonde (model 6600 V2) throughout the 2010 and 2011 seasons. The factory calibration equation was used to convert *in situ* fluorescence to total chlorophyll *a*. The sonde was moored 1 m below the water surface at site #2 from March 18, 2010, to November 15, 2011. Unattended readings were taken by the sonde every 6 h and downloaded monthly using EcoWatch (version 3.18) software. An antifouling kit was installed on the sonde. In addition, a YSI Handheld Multiparameter Probe was used ca. every two weeks to determine the vertical profiles of temperature and salinity at all three sites. The vertical profiles were recorded at every 1 m interval from the water surface to 5 m depth (Figure A-2 in Appendix A).

3.2.3 Preparation of artificial plates

Plates constructed of heavy gauge aluminum foil were mounted with push pins to a wooden plate to supply backing support. PVC plates (0.6 cm thick) were dark grey in colour. Wooden plates were fashioned from untreated pine. A hole was drilled through the centre of each plate for attachment to mooring lines. The top surface of each plate was uniquely labelled with a Dremel Tool or a pencil. Before

deployment, all plates were immersed for two weeks in a large tank that contained 30 μm filtered ambient seawater from Logy Bay, NL, Canada. This process resulted in a biofilm of marine micro-organisms on the plates, which may allow for the settlement and metamorphosis of ascidian larvae (Keough and Raimondi 1996, Wieczorek and Todd 1997, Carver et al. 2006b, Howes et al. 2007, Ruis et al. 2010).

For the 2010 season, each sampling array consisted of six polypropylene mooring lines attached to floating docks. A brick tied to the end of each mooring line ensured that the lines remained straight (Figure 3.2.2). Throughout the study, no mooring lines were entangled despite being spaced 20 cm apart. Plates were attached at three different depths on the line and secured with cable ties. Due to randomisation, the arrangement of artificial plates comprised of different substrate types was different for every array that was assembled and deployed. For each array, the random position of substrate types was determined by rolling a virtual six-faced die, which is available at http://www.random.org (Haahr 2012). Each line of an array was numbered one to six. For each depth within each array, a set of two aluminum, two PVC, and two wooden plates was sequentially assigned to the line number having the same value as the die roll outcome. The die was rolled again if the position was already assigned. This randomisation process resulted in an equal number of duplicated substrate types per depth. For the 2011 season, an array at each site consisted of two mooring lines. The arrays were assembled as in 2010 (see above) but contained only PVC plates because the utility of PVC plates was sufficient to elucidate inter-annual variability in recruitment.

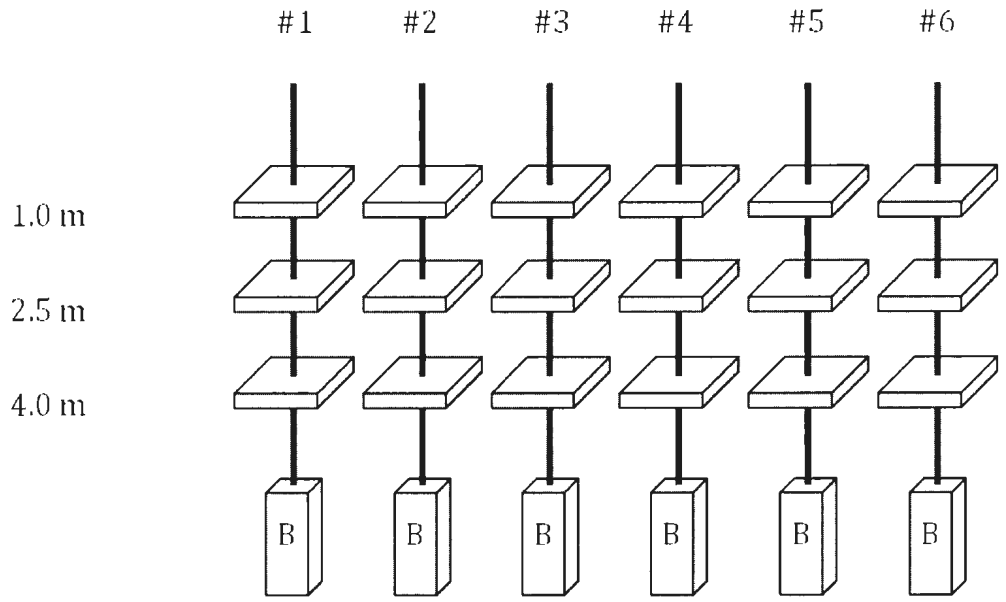

Figure 3.2.2 A sampling array of 18 artificial plates used during the 2010 sampling season. Plates were secured 1.0, 2.5, and 4.0 m below the water surface. Mooring lines were numbered one to six. B = bricks. One array was deployed at each of the three sites shown in Figure 3.2.1 such that there was a total of 54 plates per monthly deployment.

3.2.4 Deployment and retrieval of artificial plates

Plates were placed in 30 μm filtered ambient seawater in 40 L insulated containers during the 1.5 h transport from the laboratory to the study area. SCUBA divers moored three arrays per deployment, i.e., one array at each of the three sites. The six mooring lines per array were attached with rope and steel snap hooks to submerged chains. These chains are part of the floating dock structures. Thus, the depths of the plates were registered from the water surface and followed tidal cycles.

At the end of the deployment period, SCUBA divers retrieved the mooring lines with the plates. The mooring lines were disassembled in air, and individual plates were then placed with the bottom surface upwards in 0.7 L plastic containers filled with unfiltered ambient seawater. The containers were transported to the laboratory in 40 L insulated coolers. Any water originating from the field was chlorinated at a minimum of 5 ppm available chlorine for at least 12 h before disposal. All field equipment was air dried for at least 1 d before washing in freshwater. Plates were air dried and stored in boxes after image analysis.

3.2.5 Image analysis

Newly recruited *Botryllus schlosseri* (from oozooids to juvenile colonies) were observed on the bottom surfaces of artificial plates. The smallest specimens were oozooids and blastozooids that were ca. 0.6-1.2 mm and 1.2-1.7 mm in length (Appendix C). Settlement (the settling larvae) and metamorphosis, which would

require the use of microscopy, were not assessed in this study. Upon retrieval, the bottom surfaces of plates were photographed in air from above with a 10 megapixel Canon PowerShot G11 digital camera. Care was taken to avoid glare from direct sunlight. In the laboratory, the bottom surfaces of all plates were visually inspected and photographed with the same digital camera as above, mounted on a tripod. A metal ruler was placed in each frame to provide scale to the nearest 1 mm. From the images taken in the field and in the laboratory, all newly recruited *B. schlosseri* were identified and counted on a 61 cm widescreen liquid crystal display (LCD) computer monitor. The final count per plate included all recruits. Although the size of all the plates should be the same, the area of each plate was measured using the ImageJ (version 1.44).

3.2.6 Statistical analysis

Seawater temperature, salinity, chlorophyll *a* concentration, and turbidity data were smoothed by calculating the daily mean values after negative chlorophyll *a* concentration and turbidity values were converted to zeros. Unreliable chlorophyll *a* concentration data, which was caused by algal growth on the *in situ* florescence probe of the sonde, were removed from July 8 to August 11, 2010. Environmental data was missing from December 9 to 15, 2010, due to maintenance of the sonde. Reproductive degree days (DD) can take into account variation in seawater temperature over the course of the recruitment season. For each day of a given year, the reproductive DD was calculated by summing the degrees Celsius above the threshold temperature of 13°C starting on the first day of that year. The threshold

temperature of 13°C was selected because it was observed as the reproductive threshold temperature in the study of Westerman (2007). Mean values in this study are reported with ± one SD. In this study, analyses of variance (ANOVA) were generalised linear models (GLM; type III sums of squares method). A discussion of GLM can be found in Appendix B. All statistical analyses were done with SPSS 19.

Pearson product-moment correlation coefficients were calculated for $ln(x+1)$ transformed daily mean values for environmental variables. An ANOVA (normal GLM with the canonical identity link function) of the transformed environmental data tested for variability among years and months nested within year.

Recruitment data for the 2010 and 2011 sampling seasons were transformed to natural logarithms and analysed with a two-component conditional model. Analysis #1 consisted of data from the 2010 season, which were tested for variability in recruitment rates among sampling dates (time), sites, depths, and substrate types. Analysis #2 consisted of only PVC data from the 2010 and 2011 seasons, which were tested for variability in recruitment rates among years, months (nested within year), sites, and depths. Analyses #1 and #2 were balanced factorial designs with no missing data. The first component of the conditional model considered presence and absence data and the second component considered zero-truncated data. The ANOVAs for the first component and the second component were conducted with a binomial logit GLM and by a log-normal GLM (with the log link function), respectively (see Appendix B for the protocol for model selection).

ANOVAs with significant interaction effects were partitioned to interpret main effects.

3.2.7 Developmental analysis

Retardation in the development rate of the oozooid is a possible adaptation to the subarctic marine environment, which could substantially change the interpretation of recruitment patterns. Laboratory experiments were conducted to determine the sizes of larvae, oozooids, and first blastozooids of *Botryllus schlosseri*, from larval settlement through metamorphosis to the end of the first blastogenic cycle, as a function of time at 20°C. In summary, the oozooid of subarctic origin lived for 9-10 d at 20°C before undergoing the first blastogenic cycle, which is similar to the oozooid of temperate origin at the same temperature. Colonies with two or more blastozooids were first observed on days 16-17 after initial settlement. These findings suggest that there are no marked differences in the development of the oozooid of subarctic and that of temperate origin. A detailed description of this experiment can be found in Appendix C.

3.3 Results

3.3.1 The physical environment

Monthly mean seawater temperature ranged from 1.4 ± 0.4°C (March) to 16.7 ± 1.2°C (August) in 2010 and from 0.8 ± 0.7°C (February) to 15.4 ± 1.6°C (September) in 2011 (Figure 3.3.1). Late August to early September was the warmest period, with daily mean seawater temperatures ≥ 16°C. Late December to

mid-May was the coldest period, with daily mean seawater temperatures ≤ 5°C. The coldest month was February 2011, when temperatures occasionally reached below 0°C. Monthly mean salinity ranged from 30.0 ± 1.5 psu (August) to 32.0 ± 0.2 psu (April) in 2010, and from 30.6 ± 0.9 psu (July) to 31.7 ± 0.2 psu (May) in 2011 (Figure 3.3.1). Monthly mean chlorophyll *a* concentration ranged from 0.4 ± 0.3 μg L^{-1} (March and December) to 2.5 ± 4.4 μg L^{-1} (September) in 2010, and 0.4 ± 0.3 μg L^{-1} (February) to 2.8 ± 5.3 μg L^{-1} (September) in 2011 (Figure 3.3.1). The spring bloom of phytoplankton occurred in April with a mean of 1.1 ± 1.0 μg L^{-1} in 2010 and 2.8 ± 2.0 μg L^{-1} in 2011. The autumn bloom of phytoplankton occurred in September. Monthly mean turbidity ranged from 0 nephelometric turbidity units (NTU; March and April) to 11.5 ± 14.0 NTU (June) in 2010 and 0.4 ± 0.5 NTU (January) to 19.2 ± 20.3 NTU (July) in 2011 (Figure 3.3.1). Increased human activities during the lobster fishing season, including boat traffic and dockside baitfish discards, may have contributed to higher levels of turbidity in the months of May and June. In July 2011, turbidity levels peaked around the same time that a nearby pier was under reconstruction. The source(s) of elevated turbidity (mean 3.1 ± 4.9 NTU) in October and November 2011 is unknown.

Hurricane Igor passed almost directly over the study area on September 21, 2010. The seawater temperature rapidly declined to 7.7°C one week prior to the hurricane. Salinity gradually decreased to a low of 25.1 psu several days post-hurricane. Turbidity was 0 NTU before and after the hurricane. However, the daily

mean turbidity attained a maximum of 8.0 ± 11.7 NTU on the day the hurricane passed over the harbour.

The harbour exhibited significant inter-annual and seasonal variability in seawater temperature, salinity, chlorophyll *a* concentration, and turbidity, with the seasonal scale predominant (Table 3.3.1). Temperature and salinity values from the vertical profiles followed the seasonal cycle of data collected with the sonde (Figure A-2 in Appendix A). According to the data from the vertical profiles, variability in temperature and salinity was significant among months and depths but not among sites (Table A-5 in Appendix A). The correlation between each pair of the four environmental variables (temperature, salinity, chlorophyll *a* concentration, and turbidity), with the exception of chlorophyll *a* and turbidity, was statistically significant during the entire sampling period, from March 18, 2010, to November 15, 2011 (Table 3.3.2).

Table 3.3.1 Nested analysis of variance (ANOVA; generalised linear model) of *ln(x+1)* transformed daily mean seawater temperature, salinity, chlorophyll *a* concentration, and turbidity (n = 601 per environmental variable) that were recorded at 1 m depth from March 2010 to November 2011. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

	Temperature		Salinity		Chlorophyll *a*		Turbidity	
Source of variation	Wald χ^2	df	Wald χ^2	df	Wald χ^2	df	Wald χ^2	df
Intercept	$4.4 \times 10^{4***}$	1	$2.1 \times 10^{7***}$	1	$2.3 \times 10^{3***}$	1	$7.0 \times 10^{2***}$	1
Year	$1.4 \times 10^{2***}$	1	$9.7 \times 10^{0*}$	1	$8.7 \times 10^{1***}$	1	$1.8 \times 10^{1***}$	1
Month (Year)	$5.9 \times 10^{3***}$	19	$4.6 \times 10^{2***}$	19	$5.2 \times 10^{2***}$	19	$6.3 \times 10^{2***}$	19

Table 3.3.2 Pearson product-moment correlation coefficient matrix of *ln(x+1)* transformed daily mean seawater temperature, salinity, chlorophyll *a* concentration, and turbidity (n = 601 per environmental variable) recorded at 1 m depth from March 2010 to November 2011. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

	Temperature	Salinity	Chlorophyll *a*	Turbidity
Temperature	1.00	-0.48***	0.29***	0.25***
Salinity		1.00	-0.26***	-0.20***
Chlorophyll *a*			1.00	NS
Turbidity				1.00

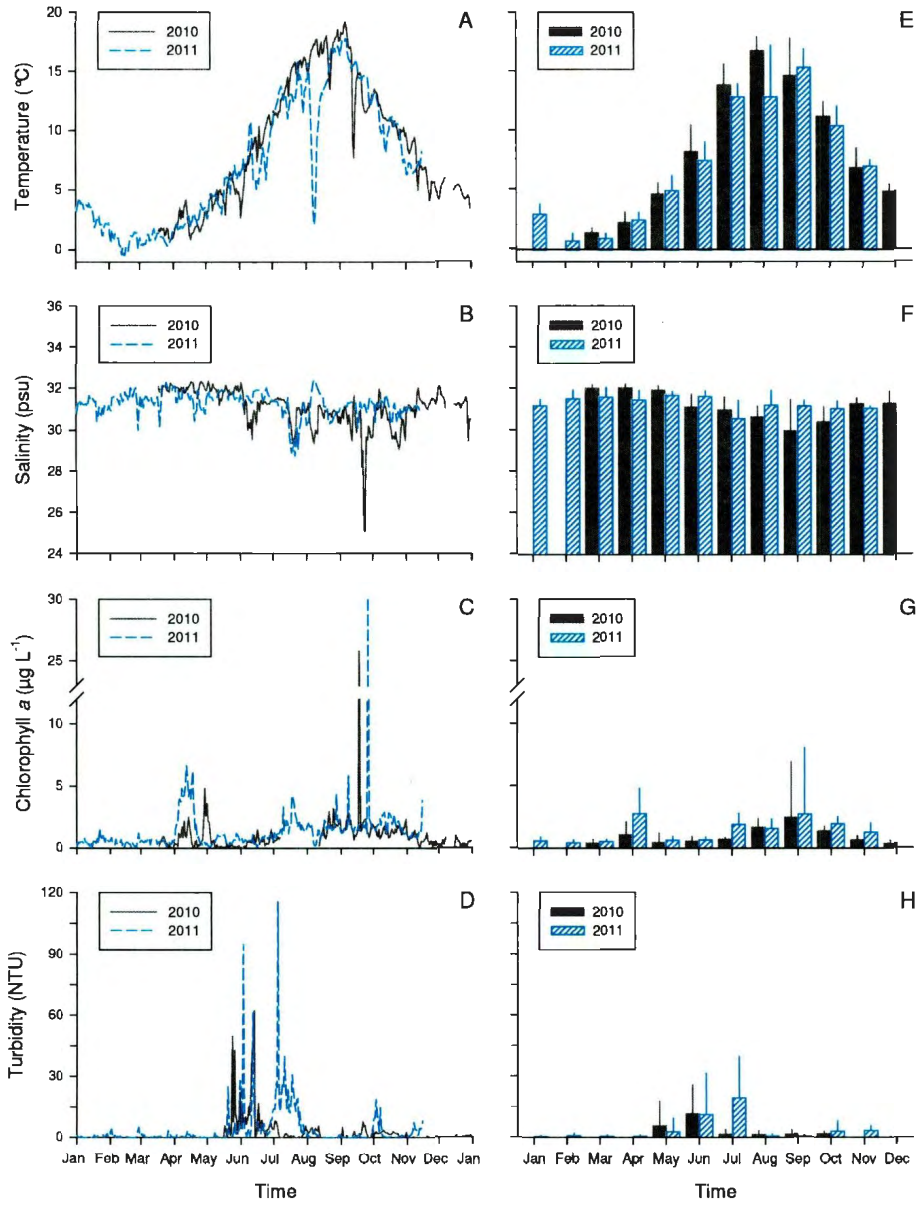

Figure 3.3.1 Environmental data recorded by the sonde at 1 m below the water surface in Arnold's Cove, Placentia Bay, NL, Canada. (A) Daily mean seawater temperature; (B) salinity; (C) chlorophyll *a* concentration; (D) turbidity. Monthly mean (E) seawater temperature; (F) salinity; (G) chlorophyll *a* concentration, and (H) turbidity. Bars represent one SD and $n = 4$ observations per day.

3.3.2 Timing of recruitment

Recruitment occurred from early August to mid-October. A single conspicuous period of recruitment was detected in each year (Figure 3.3.2). The seasonal onset of recruitment was observed on August 4, 2010, on a single supplementary PVC plate, and on August 1, 2011. The daily mean seawater temperature on the day of initial observation of recruitment was 15.6 ± 0.4°C (DD = 48°C) in 2010 and 14.4 ± 0.8 (DD = 12°C) in 2011, which is above the 13°C threshold temperature (Table 3.3.3). The seasonal peak recruitment periods were observed in September in 2010 and 2011 (Figure 3.3.2), coincident with peak seasonal temperatures and chlorophyll *a* concentrations (Figure 3.3.1). The daily mean temperatures during the peak recruitment period were 17.2 ± 2.7°C (DD = 164-213°C) in 2010, and 15.8 ± 1.3°C (DD = 55-131°C) in 2011. The daily mean chlorophyll *a* concentrations during the peak recruitment period were 1.8 ± 0.8 μg L^{-1} in 2010 and 2.8 ± 8.1 μg L^{-1} in 2011. I was able to observe the seasonal end of recruitment, which occurred from October 1 to 11, 2010. The daily mean temperature during the period of final recruitment was 12.5 ± 0.8°C (DD = 222-223°C) in 2010. The same information was not available for the 2011 season because plates were deployed and retrieved after ca. six weeks, which was not suitable for the observation of the seasonal end of recruitment.

The results of the laboratory experiments conducted to determine larval, oozooid, and first blastozooid size as a function of time suggests that recruits and newly recruited colonies should be observable on artificial plates within the

deployment period of at least four weeks (see Appendix C). Also, these laboratory experiments showed that larvae of subarctic origin settled on Petri dishes between 1-3 h after release and functional oozooids were observed as early as day 3 post-settlement.

The onset of fertilisation, back-calculated from the observed onset of recruitment by applying the model developed by Westerman et al. (2009), likely occurred on or about July 21, 2010, and July 16, 2011 (Table 3.3.3). These back-estimates are very close to the observation of embryos in colonies of *Botryllus schlosseri* in Arnold's Cove on July 20, 2010, and July 26, 2011, by Lowen et al. (2012). The mean seawater temperature associated with the seasonal onset of fertilisation, calculated for the seven days prior to and including the estimated date of fertilisation, was 14.7 ± 1.1°C (DD = 14°C) in 2010 and 12.4 ± 1.0°C (DD = 2°C) in 2011.

Table 3.3.3 Seasonal timing of recruitment of *Botryllus schlosseri*. Timing of initial fertilisation was estimated from the date and temperature of initial recruitment using the model of Westerman et al. (2009). Reproductive degree days (DD) were calculated from a threshold temperature of 13°C starting on the first day of the year. Daily mean seawater temperature (T) and chlorophyll *a* concentration are ± one SD. Mean seawater temperature and chlorophyll *a* concentration were calculated for the seven days prior to and including the estimated date of initial fertilisation.

	2010				2011			
Event	Date	DD (°C)	T (°C)	Chlorophyll *a* ($\mu g\ L^{-1}$)	Date	DD (°C)	T (°C)	Chlorophyll *a* ($\mu g\ L^{-1}$)
Observed initial recruitment	Aug. 4	48	15.6 ± 0.4	No data	Aug. 1	12	14.4 ± 0.8	1.8 ± 0.4
Observed peak recruitment	Sep. 2-13	164-213	17.2 ± 2.7	1.8 ± 0.8	Aug. 30-Sep. 27	55-131	15.8 ± 1.3	2.8 ± 8.1
Observed final recruitment	Oct. 1-11	222-223	12.5 ± 0.8	1.4 ± 0.5				
Initial day when T ≥ 13°C	Jul. 12	0	13.1 ± 0.2	No data	Jul. 7	0	13.3 ± 0.1	1.3 ± 0.7
Final day when T ≥ 13°C	Oct. 5	223	13.3 ± 0.2	1.0 ± 0.5	Oct. 4	132	13.2 ± 0.1	1.8 ± 0.7
Estimated initial fertilisation	Jul. 21	14	14.7 ± 1.1	No data	Jul. 16	2	12.4 ± 1.0	2.0 ± 1.1

3.3.3 Recruitment rate

The population of *Botryllus schlosseri* exhibited variability in the daily mean rate of recruitment seasonally and among depths but not among sites in both 2010 (three substrate types), and 2011 (PVC only). The mean recruitment rates are reported in Table A-6 (Appendix A). Maximum recruitment rate was observed in September of both years but was greater in 2011 than in 2010 on PVC (Figure 3.3.2 and 3.3.2). Mean recruitment rates were greater at 1.0 and 2.5 m than 4.0 m depth, and greater on PVC than on aluminum and wood substrates (Figure 3.3.3). Hence, the greatest recorded rates during peak recruitment in 2010 and 2011 were 29.3 ± 25.3 and 43.5 ± 25.6 $m^{-2}d^{-1}$, respectively. The maximum recorded rate on a single plate was 74.4 $m^{-2}d^{-1}$ at 1.0 m depth on PVC in September 2010.

In analysis #1, mean recruitment rates on aluminum were greatest in August 2010 (6.9 ± 5.0 $m^{-2}d^{-1}$) but mean recruitment rates on PVC and wood were greatest in September 2010 (20.0 ± 19.0 $m^{-2}d^{-1}$ on PVC and 10.2 ± 10.7 $m^{-2}d^{-1}$ on wood; Figure 3.3.2). Mean recruitment rates were greatest at 2.5 m depth on aluminum but at 1.0 m depth on PVC and wood (Figure 3.3.3; see Figure A-3 in Appendix A on vertical recruitment patterns as a function of reproductive DD).

No significant differences in recruitment rates were found among all main and interaction effects in the ANOVA of presence and absence data, but significant differences were found in the ANOVA of zero-truncated data (Table 3.3.4). Significant two-way interaction terms indicated that the relationship between time

and recruitment rate differed as a function of site, depth, and substrate type. Hence, the main effect of time on recruitment rate could not be interpreted.

Given non-significant interaction terms between site × depth, site × substrate, and depth × substrate, the ANOVA was partitioned into nine separate analyses (Table 3.3.5). The partitioned ANOVA of the zero-truncated data indicated significant temporal variability in recruitment rates at 1.0 m depth, and on aluminum and PVC substrates. Thus, there was no difference among sites on the seasonal pattern of recruitment rate, but some differences among depth and substrate types.

In analysis #2, mean recruitment rates on PVC peaked in September 2010 (see above) and 2011 (31.5 ± 15.7 $m^{-2}d^{-1}$; Figure 3.3.2). Mean recruitment rates were greatest at 1.0 m depth (Figure 3.3.3).

No significant differences in recruitment rates were found among all main and interaction effects in the ANOVA of presence and absence data but significant differences were found in the ANOVA of zero-truncated data (Table 3.3.6). Significant two-way interaction terms indicated that the relationship between year and recruitment rate differed as a function of depth, and that between month within year and recruitment rate differed as a function of both site and depth. Hence, the main effects of year and month within year on recruitment rate could not be interpreted.

Given non-significant interaction terms between year × site and between site × depth, the ANOVA was partitioned into six separate analyses (Table 3.3.7). The partitioned ANOVA of zero-truncated data indicated a significant effect of month within year on recruitment rate at all three depths, and of year at depths of 2.5 and 4.0 m. Thus, on PVC plates, there was no difference among sites on the inter-annual pattern of recruitment rate, but some differences among depths.

Table 3.3.4 Analysis of variance (ANOVA) of the two-component conditional model of daily recruitment rates of *Botryllus schlosseri* from the 2010 sampling season (analysis #1). All main and interaction effects were not significant for the first component (not shown), which consisted of presence and absence data. The second component consisted of zero-truncated data, which were natural logarithm transformed. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

Source of variation	Wald χ^2	df
Intercept	$1.8 \times 10^{1***}$	1
Time	$3.3 \times 10^{1***}$	4
Site	NS	2
Depth	$1.0 \times 10^{1**}$	2
Substrate	$2.6 \times 10^{1***}$	2
Time × Site	$2.9 \times 10^{1***}$	8
Time × Depth	$1.8 \times 10^{1**}$	6
Time × Substrate	$2.8 \times 10^{1***}$	5
Site × Depth	NS	4
Site × Substrate	NS	4
Depth × Substrate	NS	4

Table 3.3.5 Partitioned analyses of variance (ANOVAs) of the second component model of daily recruitment rates of *Botryllus schlosseri* from the 2010 sampling season (analysis #1). The second component consisted of zero-truncated data, which were natural logarithm transformed. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

Partitioned ANOVA	Source of variation	Wald χ^2	df
Depth of 1.0 m	Intercept	$4.8 \times 10^{1***}$	1
	Time	$1.7 \times 10^{1**}$	4
Depth of 2.5 m	Intercept	$1.6 \times 10^{2***}$	1
	Time	NS	3
Depth of 4.0 m	Intercept	$7.1 \times 10^{1***}$	1
	Time	NS	3
Site #1	Intercept	$1.9 \times 10^{1***}$	1
	Time	NS	4
Site #2	Intercept	$1.5 \times 10^{1***}$	1
	Time	NS	4
Site #3	Intercept	$5.3 \times 10^{1***}$	1
	Time	NS	4
Aluminum substrate	Intercept	$7.7 \times 10^{0**}$	1
	Time	$7.7 \times 10^{0*}$	2
PVC substrate	Intercept	$1.7 \times 10^{2***}$	1
	Time	$4.4 \times 10^{1***}$	4
Wood substrate	Intercept	NS	1
	Time	NS	3

Table 3.3.6 Nested analysis of variance (ANOVA) of the two-component conditional model of daily recruitment rates of *Botryllus schlosseri* on PVC from the 2010 and 2011 sampling seasons (analysis #2). All main and interaction effects were not significant for the first component (not shown), which consisted of presence and absence data. The second component consisted of zero-truncated data, which were natural logarithm transformed. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

Source of variation	Wald χ^2	df
Intercept	$8.3 \times 10^{1***}$	1
Year	NS	1
Month (Year)	$8.8 \times 10^{1***}$	6
Site	NS	2
Depth	$2.2 \times 10^{1***}$	2
Year × Site	NS	2
Year × Depth	$7.1 \times 10^{0*}$	2
Month (Year) × Site	$3.1 \times 10^{1**}$	11
Month (Year) × Depth	$3.2 \times 10^{1***}$	9
Site × Depth	NS	4

Table 3.3.7 Partitioned analyses of variance (ANOVAs) of the second component model of daily recruitment rates of *Botryllus schlosseri* on PVC from the 2010 and 2011 sampling seasons (analysis #2). The second component consisted of zero-truncated data, which were natural logarithm transformed. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

Partitioned ANOVA	Source of variation	Wald χ^2	df
Depth of 1.0 m	Intercept	8.5×10^{1}***	1
	Year	NS	1
	Month (Year)	2.5×10^{1}***	6
Depth of 2.5 m	Intercept	4.8×10^{1}***	1
	Year	4.533*	1
	Month (Year)	2.2×10^{1}**	5
Depth of 4.0 m	Intercept	4.0×10^{1}***	1
	Year	6.841**	1
	Month (Year)	7.4×10^{1}***	5
Site #1	Intercept	2.0×10^{1}***	1
	Year	NS	1
	Month (Year)	2.7×10^{1}***	6
Site #2	Intercept	4.4×10^{1}***	1
	Year	NS	1
	Month (Year)	4.0×10^{1}***	6
Site #3	Intercept	1.2×10^{1}**	1
	Year	NS	1
	Month (Year)	1.7×10^{1}**	6

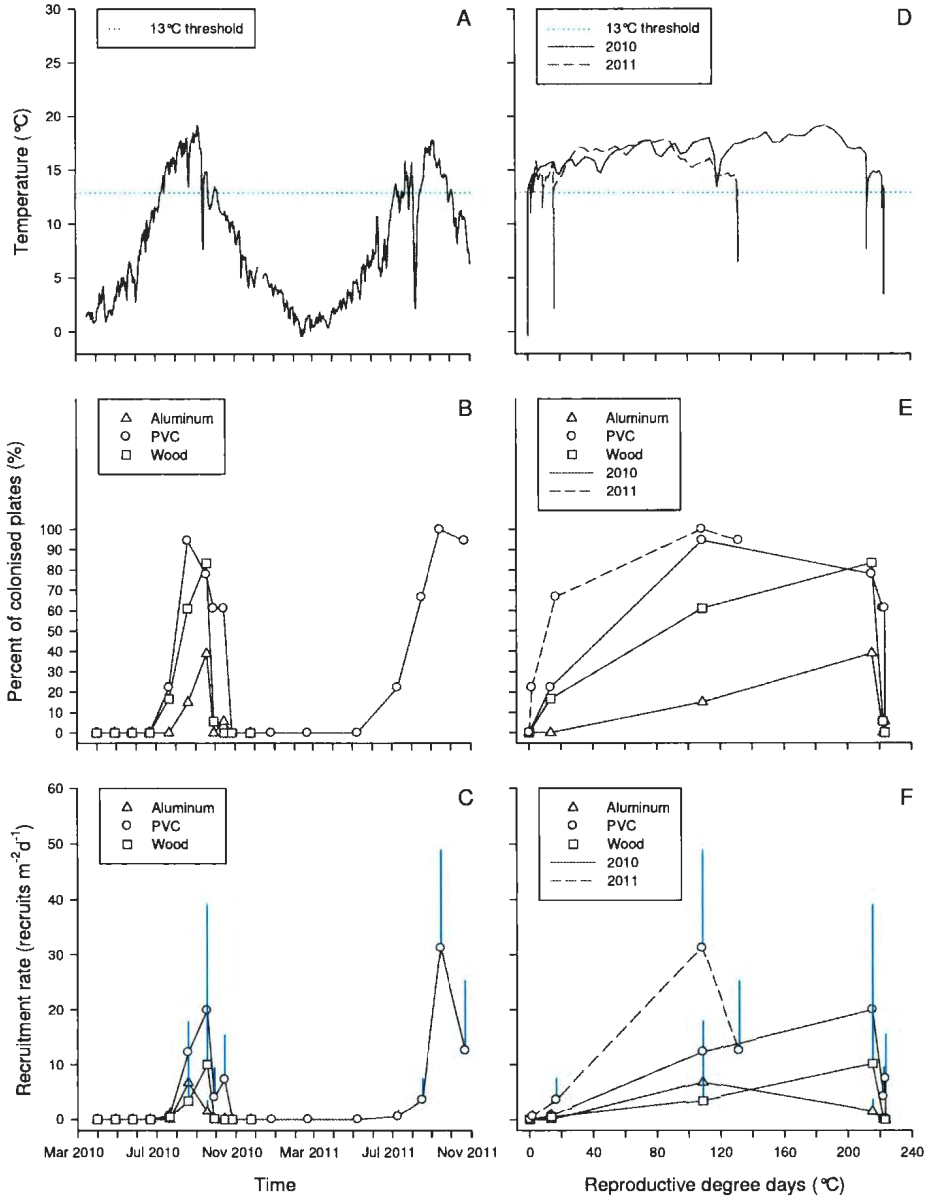

Figure 3.3.2 Recruitment patterns of *Botryllus schlosseri* over time (A-C) and reproductive degree days (D-F). (A and D) Daily mean seawater temperature; (B and E) percent of colonised plates; (C and F) mean recruitment rate. Reproductive degree days were calculated from a threshold temperature of 13°C starting on the first day of the year. Bars represent one SD and n = 18 observations per data point.

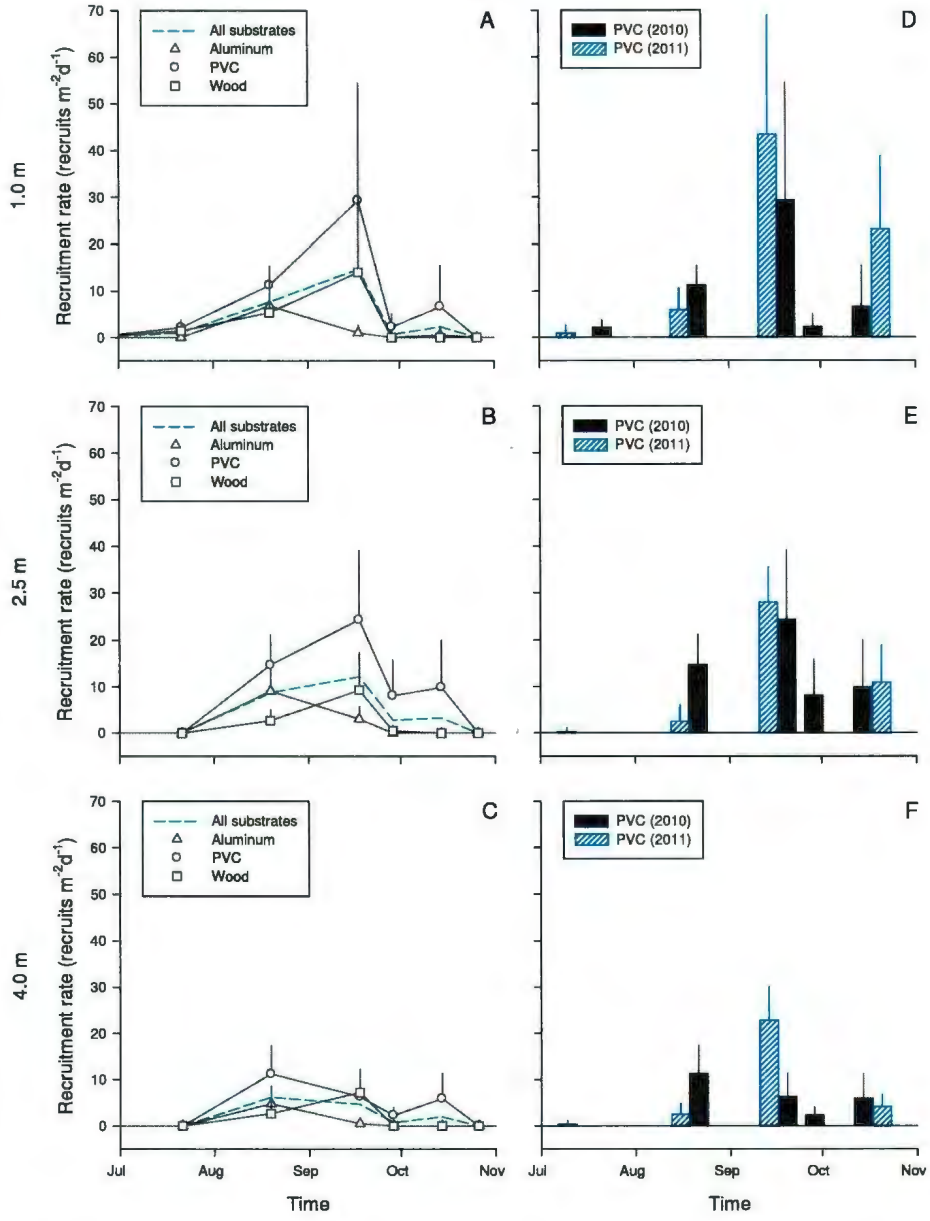

Figure 3.3.3 Vertical recruitment patterns of *Botryllus schlosseri*. Mean recruitment rates at (A) 1.0 m, (B) 2.5 m, and (C) 4.0 m depths on aluminum, PVC, and wood substrates in 2010. Mean recruitment rates on PVC at (D) 1.0 m, (E) 2.5 m, and (F) 4.0 m depth compared between years. Zero values during the non-recruitment period were excluded. Bars represent one SD and n = 6 observations per data point.

3.4 Discussion

In the temperate waters of New England, the recruitment period of *Botryllus schlosseri* is between May and October (Yund and Stires 2002). However, the recruitment period in Arnold's Cove is between early August to mid-October, which is much shorter than what has been reported in or calculated from the literature for this NIA species (see Table 3.4.1 for a summary of recruitment periods). This represents a compression of the recruitment period from six months in New England to 2.5 months in Newfoundland.

The shortened recruitment period of *Botryllus schlosseri* in Arnold's Cove is likely due to the seasonal cycle of seawater temperature. This is because temperature is a predominant seasonal cue that signals the onset of recruitment in colonial ascidians (Millar 1971, Westerman et al. 2009). In *B. schlosseri*, field observations indicate that the onset of fertilization occurs at 11.7°C (Westerman et al. 2009) and the lowest reported temperature threshold for sexual reproduction is 11°C (Brunetti et al. 1980). Laboratory observations suggest that the lowest temperature threshold for sexual reproduction is between 10-13°C, which corroborates field observations of recruitment in the Venetian Lagoon, Italy, after temperatures in April reached 13°C (Brunetti 1974, Brunetti et al. 1984). In the Damariscotta River estuary in Maine, estimations from the data of Yund and Stires (2002) suggest that the onset of recruitment occurs in mid-June when temperatures were ca. 12-15°C.

My results indicate that in Newfoundland, management of *Botryllus schlosseri* should target mitigation efforts, such as eradication, before the annual onset of sexual reproduction and recruitment in July. My findings suggest that recruitment in the subarctic population in Arnold's Cove is constrained by temperature, and that *B. schlosseri* has not adapted to settle and recruit in temperatures lower than those of its temperate range. This constraint also may indicate that *B. schlosseri* has adapted to subarctic conditions by compressing the recruitment season instead of recruiting at lower temperatures.

Recruitment rates of *Botryllus schlosseri* vary markedly among different geographic localities around the world (Table 3.4.1). In Arnold's Cove, the mean recruitment rates on PVC plates, over the recruitment period, was 8.9 $m^{-2}d^{-1}$ in 2010 and 12.1 $m^{-2}d^{-1}$ in 2011. This rate is lower than the mean recruitment rate of ca. 800 $m^{-2}d^{-1}$ from May to October over 15 years in Connecticut, which was calculated from the data of Whitlatch and Osman (2009). Calculations made from the multi-year data of Grosberg (1982) and Yund and Stires (2002) indicated annual mean recruitment rates of ca. 500-1000 $m^{-2}d^{-1}$ in Massachusetts and Maine. Similarly, peak recruitment rates of *B. schlosseri* in Arnold's Cove are lower than peak rates reported in and calculated from the literature (Table 3.4.1).

A smaller adult population size, environmental conditions, mortality in larvae and recruits, and hydrodynamics of the harbour may explain the relatively lower recruitment rates between the population of *Botryllus schlosseri* in Arnold's Cove and those of other geographic localities. Recruitment rate is partly a function of the

concentration of larvae in the water column. Thus, a population with high colony abundance coupled by optimal seawater temperatures, i.e., when the brood period is the shortest, would directly increase the release of larvae. Variability in disease and predation among different localities are sources of mortality in larvae and recruits that could affect recruitment rate. Also, tidal regimes, wind, and geographic shape of the harbour could influence the fate of larvae. For instance, differences in tidal mixing, strong winds, and unsheltered harbours could decrease the ability of larvae to disperse and recruit within the population. Comparisons drawn between studies on the recruitment rates of *B. schlosseri* should consider inherent differences in methodology such as differences in the length of deployment period, substrate type, and area of artificial plates. Notably, recruitment rates from this study are similar in magnitude to those of Australia and California ($< 100\ m^{-2}d^{-1}$; Table 3.4.1); however, these values may not be comparable because the entire recruitment period was not examined in those studies.

The timing of estimated initial fertilization and observed initial recruitment of *Botryllus schlosseri* in Arnold's Cove may be relatively constant from year-to-year. In 2010 and 2011, ca. 9 d elapsed from the first day when daily mean temperatures were ≥ 13°C until the estimated seasonal onset of fertilisation, and 23-25 d elapsed until the observed seasonal onset of recruitment.

Inter-annual variability in recruitment rate between 2010 and 2011 was not found in the population of *Botryllus schlosseri* in Arnold's Cove. However, inter-annual variability in recruitment rate may be detectable if the present study

continued for more than two years. For example, inter-annual variability in annual mean recruitment rate of *B. schlosseri* in eastern United States of America can be seen in the data of Terwin et al. (2000), which spanned the years 1991-1999 and peaked in 1996. In the same study, the colonial ascidian, *Diplosoma macdonaldi*, exhibited pronounced inter-annual fluctuations that were characterised by a high annual mean recruitment rate in 1995 but nearly absent during intervening years of 1994 and 1996 (Terwin et al. 2000).

Some populations of *Botryllus schlosseri* in eastern United States of America exhibit two annual peaks in recruitment in July and September (Grosberg 1982, Terwin et al. 2000, Yund and Stires 2002), perhaps to occupy a temporal niche in order to maximise survival of recruits. However, only one peak per annum was evident in the present study. A second peak in recruitment may be precluded by too short a temporal window of adequate temperatures. Alternatively, there may be selection against a second peak in recruitment to maximise survival of new colonies because of strong competition for space within a relatively short reproductive season.

Inter-annual variations in abundance, environmental conditions affecting development of colonies and quality of gametes and larvae, and natural disturbances are possible causes of inter-annual variability in recruitment of *Botryllus schlosseri*. Terwin et al. (2000) concluded that variations in winter seawater temperature likely influenced inter-annual variability in the recruitment of *Diplosoma macdonaldi*. In the present study, disturbances associated with Hurricane Igor, such as

environmental changes (see Section 3.3.1) and wave action, may have resulted in mass mortality of larvae and recruits in late September 2010, creating space for re-colonisation afterwards.

In Arnold's Cove, maximum recruitment rates of *Botryllus schlosseri* were observed at 1 m depth. In Florida, Dalby and Young (1992) showed that recruitment of the colonial ascidian, *Diplosoma glandulosum*, did not differ between shallow and deep depths in the water column. However, in the same study, recruitment of the solitary ascidian, *Styela plicata*, was only observed at shallow depths.

There are two possible reasons for vertical zonation of recruitment. Firstly, larval behaviour could explain the tendency of recruitment to peak at shallow depths. In the larva of *Botryllus schlosseri*, the photolith is a sensory organ situated in the head region that responds to light and gravity (Sorrentino et al. 2000). The larva is initially positively phototactic and negatively geotactic (Grave and Woodbridge 1924, Grave 1937, Crisp and Ghobashy 1971, Boyd et al. 1986). Within minutes to hours of release, the positively phototactic larva transitions to a state of being non-responsive to light (Grave and Woodbridge 1924). Prior to settlement, the larva is briefly negatively phototactic and, hence, shows a preference for dark spaces (Grave and Woodbridge 1924, Crisp and Ghobashy 1971, Schmidt 1982). Secondly, vertical zonation of recruits may be related to environmental conditions at different depths. Larvae may be sufficiently sensitive to respond to environmental cues and, thus, actively select suitable habitats at sites where temperature and salinity vary among different depths. For example, in Arnold's Cove, variability in seawater

temperature and salinity was significant among depths and not significant among sites (Table A-5 in Appendix A).

My results indicate that mitigation of *Botryllus schlosseri* should target the upper 3-4 m of the water column. In particular, these results suggest floating objects, such as floating docks, near the water surface may be colonised at a greater rate.

In Arnold's Cove, maximum recruitment rates of *Botryllus schlosseri* were observed on PVC plates. The work of Tyrrell and Byers (2007) investigated substrate preference of *B. schlosseri*, which was quantified in percent cover of colonies. Calculations from their data indicate that mean cover was greater on aluminum (ca. 20%) and wood (ca. 15%) than on scallop shells (ca. 4%) and PVC (ca. 3%). Based on their study, the low affinity for PVC suggests that monitoring programmes that use PVC have been systematically under-estimating recruitment rates and colony cover. However, my data suggests that the utility of PVC plates would maximise the detection of *B. schlosseri* within a deployment period of four weeks. Therefore, the efficacy of PVC as the substrate of choice in research studies and monitoring programmes, such as the AIS-AZMP is supported.

I found no geographic variability in recruitment rates of *Botryllus schlosseri* among three sites ca. 15-20 m apart in Arnold's Cove. Recruitment of this species can exhibit geographic variability at higher spatial scales. For example, Yund and Stires (2002) reported that larval settlement patterns varied among their five sites along the ca. 17 km Damariscotta River estuary in Maine. Similarly, some variation in recruitment rates of the solitary ascidian, *Ciona intestinalis*, was reported among

three sites ca. 1.5-2 km apart within the Brudenell estuary (Ramsay et al. 2008). My findings suggest that the monitoring of *B. schlosseri* using artificial plates at one site in Arnold's Cove would adequately represent the entire harbour.

In general, the present study is an example which the seasonal patterns of recruitment of an AIS of temperate origins in a novel subarctic environment is limited by seawater temperatures. Furthermore, I demonstrated that significant vertical patterns of recruitment of an established AIS population can be detectable between the depths of 1-4 m of the water column in a harbour. This is surprising because AIS would be expected to recruit on any available space and, in this case, on bare plates at greater depths.

Table 3.4.1 Recruitment periods and rates of *Botryllus schlosseri* that were reported in or calculated from the literature.

Recruitment period		Recruitment rate (recruits $m^{-2}d^{-1}$)			
Window	Peak(s)	Mean rate	Peak rate	Location*	References**
May-Oct.	Early Jul.; early Sep.	500-1,000	2,100	Damariscotta River, ME	[1]
		90	390	Darling Marine Center, ME	[2]
		50	220	New Castle, NH	[2]
		60	160	Salem, MA	[2]
Early Jun.-late Sep.		460-530	2,100-2,700	Woods Hole, MA	[3], [4]
May-Nov.	Aug.			CT and MA	[5]
May-Oct.		800	17,800	Avery Point, CT	[6], [7]
Jun.-late Sep.				Vancouver Island, BC	[8]
		17.9		Bodega Bay, CA	[9]
Jul.-Nov.				Ardrossan Harbour, Scotland	[10]
		307.5 (horizontal plates)		Langstone Harbour, England	[11]
Jul.-Sep.	Aug.	40-330		Island of Helgoland, Germany	[12]
Sep.-May		8.9-17.7; 53.6-89.3		Williamstown, Australia	[13], [14]
Early Aug.-mid-Oct.	Sep.	8.9-12.1 (PVC)	29.3; 43.5	Arnold's Cove, NL	Present study

* ME = Maine; NH = New Hampshire; MA = Massachusetts; CT = Connecticut; BC = British Columbia; CA = California; NL = Newfoundland and Labrador.

** References for Table 3.4.1: [1] Yund and Stires 2002; [2] Westerman 2007; [3] Grosberg 1982; [4] Grosberg 1988 [5] Osman et al. 2010; [6] Terwin et al. 2000; [7] Whitlatch and Osman 2009; [8] Epelbaum et al. 2009b; [9] Claar et al. 2011; [10] Millar 1952; [11] Schmidt 1982; [12] Harms and Anger 1983; [13] Keough and Raimondi 1996; [14] Keough 1998.

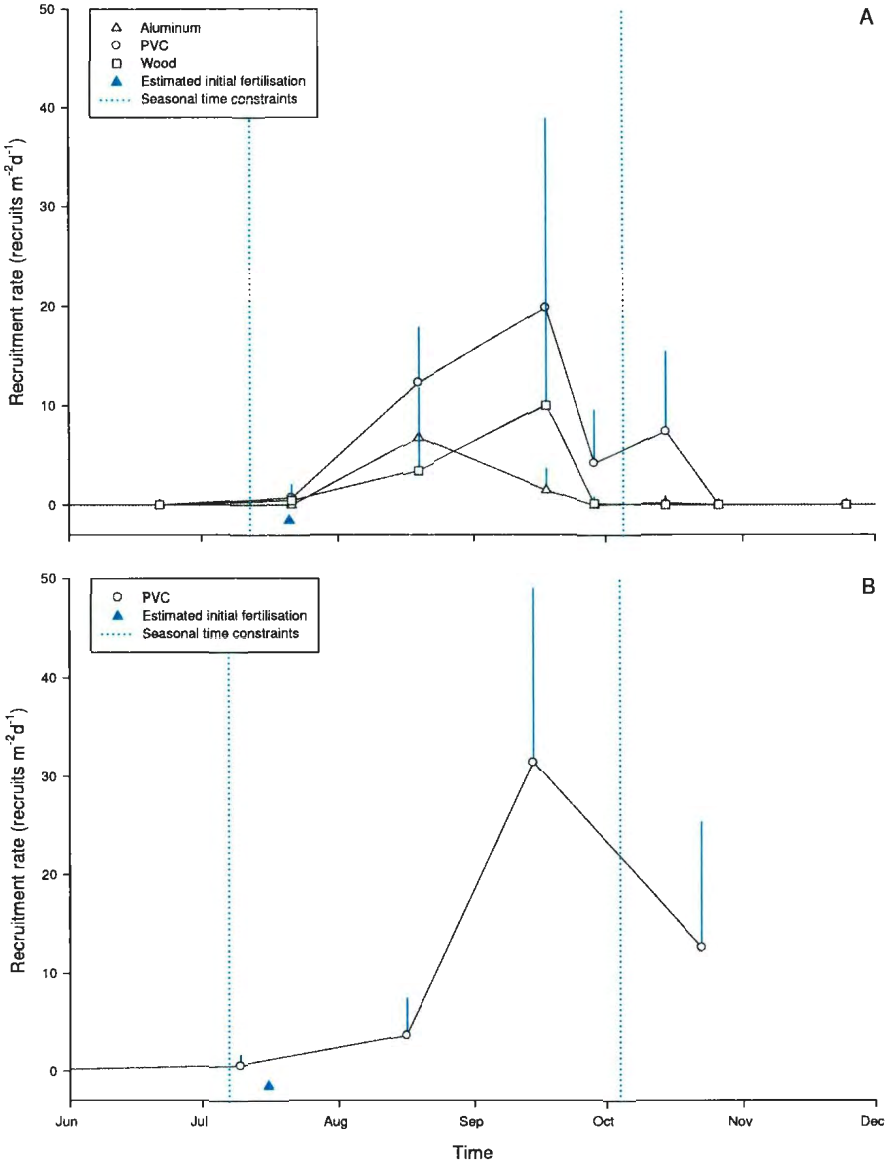

Figure 3.4.1 Seasonal window for recruitment of *Botryllus schlosseri*. (A) Mean recruitment rates in 2010; (B) mean recruitment rates in 2011. The estimated date of initial fertilisation and the seasonal time constraints (initial and final days when the daily mean temperatures were ≥ 13°C) are indicated. The graphical display of time constraints was modified from Lowen et al. (2010, 2011, 2012) to show the predicted window for recruitment. Bars represent one SD and n = 18 observations per data point.

CHAPTER 4 – PATTERNS OF ABUNDANCE OF *BOTRYLLUS SCHLOSSERI* ON WHARF PILINGS

4.1 Introduction

Fouling ascidian species can have long-term ecological impacts on benthic communities. For example, ascidians can be competitors for space (Claar et al. 2011) and can rapidly colonize economically important areas such as shellfish aquaculture sites (Carman et al. 2010). In particular, *Botryllus schlosseri* (Pallas, 1766) is a dominant competitor for space which exhibits high rates of recruitment (Anger 1978, Grosberg 1981) and inhibits the recruitment and survival of competitively less dominant species (Grosberg 1981). Consequently, competition for space between *B. schlosseri* and other species can change the biological diversity of benthic communities (Claar et al. 2011).

Botryllus schlosseri are vertically distributed from the upper subtidal zone to a depth of 75 m or more (Rinkevich et al. 1998). Colonies typically encrust substrates such as bivalves, eelgrass, solitary ascidian species, stones, ship hulls, and wharf structures (Visscher 1928, Berrill 1950, Milkman 1967, Tyrrell and Byers 2007, Carman et al. 2009, Ben-Shlomo et al. 2010). In the eastern Mediterranean Sea, colonies are present year-round but abundance fluctuates seasonally (Rinkevich et al. 1998). *B. schlosseri* on stones in the eastern Mediterranean Sea exhibited a maximum peak cover of ca. 1.5%, coincident with the seasonal maximum in seawater temperature (Rinkevich et al. 1998). Dijkstra et al. (2007) found that peak

abundance of non-indigenous colonial ascidians in coastal waters of the Gulf of Maine was correlated with seasonal changes in seawater temperature. However, maximum abundance was observed during the winter of 1979-1980 and during the spring of 2003-2005 in the Gulf of Maine (Dijkstra et al. 2007). This peak in abundance during the winter may have been related to the absence of natural predators. For instance, maximum abundance was observed between December and February when predators were experimentally excluded using 0.56 cm mesh cages in Lynnhaven Bay, Virginia (Otsuka and Dauer 1982).

Botryllus schlosseri was recently reported from a survey of the south coast of insular Newfoundland (Callahan et al. 2010). It is unknown if the abundance of *B. schlosseri* fluctuates seasonally or if colonies are present year-round. The hypotheses tested in this study were that peak abundance and biomass of colonies established on wharf pilings corresponds to the seasonal maximum in seawater temperature, and that colonies experience 100% mortality during winter with re-colonization from outside the harbour each spring.

4.2 Materials and methods

4.2.1 Study area and study population

Data were obtained from Arnold's Cove, Placentia Bay, NL, Canada. The geographic and physical description of the harbour is given in Section 3.2.1 in Chapter 3. An extensive population of *Botryllus schlosseri* was examined, which is fouling a subtidal section of a fixed wharf structure (Figure 4.2.1).

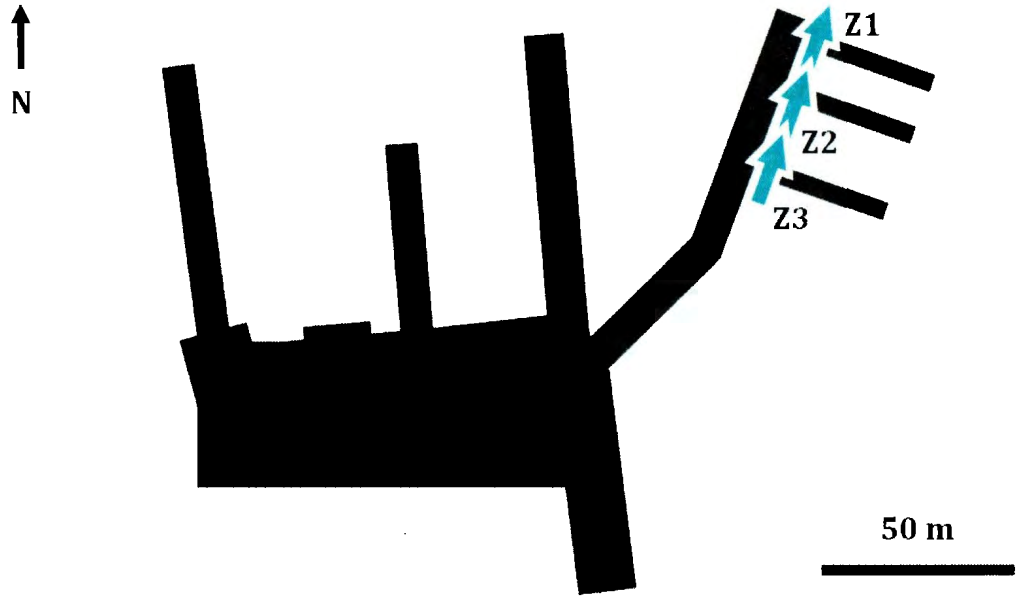

Figure 4.2.1 Schematic map of the government wharf in Arnold's Cove, Placentia Bay, NL, Canada. The belt transect was partitioned *post hoc* into 3 zones: Z1 = zone #1, Z2 = zone #2, Z3 = zone #3. Cross hatched area indicates land. Solid black area indicates permanent wharf structure and floating docks. Scale bar = 50 m.

4.2.2 Sampling design

Twenty-eight video surveys were made ca. every two weeks along a subtidal belt transect (video belt transect method; Lam et al. 2006) of a wharf from March 18, 2010, to May 11, 2011. The transect consisted of 141 pilings along a total length of 50 m (Figure 4.2.2). Every tenth piling was marked with a stainless steel screw-eye for reference. The markers were fixed at the low water mark. While swimming slowly, SCUBA divers used an HD video camera recorder (SONY HVR-V1U; 30 frames per second) housed inside a waterproof housing (Amphibico Endeavor) to film the pilings at a fixed distance of ca. 30 cm from the lens. An underwater light system (Amphibico Discovery G3 arc light) provided uniform illumination. Up to three passes along the transect were made on each sampling date. The first pass covered the intertidal zone (above the markers), the second pass the subtidal zone (below the markers), and, if time permitted, a third pass covered greater depths, just below the second pass. Each pass required ca. 15 minutes to cover the 50 m transect (i.e., a swimming velocity of ca. 5.6 cm s^{-1}). For scale, two green laser pointers (wavelength = 532 nm; BALP-LG05-B150) were affixed 30.0 cm apart on the underwater housing.

Seawater temperature, salinity, *in situ* fluorescence, and turbidity were monitored with a YSI Multiparameter Sonde (6600 V2) throughout the study period. The factory calibration equation was used to convert *in situ* fluorescence to total chlorophyll *a*. The sonde was moored 1 m below the water surface at site #2 from March 18, 2010, to November 15, 2011 (see Figure 3.2.1 in Chapter 3 for the location of site #2). Data were recorded by the unattended sonde every 6 h and downloaded

monthly using EcoWatch (version 3.18) software. An antifouling kit was installed on the sonde.

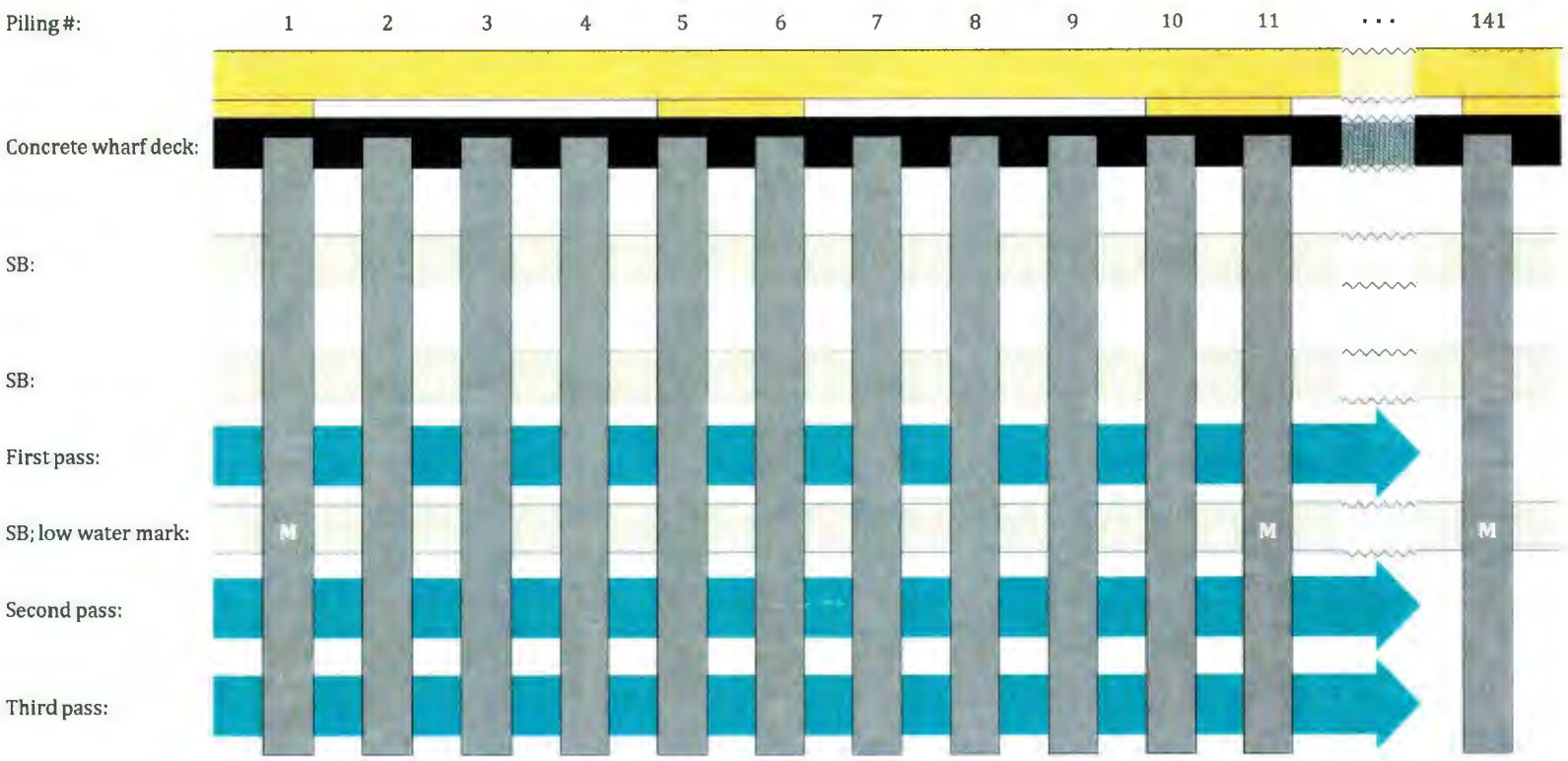

Figure 4.2.2 Schematic drawing of the belt transect of 141 wharf pilings. Up to three video survey passes were made along the transect at different depths. M = reference marker, which was fixed at the low water mark on every tenth piling. SB = horizontal wooden stringer board.

4.2.3 Video and image analysis

All video sequences were digitised, downloaded into Pinnacle Studio 12, and converted into the MPEG file format (HD; 1700 dpi) for backup. Colonies of *Botryllus schlosseri* were generally present at subtidal depths. Therefore, only the video sequences of the second pass made at a subtidal depth were analysed. Video from the third pass made at a subtidal depth were not analysed because they were not made on each sampling date. After screening video quality, 10 of the total of 28 video surveys were analysed. For example, sampling dates with highly turbid water or high macro-algal cover on pilings were excluded from analysis.

For each survey, a random order of the 141 pilings was generated in MS Excel. The number of replicates for analysis was determined by calculating the coefficient of variation (CV) using colony cover data. A range of 21-44 pilings, depending on the sampling date, were required for the change in the CV of mean cover to remain at ≤ 5% for at least four consecutive pilings. Consequently, at least 45 randomised pilings per sampling date were analysed for all 10 surveys.

A still image of each of the randomly selected pilings was directly extracted from the videos using Pinnacle Studio 12 software and converted into the JPEG file format. Image size and quality were not modified. Images were analysed with ImageJ (version 1.44) and viewed on a 61 cm widescreen liquid crystal display (LCD) computer monitor. From the lasers scale reference, the mean width of the pilings was 15.5 ± 1.0 cm (n = 15). For convenience, the piling width of 15.5 cm was also used as the primary scale in the images, with the laser pointers as backup. After the

scale was determined for each image, a virtual quadrat (15.5 × 23.3 cm in the real world) was superimposed on each piling. The left and right edges of the virtual quadrat were determined by the vertical edges of the piling, and the top and bottom edges were haphazardly determined by generating two horizontal lines spaced 23.3 cm apart within the image frame and positioned by default by the imaging software. Colonies were identified, the number of colonies was counted, and the surface area per colony was measured within the virtual quadrat. Frame-by-frame re-plays of the video sequences were utilised to facilitate colony identification if there was uncertainty. The substrate type for each colony was recorded (alga or mussel). The surface area per colony was determined by hand-tracing the perimeter of the colony on a high-resolution graphics tablet (Wacom). Colonies smaller than 4 cm^2 could have been difficult to detect using this technique. Nonetheless, the smallest colony that was positively identified was 0.4 cm^2 on a mussel (*Mytilus* sp.) in May, 2011.

4.2.4 Elemental analysis

Sixty-one colonies of *Botryllus schlosseri* (n = 2-5 per sampling date) were collected over 19 sampling dates from April 28, 2010, to June 29, 2011, to determine seasonal variability in carbon to nitrogen ratios (C:N) and biomass. Colonies were collected off floating docks in the vicinity of the wharf pilings. Substrates included algae, mussels, and man-made objects such as boat hulls and buoys. Colonies were carefully removed from the substrate with a scalpel. Bryozoans, hydroids, mussel spat, skeleton shrimps, small pebbles, etc. were removed from the colonies with a pair of forceps. A subsample of tissue (ca. 1 × 1 cm) was dissected from each colony

with a razor blade. Colonies and tissue subsamples were photographed with a 10 megapixel Canon PowerShot G11 digital camera mounted on a tripod. A metal ruler was placed in each frame to provide scale to the nearest 1 mm. The area of each tissue subsample was measured from the photographs in ImageJ by hand-tracing the perimeter of the colony on a high-resolution graphics tablet (Wacom). Tissue subsamples were stored in a -80°C freezer until analysis.

Frozen tissue subsamples were rinsed with distilled water for 10 seconds to remove adherent salt and individually placed in pre-weighed aluminum cups. The tissue subsamples were dried for 48 h in an oven at 60°C and then stored in a desiccator for 24 h. The dry weight of each tissue subsample was determined with a balance (Mettler Toledo UMT 2) after subtracting the known weight of the aluminum cup. Each tissue subsample was crushed with a mortar and pestle and a weighed subsample (at least 2 mg) was placed in an aluminum cup. A preliminary assessment indicated that tissue subsamples did not need to be fumed with hydrochloric acid prior to analysis (data not shown). Blank and acetanilide standard samples were prepared to calibrate and standardise the CHN analyser (PE 2400 Series II) and then the 2 mg tissue subsamples were analysed.

4.2.5 Statistical analyses

Seawater temperature, salinity, chlorophyll *a* concentration, and turbidity data were smoothed by calculating the daily mean values after negative chlorophyll *a* concentration and turbidity values were converted to zeros. Unreliable chlorophyll *a* concentration data, which was caused by algal growth on the *in situ* florescence

probe of the sonde, were removed from July 8 to August 11, 2010. Environmental data was missing from December 9 to 15, 2010, due to maintenance of the sonde.

For each day of a given year, the growing degree day (DD) was calculated by summing the degrees Celsius above the threshold temperature of 6°C starting on the first day of that year. The conservative threshold temperature of 6°C was selected because the lower temperature threshold for growth exceeded 5°C (Epelbaum et al. 2009c). Mean values in this study are reported ± one SD. Analyses of variance (ANOVA) were generalised linear models (GLM; type III sums of squares method). A discussion of GLM can be found in Appendix B. All statistical analyses were carried out with SPSS 19.

Pearson product-moment correlation coefficients were calculated for $ln(x+1)$ transformed daily mean environmental data. An ANOVA (normal GLM with the canonical identity link function) of the transformed environmental data was used to test variability among years and months (nested within year).

The belt transect was partitioned *post hoc* into three zones to test for spatial variability in abundance within the 50 m length, given that zone #1 was relatively exposed and zone #3 was relatively sheltered. Each zone was composed of 47 pilings (Figure 4.2.1). Densities of colonies and percent cover were transformed to natural logarithms and analysed with a two-component conditional model, which tested for variability among sampling dates (time) and zones. The first component of the conditional model considered presence and absence data and the second component considered zero-truncated data. The ANOVAs for the first component and the second

component were conducted with a binomial logit GLM and by a log-normal GLM (with the log link function), respectively (see Appendix B for the protocol for model selection).

C:N ratios and biomass (dry weight per unit area) of tissue subsamples that were greater than three SDs from the mean were considered outliers and these values were removed from any statistical analyses. Colony size (surface area per colony), C:N ratios, and biomass were transformed to natural logarithms. An ANOVA (log-normal GLM) of the transformed colony size data was used to determine variability among sampling dates (time), zones, and substrate types. An ANOVA (log-normal GLM) of the transformed C:N ratios and biomass data was employed to test for variability among sampling dates (time) and substrate types. ANOVAs with significant interaction effects were partitioned to interpret main effects.

4.3 Results

4.3.1 The physical environment

A description of the physical environment is given in Chapter 3 (see Section 3.3.1). The harbour exhibited significant seasonal and inter-annual variability in temperature, salinity, and turbidity, with the seasonal scale predominant (Table 4.3.1). There was significant seasonal variability in chlorophyll *a* concentration but no inter-annual variability (Table 4.3.1). The correlations between each pair of the four environmental variables (temperature, salinity, chlorophyll *a* concentration, and turbidity), with the exception of chlorophyll *a* and turbidity, were statistically

significant during the entire sampling season from March 18, 2010, to May 11, 2011 (Table 4.3.2).

Table 4.3.1 Nested analysis of variance (ANOVA; generalised linear model) of *ln(x+1)* transformed daily mean seawater temperature, salinity, chlorophyll *a* concentration, and turbidity (n = 413 per environmental variable) recorded at 1 m depth from March 2010 to May 2011. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

	Temperature		Salinity		Chlorophyll *a*		Turbidity	
Source of variation	Wald χ^2	df	Wald χ^2	df	Wald χ^2	df	Wald χ^2	df
Intercept	$1.7 \times 10^{4***}$	1	$1.1 \times 10^{7***}$	1	$8.9 \times 10^{2***}$	1	$2.1 \times 10^{2***}$	1
Year	$1.6 \times 10^{3***}$	1	$2.4 \times 10^{1***}$	1	NS	1	$1.9 \times 10^{1***}$	1
Month (Year)	$2.6 \times 10^{3***}$	13	$3.3 \times 10^{2***}$	13	$3.3 \times 10^{2***}$	13	$2.9 \times 10^{2***}$	13

Table 4.3.2 Pearson product-moment correlation coefficient matrix of *ln(x+1)* transformed daily mean seawater temperature, salinity, chlorophyll *a* concentration, and turbidity (n = 413 per environmental variable) recorded at 1 m depth from March 2010 to May 2011. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

	Temperature	Salinity	Chlorophyll *a*	Turbidity
Temperature	1.00	-0.52***	0.11*	0.26***
Salinity		1.00	-0.20***	-0.25***
Chlorophyll *a*			1.00	NS
Turbidity				1.00

4.3.2 Density and cover of colonies

The population of *Botryllus schlosseri* exhibited variability in density and cover of colonies on wharf pilings seasonally (among months), among zones, and between years. In Arnold's Cove, colonies were observed on each sampling date throughout the study period, and were common in the subtidal zone—just below the low water mark—on wharf pilings and floating docks. To date, no colonies in Arnold's Cove have been observed on the seafloor. The mean percent of pilings colonised was 44.8 ± 17.3% per survey date (n = 10), mean density was 27.1 ± 13.5 colonies m^{-2} (n = 10), and mean colony cover was 1.6 ± 0.8% (n = 10). The maximum percent of pilings colonised was 68.9% of pilings, peak mean density was 47.5 ± 48.7 colonies m^{-2}, and peak mean colony cover was 2.8 ± 2.9% in October (Figure 4.3.1). The daily mean temperature during this period in October 2010 was 11.2 ± 1.2°C (DD = 916-1069°C). The maximum recorded density and cover on a single piling were 250 colonies m^{-2} and 20.3%, respectively, which occurred in mid-September 2010 in zone #1. A sharp decrease in abundance was observed in November 2010. The lowest percent of piling colonies was 16.3%, the lowest mean density was 6.8 ± 19.2 colonies m^{-2}, and the lowest mean colony cover was 0.6 ± 1.9% in May 2010. The daily mean temperature during this period in May was 4.6 ± 1.0°C (DD = 0-1°C).

There were significant differences in density and cover of colonies in the first component of the conditional model but no significant differences were found among all main and interaction effects in the second component (Table 4.3.3).

Significant variability was found among sampling dates (time) and zones but there was no interaction effect.

The abundance of colonies was greater in May 2011 than in May 2010 (Figure 4.3.1), with inter-annual increases of 290% in mean density, 222% in mean colony cover, and 231% in mean percent of colonised pilings.

Table 4.3.3 Analyses of variance (ANOVAs) of the two-component conditional model of density and cover of colonies of *Botryllus schlosseri* on wharf pilings from March 2010 to May 2011. The first component consisted of presence and absence data. The second component consisted of zero-truncated data, which were natural logarithm transformed. All main and interaction effects were not significant for the second component (not shown). *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

Source of variation	Wald χ^2	df
Intercept	NS	1
Time	3.2×10^{1}***	9
Zone	6.8×10^{0}*	2
Time × Zone	NS	18

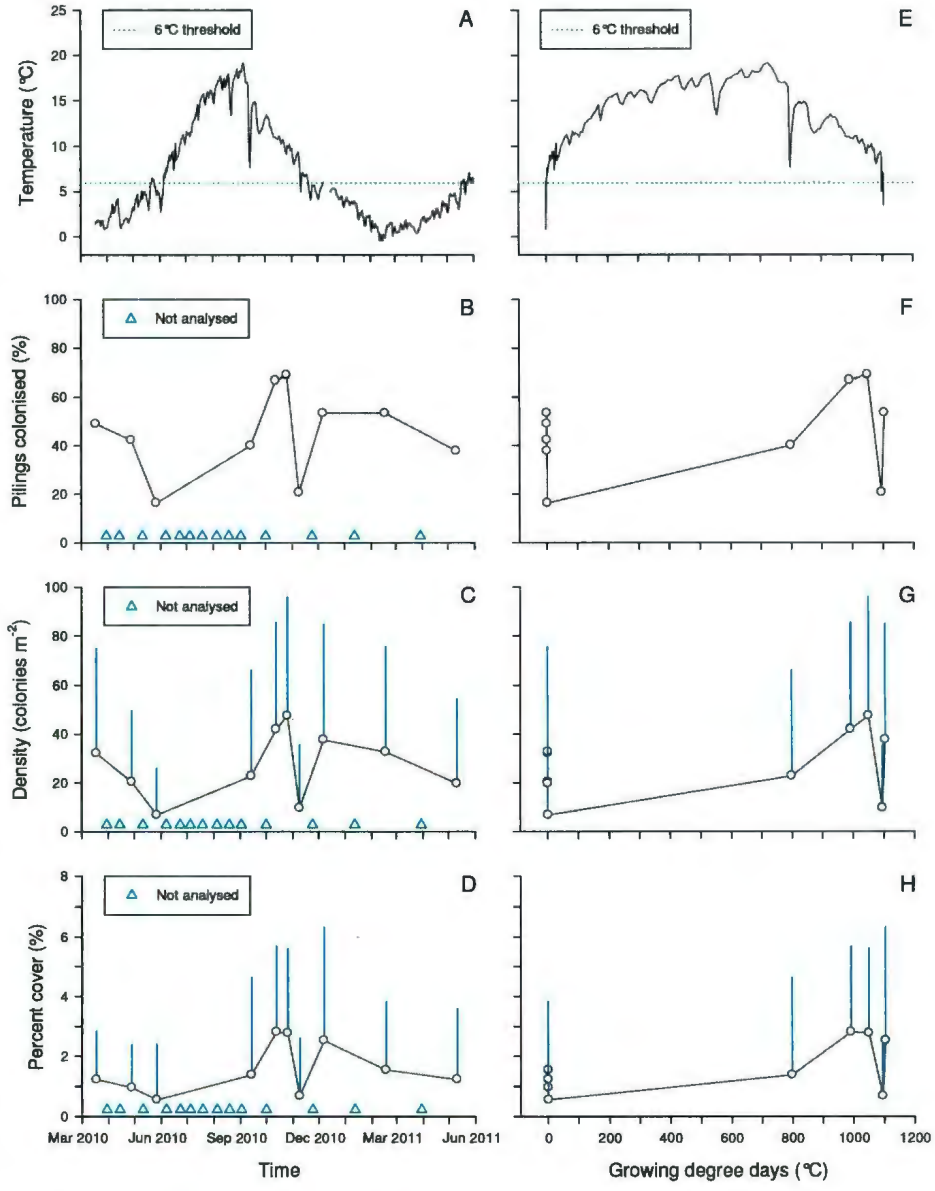

Figure 4.3.1 Abundance patterns of *Botryllus schlosseri* over time (A-D) and growing degree days (E-H). (A and E) Daily mean seawater temperature; (B and F) percent of colonised pilings; (C and G) density; (D and H) percent cover. Growing degree days were calculated from a threshold temperature of 6°C starting on the first day of the year. Bars represent one SD and $n \geq 45$ observations per data point.

4.3.3 Colony size, carbon to nitrogen ratios, and biomass

Significant variability in colony size was found among sampling dates (time) but not among zones and substrate types (alga and mussel; Table 4.3.4). There was no interaction effect. However, the seasonal cycle of colony size did not exhibit any conspicuous peaks and troughs throughout the study period (Figure 4.3.2; see Figure A-4 in Appendix A on the distribution of colony size). Mean colony size ranged from 3.8-8.2 cm^2 with a mean of 5.8 ± 4.1 cm^2.

No variability in C:N ratios was found among sampling dates (time) and substrate types, and there was no interaction effect (Table 4.3.5). Daily mean C:N ratios ranged from 3.9-4.6 (w/w) with a mean of 4.2 ± 0.3 (w/w). Daily mean biomass ranged from 66.4-273.7 g dry weight m^{-2} with a mean of 119.9 ± 55.2 g dry weight m^{-2}.

Significant differences in biomass of tissue subsamples were found among all main and interaction effects in the ANOVA (Table. 4.3.5). A significant two-way interaction term indicated that the relationship between time and biomass differed as a function of substrate type. Hence, the main effect of time on biomass could not be interpreted.

Given non-significant interaction terms between time × substrate, the ANOVA was partitioned into three separate analyses (Table. 4.3.6). The partitioned ANOVA indicated significant temporal variability in biomass on algal, anthropogenic, and mussel substrates. The effect of substrate type on biomass cannot be interpreted

because the data was not collected in a manner that can permit the partitioning of the time variable.

In the video analysis of the second pass made at a subtidal depth, colonies were only detected on algae (mean 23.1 ± 11.1% of all colonies digitised per survey) and mussels (mean 76.9 ± 11.1%) throughout the study period (Figure A-5 in Appendix A). Nevertheless, a visual review of the video sequences of the third pass made at a greater depth indicated that colonies were occasionally attached to the surface of pilings.

Table 4.3.4 Analysis of variance (ANOVA; generalised linear model) of colony size of *Botryllus schlosseri* from March 2010 to May 2011. Data were transformed to natural logarithms. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

Source of variation	Wald χ^2	df
Intercept	$6.8 \times 10^{1***}$	1
Time	$1.8 \times 10^{1*}$	9
Zone	NS	2
Substrate	NS	1
Time × Zone	NS	17
Time × Substrate	NS	9
Zone × Substrate	NS	2
Time × Zone × Substrate	NS	16

Table 4.3.5 Analyses of variance (ANOVAs; generalised linear model) of C:N ratios and biomass of tissue subsamples of *Botryllus schlosseri* from April 2010 to June 2011. Data were transformed to natural logarithms. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

	C:N ratios		Biomass	
Source of variation	Wald χ^2	df	Wald χ^2	df
Intercept	$3.8 \times 10^{3***}$	1	$5.1 \times 10^{4***}$	1
Time	NS	17	$8.4 \times 10^{1***}$	17
Substrate	NS	1	$4.5 \times 10^{0*}$	1
Time × Substrate	NS	5	$1.2 \times 10^{1*}$	5

Table 4.3.6 Partitioned analyses of variance (ANOVAs; generalised linear model) of biomass of tissue subsamples of *Botryllus schlosseri* from April 2010 to June 2011. Data were transformed to natural logarithms. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

Partitioned ANOVA	Source of variation	Wald χ^2	df
Algal substrate	Intercept	$1.4 \times 10^{4***}$	1
	Time	$1.9 \times 10^{1***}$	7
Anthropogenic substrate	Intercept	$1.8 \times 10^{4***}$	1
	Time	$2.8 \times 10^{1***}$	6
Mussel substrate	Intercept	$2.2 \times 10^{4***}$	1
	Time	$5.9 \times 10^{1***}$	9

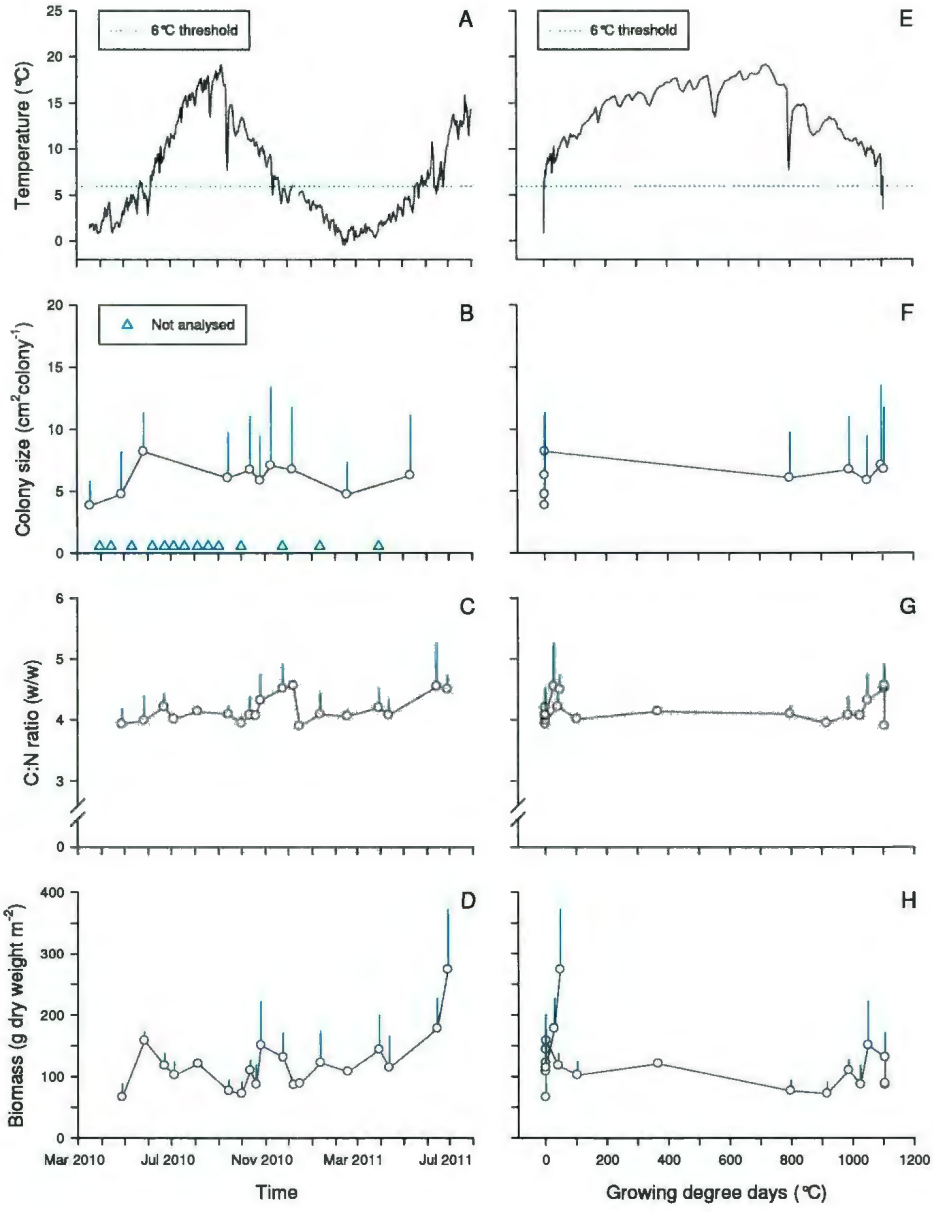

Figure 4.3.2 Colony size and biomass patterns of *Botryllus schlosseri* over time (A-D) and growing degree days (E-H). (A and E) Daily mean seawater temperature; (B and F) colony size; (C and G) C:N ratio; (D and H) biomass. Growing degree days were calculated from a threshold temperature of 6°C starting on the first day of the year. Bars represent one SD. *n* ranged from 12-77 colonies per data point for panels (B) and (F), and 2-5 tissue subsamples per data point for panels (C), (D), (G), and (H).

4.4 Discussion

The density of *Botryllus schlosseri* along a distance of 50 m of wharf pilings in Arnold's Cove ranged from 6.8-47.5 colonies m^{-2} and the annual mean density was 27.1 colonies m^{-2}. Percent colony cover ranged from 0.6-2.8% and the annual mean cover was 1.6%. These values are much lower than in other regions of the world where *B. schlosseri* is established as an NIA species, but are comparable to those in its native range in the Mediterranean Sea. Density on natural stone substrates in Israel ranged from a minimum of ca. 10-20 colonies m^{-2} to a maximum of ca. 40-70 colonies m^{-2}, and colony cover ranged from ca. 0-1.5% (Rinkevich et al. 1998). Outside the native range, the mean colony cover of *B. schlosseri* varies among geographic localities. In particular, along the coast of Maine, mean colony cover on artificial substrates is ca. 0% in Boothbay, Freeport, and Opeeche Island, ca. 2-2.5% in Medomak and Portland, ca. 7-10% in Eastport and Orrs Island, and ca. 25% in Blue Hill (McNaught and Norden 2011). After five months of deployment, the cover of *B. schlosseri* on artificial substrates ranged from minimal (< 25%) to very abundant (> 75%) in Dipper Harbour, New Brunswick (LeGresley et al. 2008). The variability of these values among localities within the same region suggests that colony cover, and perhaps density, may be highly variable at other sites in insular Newfoundland where *B. schlosseri* is present.

Density and colony cover of *Botryllus schlosseri* are likely controlled by seawater temperatures and competition for space with other fouling species. For example, Rinkevich et al. (1998) found that mean seawater temperature was

significantly correlated to the number of stones that were colonised by *B. schlosseri*, and the density of colonies. Dijkstra et al. (2007) reported that mean colony cover of *B. schlosseri* was ca. 6% (maximum ca. 11%) on artificial substrates in 1979-1980 when it was the only fouling ascidian in Maine, but declined to a mean of ca. 1% (maximum 2.5%) in 2003-2005 when three additional NIA species were established. Also, the ecological succession of the fouling community and the length of the deployment period of artificial monitoring plates can influence density and cover of colonies over time. For instance, mean colony cover of *B. schlosseri* on artificial substrates in The Netherlands was 53% after three months of deployment, 37% after six months, and 20% after nine months (Gittenberger and van der Stelt 2011).

In this study, the greatest cover of *Botryllus schlosseri* on any single piling was 20.3%, which occurred in mid-September 2010 in zone #1, which had the lowest cover of macro-algae. Colonies tend to aggregate, which can explain the phenomenon of locally high abundances. For instance, I qualitatively estimated that up to ca. 70-80% colony cover on localised sections of floating docks was observed in Foxtrap on the east coast of insular Newfoundland, prior to mitigation efforts in December 2011. Grosberg (1991) also noted that colonies of *B. schlosseri* are abundant on floating docks, reaching more than 50% colony cover (sometimes nearly 100%) in June and July in Woods Hole, Massachusetts.

In Arnold's Cove, a greater proportion of colonies of *Botryllus schlosseri* was found on mussels (*Mytilus* sp.; 76%) than on algae (11%). Also, biomass was greater on mussels than on algae, e.g., kelp might release chemicals to deter organisms from

fouling and overgrowing (Dobretsov 1999, Dworjanyn et al. 2006). Colonies on mussels and the stipe of kelp were thicker and more lobate, whereas those colonies on kelp blades were thinner and more flat. High colony cover of *B. schlosseri* on mussels may increase competition for food. High colony cover on algae may reduce algal growth and survival by obstructing light transmission (Wahl 1989). For instance, colonial animals such as *B. schlosseri*, *Botrylloides violaceus*, and *Halichondria panacea* (the indigenous breadcrumb sponge) significantly decrease chlorophyll *a* concentrations in the blades of eelgrass, *Zostera marina*, in Nova Scotia (Wong and Vercaemer 2012).

Colonies of *Botryllus schlosseri* in Arnold's Cove ranged from 0.4-32.1 cm^2 with a mean of 5.8 cm^2, C:N ratios of tissue samples ranged from 3.9-4.6 (w/w) with a mean of 4.2 (w/w), and biomass ranged from 66.4-273.7 g dry weight $m^{-2}$with a mean of 119.9 g dry weight m^{-2}. With the exception of colony size values, there appears to be a lack of comparable C:N ratios and biomass values for colonial ascidians published in the literature. The range of C:N ratios suggests that tissues of *B. schlosseri* are high in proteins and low in lipids. In Scotland, *B. schlosseri* ranged from ca. 0.25-8 cm^2 in colony area (Millar 1952). However, Rinkevich et al. (1998) determined colony size in terms of the number of zooids per colony, and found a significant relationship between colony surface area and the number of zooids (formula: colony area = 0.0292117 × number of zooids). Hence, in Israel, *B. schlosseri* ranged from 1-1155 zooids, where 98% of colonies had < 500 zooids. Calculations

made from the data of Rinkevich et al. (1998) indicated that colonies in Israel ranged from ca. 0.3-33.7 cm^2, which are similar to the values reported in the present study.

In Arnold's Cove, the number of pilings colonised, colony size, and biomass of *Botryllus schlosseri* fluctuated seasonally. Annual mean density and cover were not significantly different as a function of time. Only the presence and absence of colonies on wharf pilings had a significant seasonal pattern. In 2010, maximum values of density, cover, and percent of pilings colonised were observed in October, and minimum values in May. Abundance decreased in May 2011 following earlier video surveys in December 2010 and February 2011.

Other populations of *Botryllus schlosseri* also exhibit seasonal variation in colony abundance (Otsuka and Dauer 1982, Rinkevich et al. 1998, Yund and Stires 2002, Dijkstra et al. 2007), which may be related to seasonal cycles of recruitment and colony mortality, interspecific interactions (competition for space, predation, etc.), or disturbances to the population (anthropogenic and natural disturbances). In Chapter 3, I detected significant seasonal and some inter-annual variability in recruitment rates (Tables 3.3.5 and 3.3.7 in Chapter 3).

An inter-annual comparison can be made in this study between data collected in May of 2010 and 2011. Abundance of *Botryllus schlosseri* in Arnold's Cove was greater in May 2011 than in May 2010, which may be related to the observed greater monthly mean seawater temperature in May 2011 (Figure 3.3.1 in Chapter 3) and greater mean and maximum recruitment rates in 2011 than in 2010 (Figure 3.3.2 in Chapter 3). In 2010 and 2011, the annual mean rates were 8.9 and 12.1

recruits $m^{-2}d^{-1}$, respectively, and maximum rates were 29.3 and 43.5 recruits $m^{-2}d^{-1}$, respectively (Table 3.4.1 in Chapter 3).

Colonies of *Botryllus schlosseri* are present year-round in Arnold's Cove and the population is sustained because of a relatively high cover of overwintering colonies in December. Lowen et al. (2011, 2012) observed that these overwintering colonies survive to reproduce sexually the following spring. My findings suggest that *B. schlosseri* is well adapted to the short productive season and long winter in subarctic waters. Its presence in the winter may represent a lack of predators or an adaptive response to survive in subarctic waters, i.e., colonies produced late in the recruitment season may be adapted to greater overwintering survival (Lowen et al. 2011, 2012) to sustain the population from one year to the next. The difference in the overwintering responses of colonies from subarctic and temperate populations can be the subject of future work. Colonies are also present year-round in the native range of *B. schlosseri* in the Mediterranean Sea (Rinkevich et al. 1998), which may suggest that the subarctic population has been able to adapt to the short growing season in Newfoundland. This explanation is plausible, despite temporal gaps in the occurrence of *B. schlosseri* in Newfoundland, given that this species was first reported in Argentia ca. 60 years ago (United States Navy 1951).

CHAPTER 5 – CONCLUSION

5.1 Summary

In contrast to a broader array of studies elsewhere, past research on non-indigenous ascidian (NIA) species in Newfoundland can be broadly categorised into two themes: monitoring of high risk harbours and phylogenetic analyses of the cytochrome *c* oxidase subunit I (COI) gene of mitochondrial DNA. Firstly, the presence and absence of *Botryllus schlosseri* and *Botrylloides violaceus* was determined from the targeted efforts in search of NIA species (Callahan et al. 2010, Fisheries and Oceans Canada 2011b, 2011c). Secondly, the analysis of the COI gene indicated that the probable origin of *B. schlosseri* in insular Newfoundland was the Atlantic and Mediterranean Sea coasts of France and Spain (Callahan 2009).

The research described in this thesis builds on the previous work on NIA species in insular Newfoundland. In particular, I addressed the following questions: (1) Which indigenous ascidian species have been reported in insular Newfoundland and how do the indigenous and non-indigenous ascidian fauna in Newfoundland compare with those of neighbouring regions (Chapter 2)? (2) What is the inter-annual, seasonal, and small-scale spatial variability of recruitment in the non-indigenous ascidian, *Botryllus schlosseri*, in a subarctic harbour in insular Newfoundland (Chapter 3)? (3) How does recruitment rate compare among different substrate types (Chapter 3)? And (4), what is the seasonal maximum and

minimum colony cover of *B. schlosseri* on wharf structures in a subarctic harbour in insular Newfoundland (Chapter 4)?

In Chapter 2, a checklist of extant indigenous and non-indigenous ascidian species of eastern Canada was compiled. The checklist allowed for comparisons of the ascidian faunal composition among regions of eastern Canada, including insular Newfoundland. The ascidian faunal composition of insular Newfoundland was most similar to that of the Gulf of St. Lawrence coast of Quebec, and was most dissimilar to that of the southern Gulf of St. Lawrence coasts of Prince Edward Island, New Brunswick, and Nova Scotia. The dissimilarity, together with low numbers of reported species, suggests that the ascidian fauna of the southern Gulf coasts may be under-described. However, Prince Edward Island, which is the province with one of the greatest number of ascidian species at invasive levels of abundance in eastern Canada, currently has four NIA species (*Botrylloides violaceus*, *Botryllus schlosseri*, *Ciona intestinalis*, and *Styela clava*).

In Chapter 3, I studied the temporal and spatial patterns of recruitment in a population of *Botryllus schlosseri* in Arnold's Cove, NL, Canada. Recruitment occurred from early August to mid-October, so future management should target mitigation before the annual onset of sexual reproduction and larval settlement and recruitment in July. Recruitment was constrained by a temperature threshold of 13°C, as predicted from the temperature threshold for sexual reproduction of temperate populations of this species in the literature. This threshold suggests that the Newfoundland population of *B. schlosseri* has not yet adapted to recruit at lower

temperatures than is typical of its native range. My recruitment results agree with the data of Lowen et al. (2011, 2012) on the sexual reproduction and somatic growth constraints of *B. schlosseri* at the same site.

Small-scale spatial variation in patterns of recruitment was reported in Chapter 3. Recruitment rates were greater near the water surface than in deeper water. This difference suggests that submerged objects in the upper 3-4 m of the water column are most likely to be colonised. However, recruitment rates were not significantly different within the harbour at a scale of tens of metres and were greater on PVC than on aluminum and wood substrates. This preference supports the efficacy of using PVC to monitor the occurrence of *Botryllus schlosseri* and possibly other closely related NIA species in eastern Canada, since PVC is the artificial substrate of choice in the AIS-AZMP.

In Chapter 4, the seasonal cycle of colony abundance of *Botryllus schlosseri* was determined in Arnold's Cove, NL. The data suggested that colonies were present year-round on wharf structures. Minimum cover of 0.6% was observed in May and maximum cover of 2.8% in October. The decrease in abundance in May suggests that mortality and regression of autumn-born colonies were significant. Furthermore, abundance of pre-reproductive colonies was greater in May 2011 than in May 2010. However, I was unable to test that greater abundance of pre-reproductive colonies contributed to a larger population of sexually reproductive colonies in 2011. This prediction is supported by an increase in mean and maximum recruitment rates in 2011 over the preceding year.

5.2 Discussion

The processes of natality, mortality, immigration, and emigration affect the abundance (size or density) in an open population over time (Krebs 2001), including the population of *Botryllus schlosseri* investigated in this thesis. The relationship of these four population variables with abundance can be expressed with a simple stochastic population model, such as the birth-immigration-death-emigration (BIDE) model (Cohen 1969, Pulliam 1988). Natality and immigration result in an addition of new individuals to the population and an increase in abundance. Mortality and emigration result in a loss of individuals in a population and a decrease in abundance. In *B. schlosseri*, natality can be discussed with reference to my data on recruitment (Chapter 3). Mortality can be discussed with reference to possible sources of natural predation and to the data of Lowen et al. (2011, 2012) on colony survival, immigration and emigration with reference to the observations of ship hull fouling, and the overall change in abundance with reference to my data on colony abundance (Chapter 4).

Natality at the colony level can be defined as the addition of new colonies to the population. New colonies arise from either recruitment or fragmentation. I determined that the mean recruitment rate on PVC during the recruitment season was 8.9 $m^{-2}d^{-1}$ in 2010 and 12.1 $m^{-2}d^{-1}$ in 2011, which represents an increase of 136% (Table 3.4.1 in Chapter 3). Moreover, the maximum mean recruitment rate on PVC was 29.3 $m^{-2}d^{-1}$ in 2010 and 43.5 $m^{-2}d^{-1}$ in 2011, which represents an increase of 148% (Table 3.3.1 in Chapter 3).

Mortality at the colony level is difficult to evaluate because colonies often enter a state of regression for an extended period of time before death. Mortality due to predators in Arnold's Cove is unknown, but is likely to be negligible in a novel environment. Colony survival data of Lowen et al. (2011, 2012) indicate that many of the colonies born later in the recruitment season (between September and early October) did not survive to maturity, which may explain the marked decrease in abundance in May in comparison to the preceding months. A decrease in abundance in November 2010 may have been an artefact of colony regression due to disturbance of the study site by Hurricane Igor on September 21, 2010. Lowen et al. (2011, 2012) provided evidence for temperature-dependent programmed colony death in which summer-born colonies tend to exhibit a lifespan of ca. 25-50 d and autumn-born colonies a longer lifespan of ca. 75-275 d. This data indicates that the natural lifespan of a colony does not exceed one year.

Ship hull fouling is the predominant vector for the introduction of NIA species to novel systems and, perhaps, a continued source of re-introductions to already invaded sites. The colonies of *Botryllus schlosseri* on a fouled ship hull can be defined as immigrants if the colonies were originally from a different population. New colonies recruited on the hull of a ship that is leaving the site of the study population can be defined as emigrants. Unfortunately, the rates of immigration and emigration are unknown for my study population, despite the fact that hulls of ships moored in Arnold's Cove were fouled by colonies of *B. schlosseri* (Figure 5.2.1). The determination of the rates of immigration and emigration was beyond the scope of

this thesis, but can be the subject of future research. However, multiple censuses (n = 29) of the number of vessels (e.g., fishing vessels, recreational vessels, and dories) tied up to the floating docks and vicinity were conducted in Arnold's Cove from March to October 2010. The annual mean number of vessels in 2010 at the study site was 40.4 ± 6.0 (Figure 5.2.2).

Inter-annually, the overall percent change in abundance was estimated by comparing the abundance of pre-reproductive colonies in May 2010 and in May 2011. Mean density increased by 290%, cover by 222%, and percent of colonised pilings by 231%. However, high algal mats and variability in turbidity can make it difficult to detect colonies on wharf pilings in May. In addition, the natural rate of chimera formation is not known, so the abundance of chimeric colonies cannot be considered. Video survey data of wharf structures made in October 2011 can be analysed in the future to contribute to this discussion.

Figure 5.2.1 An example of a ship hull fouled by colonies of *Botryllus schlosseri* in Arnold's Cove, Placentia Bay, NL, Canada. Photograph was taken by SCUBA divers in March 2010.

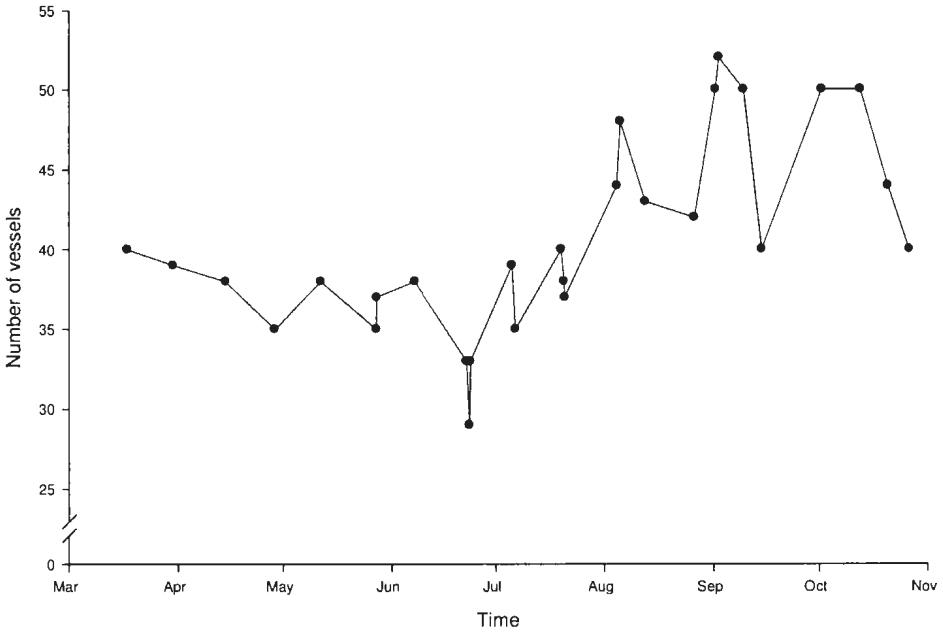

Figure 5.2.2 Multiple censuses (n = 29) of the number of vessels tied up to the floating docks and vicinity in Arnold's Cove, Placentia Bay, NL, Canada, from March to October 2010.

5.3 Conclusion

In this thesis, I investigated the dynamics of a non-indigenous population of *Botryllus schlosseri* in Arnold's Cove, particularly temporal and small-scale spatial patterns of recruitment (Chapter 3), and colony abundance (Chapter 4). The persistence of extensive populations represents a risk of northward range expansion of this invasive species and its spread to unaffected harbours and bivalve aquaculture sites in the province of Newfoundland and Labrador. This would be especially problematic if recruitment rates and colony abundance were to increase above current levels.

Since 2006, industry, management, and policymakers in this province have been successfully applying the precautionary principle to the management of NIA species. This strategy involves concerted efforts to prevent biological invasions of NIA species in insular Newfoundland and to respond rapidly when they do occur. However, the systematics of ascidians is poorly understood due to a lack of taxonomic expertise. Therefore, I reviewed the literature to compile a checklist of indigenous and non-indigenous ascidian species of eastern Canada, including insular Newfoundland, to improve our understanding of the ascidian fauna (Chapter 2). Monitoring efforts such as AIS-AZMP, Rapid Assessment Surveys of high risk harbours, provincial monitoring programmes, and reports from concerned citizens represent a vital source of up-to-date information on the status of NIA species in this province.

Mitigation efforts are important to prevent NIA species from reaching invasive levels of abundance. Presently, only six non-indigenous macro-invertebrate species have been reported in insular Newfoundland. They include *Botrylloides violaceus* (the violet tunicate), *Botryllus schlosseri* (the golden star tunicate), *Caprella mutica* (the Japanese skeleton shrimp), *Carcinus maenas* (the European green crab), *Ciona intestinalis* (the vase tunicate), and *Membranipora membranacea* (the coffin box bryozoan). However, Newfoundland and Labrador is still susceptible to biological invasions of macro-invertebrate species, which may result in negative ecological and economic consequences, particularly in the event of human-mediated global environmental change.

APPENDIX A – SUPPLEMENTAL TABLES AND FIGURES

Supplemental tables and figures are listed in the order that they were referenced in the main text of this thesis.

A-1 Supplemental tables

Table A-1 Records of ascidian species collected in waters of insular Newfoundland during the Rapid Assessment Survey (RAS) from September 2006 to October 2008 (Callahan et al. 2010) and additional field surveys (May 2009 to December 2011). GL = selected preserved specimens that were sent to, and identified by Gretchen Lambert* (personal communication); PS = live specimens collected and identified by Philip Sargent (personal communication).

Species	Location	Latitude	Longitude	Collection date	Identifier	*n*	Remarks
Aplidium glabrum	Bauline	47°44' N	52°50' W	June 7, 2007		1	RAS
Aplidium glabrum	Gadd's Harbour	49°31' N	57°52' W	April 26, 2007		1	RAS
Aplidium glabrum	Logy Bay	47°38' N	52°39' W	August, 2007		1	RAS
Aplidium glabrum	Logy Bay	47°38' N	52°39' W	February 2, 2010	PS	4	
Ascidia callosa	Bauline	47°44' N	52°50' W	June 7, 2007		1	RAS
Ascidia callosa	Logy Bay	47°38' N	52°39' W	August, 2007		1	RAS
Ascidia callosa	Logy Bay	47°38' N	52°39' W	February 2, 2010	PS	8	
Ascidia prunum	Harbour Breton	47°28' N	55°50' W	October 23, 2007	GL	1	RAS
Ascidia prunum	Hermitage	47°34' N	55°56' W	October 23, 2007	GL	1	RAS
Boltenia echinata	Bauline	47°44' N	52°50' W	June 7, 2007		1	RAS
Boltenia echinata	Belleoram	47°31' N	55°24' W	November 1, 2007		15	RAS
Boltenia echinata	Harbour Breton	47°28' N	55°50' W	September 20, 2007		1	RAS

Table A-1 Continued.

Species	Location	Latitude	Longitude	Collection date	Identifier	*n*	Remarks
Boltenia echinata	Harbour Breton	47°28' N	55°50' W	October 23, 2007		8	RAS
Boltenia echinata	Logy Bay	47°38' N	52°39' W	August, 2007		7	RAS
Boltenia echinata	Logy Bay	47°38' N	52°39' W	February 2, 2010	PS	3	
Boltenia echinata	Port aux Basques	47°34' N	59°09' W	November 6, 2006		1	RAS
Boltenia echinata	Port aux Basques	47°34' N	59°09' W	July 16, 2007		2	RAS
Boltenia echinata	Southern Harbour	47°43' N	53°58' W	September 19, 2007		3	RAS
Botrylloides violaceus	Belleoram	47°31' N	55°24' W	October 24, 2007		18	RAS
Botrylloides violaceus	Belleoram	47°31' N	55°24' W	October 28, 2007		6	RAS
Botrylloides violaceus	Belleoram	47°31' N	55°24' W	November 1, 2007		12	RAS
Botrylloides violaceus	Belleoram	47°31' N	55°24' W	March 11, 2008		10	RAS
Botrylloides violaceus	Belleoram	47°31' N	55°24' W	March 10, 2010		9	
Botryllus schlosseri	Argentia	47°18' N	53°59' W	December 7, 2006		4	RAS
Botryllus schlosseri	Arnold's Cove	47°45' N	54°00' W	September 16, 2007		1	RAS
Botryllus schlosseri	Arnold's Cove	47°45' N	54°00' W	September 19, 2007		1	RAS
Botryllus schlosseri	Arnold's Cove	47°45' N	54°00' W	October 13, 2009		7	

Table A-1 Continued.

Species	Location	Latitude	Longitude	Collection date	Identifier	*n*	Remarks
Botryllus schlosseri	Arnold's Cove	47°45' N	54°00' W	October 27, 2009		7	
Botryllus schlosseri	Arnold's Cove	47°45' N	54°00' W	November 24, 2009	GL	24	Appendix D
Botryllus schlosseri	Arnold's Cove	47°45' N	54°00' W	February 15, 2010		4	
Botryllus schlosseri	Arnold's Cove	47°45' N	54°00' W	October 12, 2010		77	Appendix D
Botryllus schlosseri	Arnold's Cove	47°45' N	54°00' W	July 15, 2011		3	Appendix C
Botryllus schlosseri	Arnold's Cove	47°45' N	54°00' W	August 1, 2011		1	Appendix C
Botryllus schlosseri	Foxtrap	47°31' N	53°00' W	December 5, 2011		29	Appendix D
Botryllus schlosseri	Hermitage	47°34' N	55°56' W	September 19, 2007		1	RAS
Botryllus schlosseri	Hermitage	47°34' N	55°56' W	October 23, 2007		1	RAS
Botryllus schlosseri	Long Harbour	47°25' N	53°50' W	February 22, 2007		1	RAS
Botryllus schlosseri	North Harbour	47°51' N	54°06' W	September 11, 2007		14	RAS
Dendrodoa carnea	Salmonier Arm	47°09' N	53°28' W	September 22, 2009	PS	3	
Didemnum albidum	Bauline	47°44' N	52°50' W	2006		1	RAS
Didemnum albidum	Bauline	47°44' N	52°50' W	June 7, 2007		1	RAS
Didemnum albidum	Gadd's Harbour	49°31' N	57°52' W	April 26, 2007		1	RAS

Table A-1 Continued.

Species	Location	Latitude	Longitude	Collection date	Identifier	*n*	Remarks
Didemnum albidum	Logy Bay	47°38' N	52°39' W	August, 2007	GL	3	RAS
Didemnum albidum	Logy Bay	47°38' N	52°39' W	February 2, 2010	PS	1	
Halocynthia pyriformis	Bauline	47°44' N	52°50' W	June 7, 2007		3	RAS
Halocynthia pyriformis	Logy Bay	47°38' N	52°39' W	August, 2007		4	RAS
Halocynthia pyriformis	Logy Bay	47°38' N	52°39' W	February 2, 2010	PS	4	
Halocynthia pyriformis	Port aux Basques	47°34' N	59°09' W	November 6, 2006		26	RAS
Halocynthia pyriformis	Port aux Basques	47°34' N	59°09' W	July 16, 2007		3	RAS
Molgula griffithsii	Gadd's Harbour	49°31'N	57°52' W	May 5, 2009	GL	1	
Molgula sp.	Arnold's Cove	47°45' N	54°00' W	March, 2010		2	
Molgula sp.	Foxtrap	47°31' N	53°00' W	October 12, 2007		17	RAS
Molgula sp.	Logy Bay	47°38' N	52°39' W	February 2, 2010	PS	1	

* In 2010, preserved specimens were sent by Dr. J. Ben Lowen to Gretchen Lambert for taxonomic identification. Two specimens of *Botrylloides violaceus* collected in Belleoram were identified by G. Lambert in addition to the records listed in Table A-1. The address of G. Lambert is 12001 11th Avenue NW, Seattle, Washington, 98177, United States of America.

Table A-2 Number of ascidian species reported or cited per region per source of information. QC = Quebec; PEI = Prince Edward Island; NB = New Brunswick; NL = Newfoundland; NS = Nova Scotia.

	Region									
					Gulf of St. Lawrence				Bay of Fundy	
Source	Arctic	Labrador	Insular NL	Atlantic NS	QC	PEI	NB	NS	NB	NS
Aitken and Fournier (1993)	1									
Atkinson and Wacasey (1989)	23									
Berrill (1928)									3	
Bock (2010)				2		1		1	1	
Brunel et al. (1998)			12		29	11				
Caddy (1970)										2
Callahan (2009)			4			2				
Callahan et al. (2010)			4			2				
Carman et al. (2010)						6				
Carver et al. (2006a)			1	2	1	2				1
Carver et al. (2006b)	1			1	1	2			1	
Clarke and Therriault (2007)						1				
Cole (1989)			1	1						
Ganong (1885)									1	
GBIF (2012)	16	20	29	25	9		5		17	22
Gould (1870)		6	1						9	
Hatfield et al. (1992)									7	
Hooper (1975)			11							
Huntsman (1912)									21	
Huntsman (1922a)	5	2	2							

Table A-2 Continued.

Source	Region									
	Arctic	Labrador	Insular NL	Atlantic NS	Gulf of St. Lawrence				Bay of Fundy	
					QC	PEI	NB	NS	NB	NS
Huntsman (1922b)	13		1							
Kindle (1917)										1
Korczynski (1989)	1									
LeGresley et al. (2008)									2	
Lejeusne et al. (2011)				2		1				
Linkletter (1977)									29	
Locke et al. (2007)				3		4		1	2	
Logan (1988)									3	
Logan et al. (1984)									7	
Mackenzie (2011)					1					
Martin et al. (2011)									3	
Millar (1966)	21		27	19	15				14	
Millar (1970)	9									
Moore and Ma (unpub. data)				3						
Nadeau (2008)					2					
Packard (1863)		4								
Packard (1867)		8	8						1	
Sargent et al. (in preparation)			1							
Sephton (pers. comm.)									1	
Sephton et al. (2011)				3				3		2
Stafford (1906)					6					
Stimpson (1852)									6	

Table A-2 Continued.

Source	Region									
	Arctic	Labrador	Insular NL	Atlantic NS	Gulf of St. Lawrence				Bay of Fundy	
					QC	PEI	NB	NS	NB	NS
Stimpson (1854)									8	
Therriault and Herborg 2008a			1	3	2	4		2	1	2
Trason (1964)	27									
United States Navy (1951)			1							
Vanden Berghe (2007)	12	19	25	23	1					31
Van Name (1910)	1	1	10	4	4	1			4	
Van Name (1912)	11	7	10	8	11	3			14	
Van Name (1921)	3								1	
Van Name (1945)	29	4	18	5					12	
Verrill (1871)		5	6		1				9	
Whiteaves (1901)		8	2	9	10	8		8	14	
Willis et al. (2011)					1	4				
Present study			10							
Number of sources	14	11	21	16	15	15	1	5	25	8

Table A-3 List of 56 extant ascidian species of eastern Canada and their authority name and year, distribution, and status. AS = restricted to arctic and or subarctic waters; CA = with a continuous amphi-Atlantic distribution; DA = with a disjunct amphi-Atlantic distribution; NA = restricted to the northeast coast of North America.

Species	Authority	Distribution*	Status
Aplidium glabrum	(Verrill, 1871)	CA	Indigenous
Aplidium mutabile	(Sars, 1851)	AS	Indigenous
Aplidium pallidum	(Verrill, 1871)	CA	Indigenous
Aplidium spitzbergense	Hartmeyer, 1903	CA	Indigenous
Aplidium stellatum	(Verrill, 1871)	NA	Indigenous
Ascidia callosa	Stimpson, 1852	AS	Indigenous
Ascidia obliqua	Alder, 1863	CA	Indigenous
Ascidia prunum	Müller, 1776	CA	Indigenous
Ascidia dijmphniana	(Traustedt, 1886)	AS	Indigenous
Ascidiella aspersa	(Müller, 1776)	DA	Non-indigenous
Bostrichobranchus pilularis	(Verrill, 1871)	NA	Indigenous
Boltenia echinata	(Linnaeus, 1767)	CA	Indigenous
Boltenia ovifera	(Linnaeus, 1767)	AS	Indigenous
Botrylloides aureum	(Sars, 1851)	AS	Indigenous
Botrylloides violaceus	Oka, 1927	DA	Non-indigenous
Botryllus schlosseri	(Pallas, 1766)	DA	Non-indigenous
Chelyosoma macleayanum	Broderip & Sowerby, 1830	AS	Indigenous
Ciona intestinalis	(Linnaeus, 1767)	CA	Cryptogenic
Cnemidocarpa finmarkiensis	(Kiaer, 1893)	AS	Indigenous
Cnemidocarpa mollis	(Stimpson, 1852)	DA	Indigenous
Cnemidocarpa mortenseni	(Hartmeyer, 1912)	AS	Indigenous
Cnemidocarpa rhizopus	(Redikorzev, 1907)	AS	Indigenous

Table A-3 Continued.

Species	Authority	Distribution*	Status
Corella borealis	Traustedt, 1886	AS	Indigenous
Dendrodoa aggregata	Müller, 1776	AS	Indigenous
Dendrodoa carnea	(Rathke, 1806)	DA	Indigenous
Dendrodoa grossularia	(Van Beneden, 1846)	CA	Indigenous
Dendrodoa pulchella	(Rathke, 1806)	AS	Indigenous
Didemnum albidum	(Verrill, 1871)	AS	Indigenous
Didemnum candidum	Savigny, 1816	DA	Indigenous
Diplosoma listerianum	(Milne-Edwards, 1841)	DA	Non-indigenous
Distaplia clavata	(Sars, 1851)	AS	Indigenous
Eudistoma vitreum	(Sars, 1851)	AS	Indigenous
Eugyra glutinans	(Moeller, 1842)	AS	Indigenous
Halocynthia pyriformis	(Rathke, 1806)	AS	Indigenous
Hartmeyeria arctica	Korczynski, 1989	AS**	Indigenous
Kukenthalia borealis	(Gottschaldt, 1894)	AS	Indigenous
Leptoclinides faeroensis	Bjerkan, 1905	AS	Indigenous
Lissoclinum aureum	Verrill, 1871	AS	Indigenous
Microcosmus glacialis	(Sars, 1859)	CA	Indigenous
Molgula arenata	Stimpson, 1852	NA	Indigenous
Molgula citrina	Alder & Hancock, 1848	CA	Indigenous
Molgula complanata	Alder & Hancock, 1870	CA	Indigenous
Molgula griffithsii	(MacLeay, 1825)	AS	Indigenous
Molgula manhattensis	(De Kay, 1843)	DA	Indigenous
Molgula provisionalis	Van Name, 1945	NA	Indigenous
Molgula retortiformis	Verrill, 1871	AS	Indigenous
Molgula siphonalis	Kiaer, 1896	AS	Indigenous

Table A-3 Continued.

Species	Authority	Distribution*	Status
Pelonaia corrugate	Goodsir & Forbes, 1841	CA	Indigenous
Polycarpa fibrosa	(Stimpson, 1852)	CA	Indigenous
Rhizomolgula globularis	(Pallas, 1776)	AS	Indigenous
Styela canopus	(Savigny, 1816)	DA	Indigenous
Styela clava	Herdman, 1881	DA	Non-indigenous
Styela coriacea	(Alder & Hancock, 1848)	CA	Indigenous
Styela rustica	Linnaeus, 1767	AS	Indigenous
Synoicum pulmonaria	(Ellis & Solander, 1786)	CA	Indigenous
Tridemnum tenerum	(Verrill, 1871)	CA	Indigenous

* Listed in this table are the distributions for 55 ascidian species that were reviewed and categorised by Haydar (2010).

** Distribution of *Hartmeyeria arctica* is based on Korczynski (1989).

Table A-4 List of 18 contiguous deployment periods of artificial plates in Arnold's Cove, Placentia Bay, NL, Canada.

Deployment start date	Deployment end date
March 17, 2010	April 14, 2010
April 14, 2010	May 11, 2010
May 11, 2010	June 7, 2010
June 7, 2010	July 5, 2010
July 5, 2010	August 5, 2010
August 5, 2010	September 2, 2010
September 2, 2010	October 1, 2010
September 14, 2010	October 12, 2010
October 1, 2010	October 26, 2010
October 12, 2010	November 9, 2010
November 9, 2010	December 8, 2010
December 8, 2010	January 12, 2011
January 12, 2011	March 30, 2011
March 30, 2011	June 15, 2011
June 15, 2011	August 1, 2011
August 1, 2011	August 30, 2011
August 30, 2011	September 28, 2011
September 28, 2011	November 15, 2011

Table A-5 Analysis of variance (ANOVA; generalised linear model) of *ln(x+1)* transformed daily mean seawater temperature and salinity recorded every 1 m interval from the water surface to 5 m depth from March 2010 to June 2011 in Arnold's Cove, Placentia Bay, NL, Canada. *** = significant at $p < 0.001$; ** = at $p < 0.01$; * = at $p < 0.05$; NS = not significant.

	Temperature		Salinity	
Source of variation	Wald χ^2	df	Wald χ^2	df
Intercept	$5.9 \times 10^{4***}$	1	$1.7 \times 10^{7***}$	1
Month	$8.2 \times 10^{3***}$	12	$6.6 \times 10^{2***}$	12
Site	NS	2	NS	2
Depth	$2.1 \times 10^{1**}$	5	$2.1 \times 10^{1**}$	5
Month × Site	NS	16	NS	16
Month × Depth	NS	60	NS	60
Site × Depth	NS	10	NS	10
Month × Site × Depth	NS	79	NS	79

Table A-6 Mean daily recruitment rate (± one SD) per deployment period (mean 32.7 d), depth (1.0, 2.5, 4.0 m from the water surface), and substrate type (aluminum, PVC, and wood). Zero values during the non-recruitment period were excluded.

		Mean daily recruitment rate (recruits $m^{-2}d^{-1}$)								
		2010					2011			
Substrate	Depth (m)	Jul.	Aug.	Sep.	Late Sep.	Oct.	Early Jul.	Aug.	Sep.	Late Oct.
Aluminum	1.0	0	6.8 ± 6.0	1.0 ± 1.6	0	0.6 ± 1.4				
	2.5	0	9.0 ± 4.7	3.1 ± 2.7	0	0				
	4.0	0	4.8 ± 3.9	0.5 ± 1.3	0	0				
	All depths	0	6.9 ± 5.0	1.5 ± 2.2	0	0.2 ± 0.8				
PVC	1.0	2.1 ± 1.7	11.2 ± 4.1	29.3 ± 25.3	2.3 ± 2.8	6.6 ± 8.9	1.0 ± 1.7	6.0 ± 4.7	43.5 ± 25.6	23.2 ± 15.7
	2.5	0	14.7 ± 6.5	24.4 ± 14.8	8.0 ± 7.8	9.8 ± 10.1	0.3 ± 0.8	2.6 ± 3.6	28.2 ± 7.4	11.0 ± 8.0
	4.0	0	11.2 ± 6.1	6.3 ± 5.0	2.3 ± 1.8	5.9 ± 5.4	0.3 ± 0.8	2.6 ± 2.3	22.8 ± 7.4	4.1 ± 2.6
	All depths	0.7 ± 1.4	12.4 ± 5.6	20.0 ± 19.0	4.2 ± 5.4	7.4 ± 8.1	0.6 ± 1.2	3.7 ± 3.8	31.5 ± 17.5	12.6 ± 12.7
Wood	1.0	1.4 ± 1.6	5.4 ± 7.3	14.0 ± 16.5	0	0				
	2.5	0	2.7 ± 2.4	9.2 ± 8.1	0.5 ± 1.2	0				
	4.0	0	2.7 ± 2.4	7.2 ± 5.1	0	0				
	All depths	0.5 ± 1.1	3.6 ± 4.5	10.2 ± 10.7	0.2 ± 0.7	0				

A-2 Supplemental figures

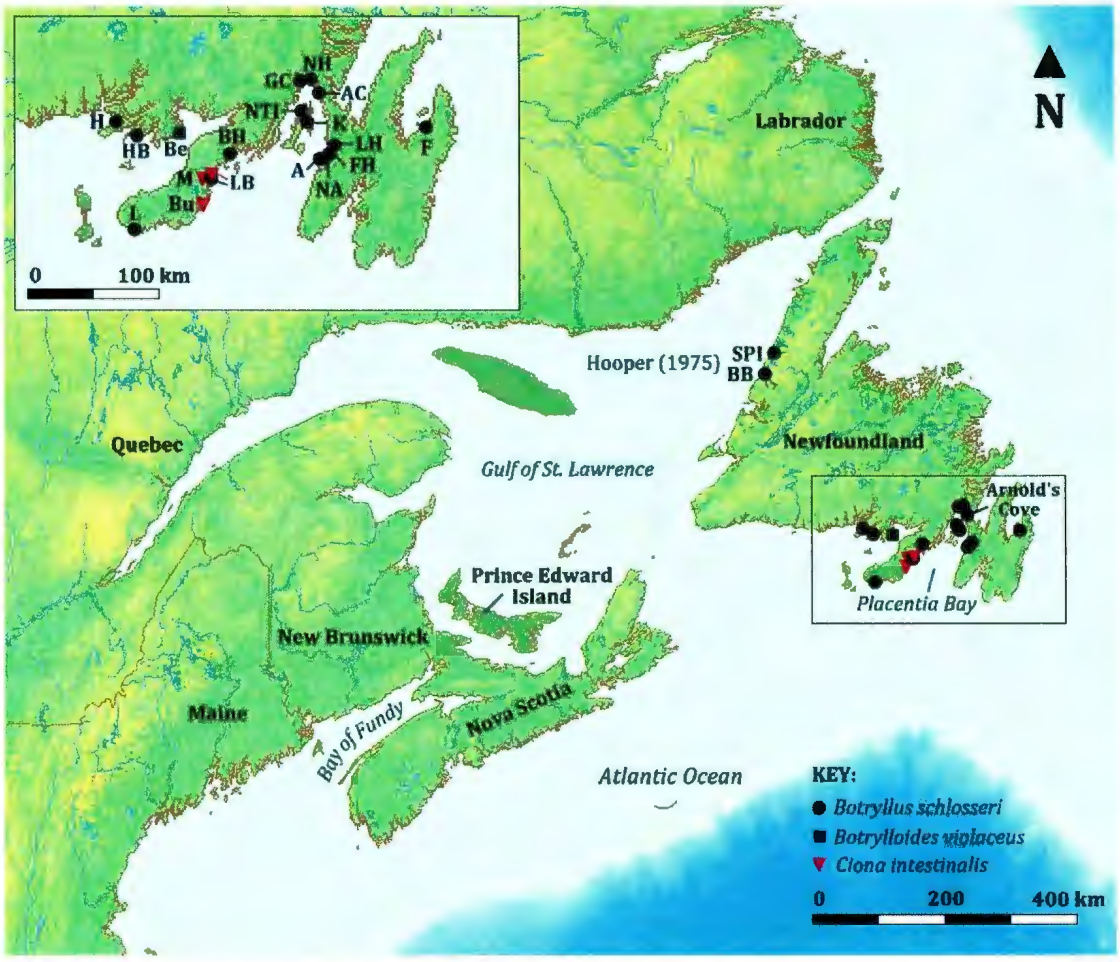

Figure A-1 Map of eastern Canada and geographical distribution of *Botryllus schlosseri*, *Botrylloides violaceus*, and *Ciona intestinalis* in insular Newfoundland. A = Argentia; AC = Arnold's Cove; BB = Bonne Bay; Be = Belleoram; BH = Baine Harbour; Bu = Burin; F = Foxtrap; FH = Fox Harbour; GC = Garden Cove; H = Hermitage; HB = Harbour Breton; K = vicinity of Kingwell; L = Lamaline; LB = Little Bay; LH = Long Harbour-Mount Arlington Heights; M = Marystown; NA = Northeast Arm; NH = North Harbour; NTI = vicinity of North Tilt Island; SPI = St. Paul's Inlet. Sources of data: Hooper (1975), Cynthia H. McKenzie (personal communication), Sargent et al. (in preparation), and Table A-1 (the present study).

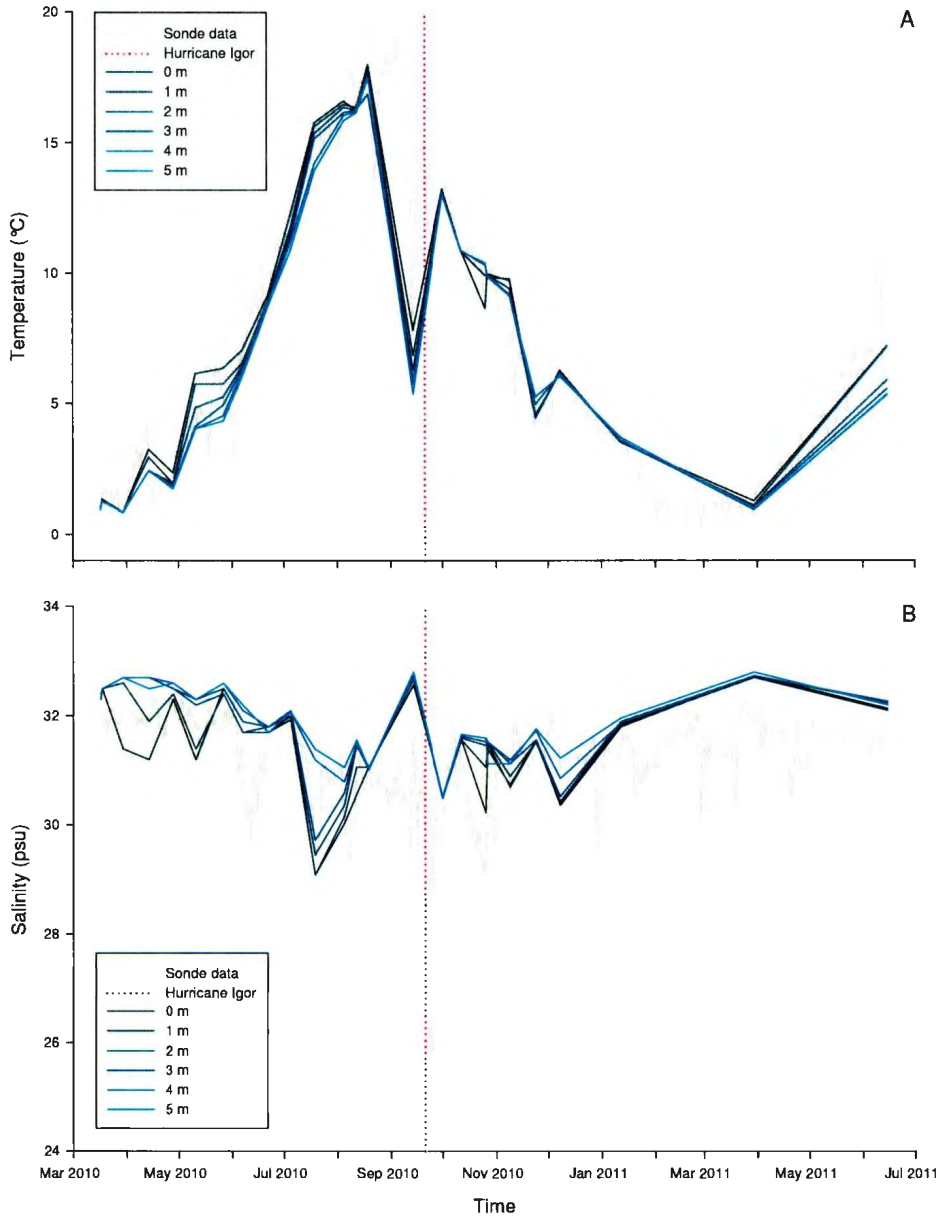

Figure A-2 Environmental data recorded every 1 m interval from the water surface to 5 m depth in Arnold's Cove, Placentia Bay, NL, Canada. (A) Daily mean seawater temperature; (B) daily mean salinity. Data from the sonde is shown in dashed grey lines. A vertical dotted red line marks the passage of Hurricane Igor over the study area on September 21, 2010.

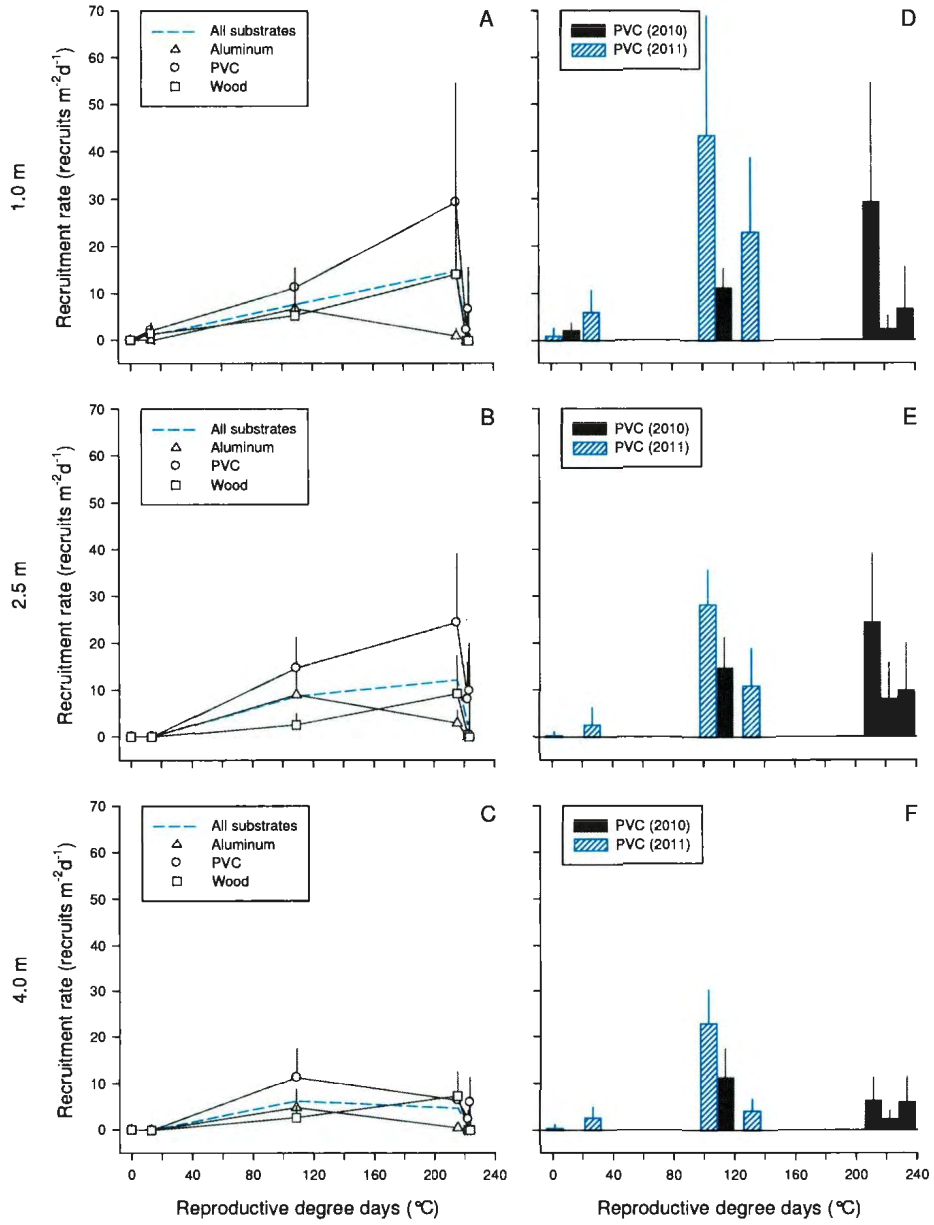

Figure A-3 Vertical recruitment patterns of *Botryllus schlosseri* as a function of reproductive degree days. Mean recruitment rates at (A) 1.0 m, (B) 2.5 m, and (C) 4.0 m depth compared among aluminum, PVC, and wood in 2010. Mean recruitment rates on PVC at (D) 1.0 m, (E) 2.5 m, and (F) 4.0 m depths compared between years. Zero values during the non-recruitment period were excluded. Bars represent one SD and n = 6 observations per data point.

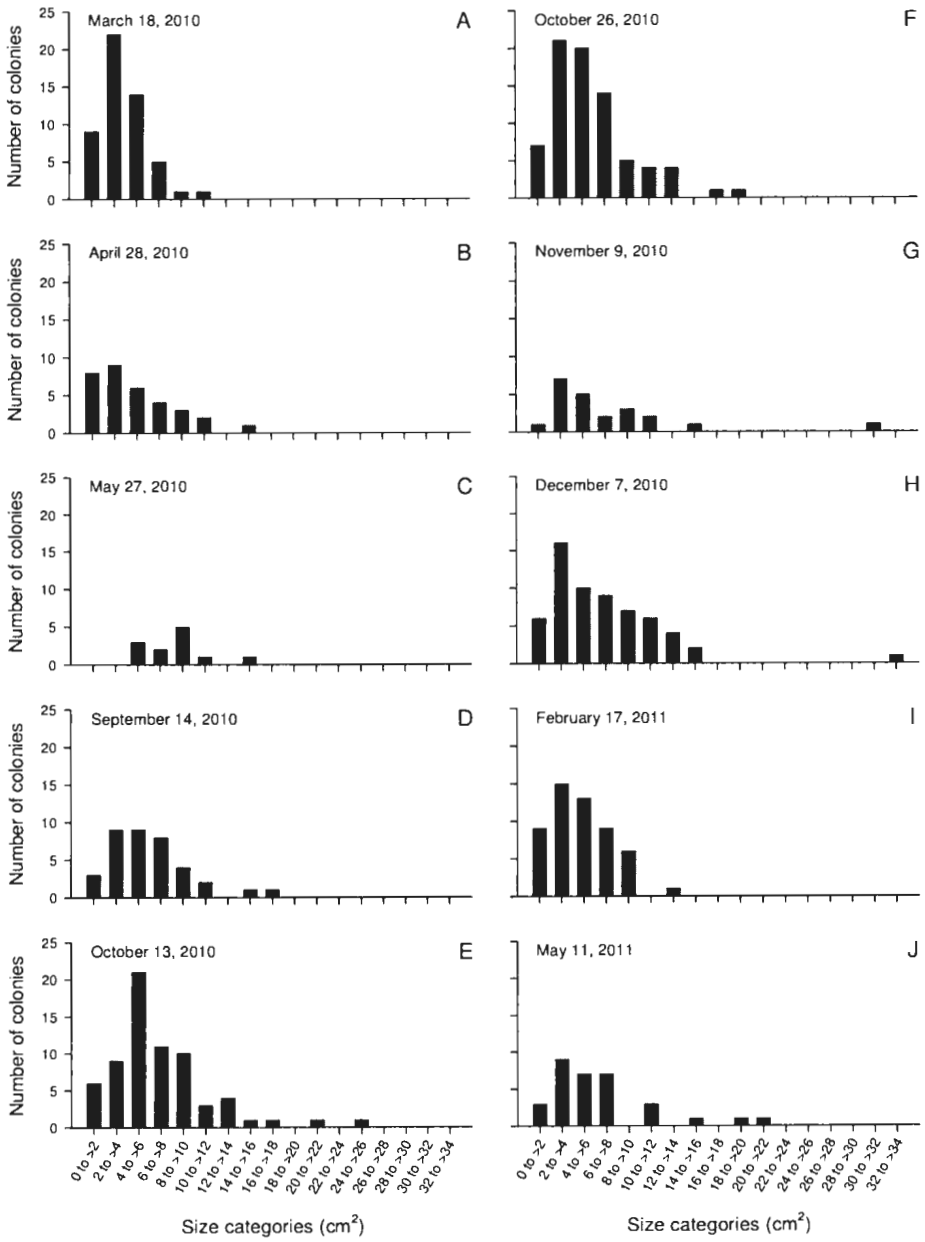

Figure A-4 Distribution of colony size of *Botryllus schlosseri*. (A) March 18, 2010, *n* = 52 colonies; (B) April 28, 2010, *n* = 33; (C) May 27, 2010, *n* = 12; (D) September 14, 2010, *n* = 37; (E) October 13, 2010, *n* = 68; (F) October 26, 2010, *n* = 77; (G) November 9, 2010, *n* = 22; (H) December 8, 2010, *n* = 61; (I) February 17, 2011, *n* = 53; (J) May 11, 2011, *n* = 32.

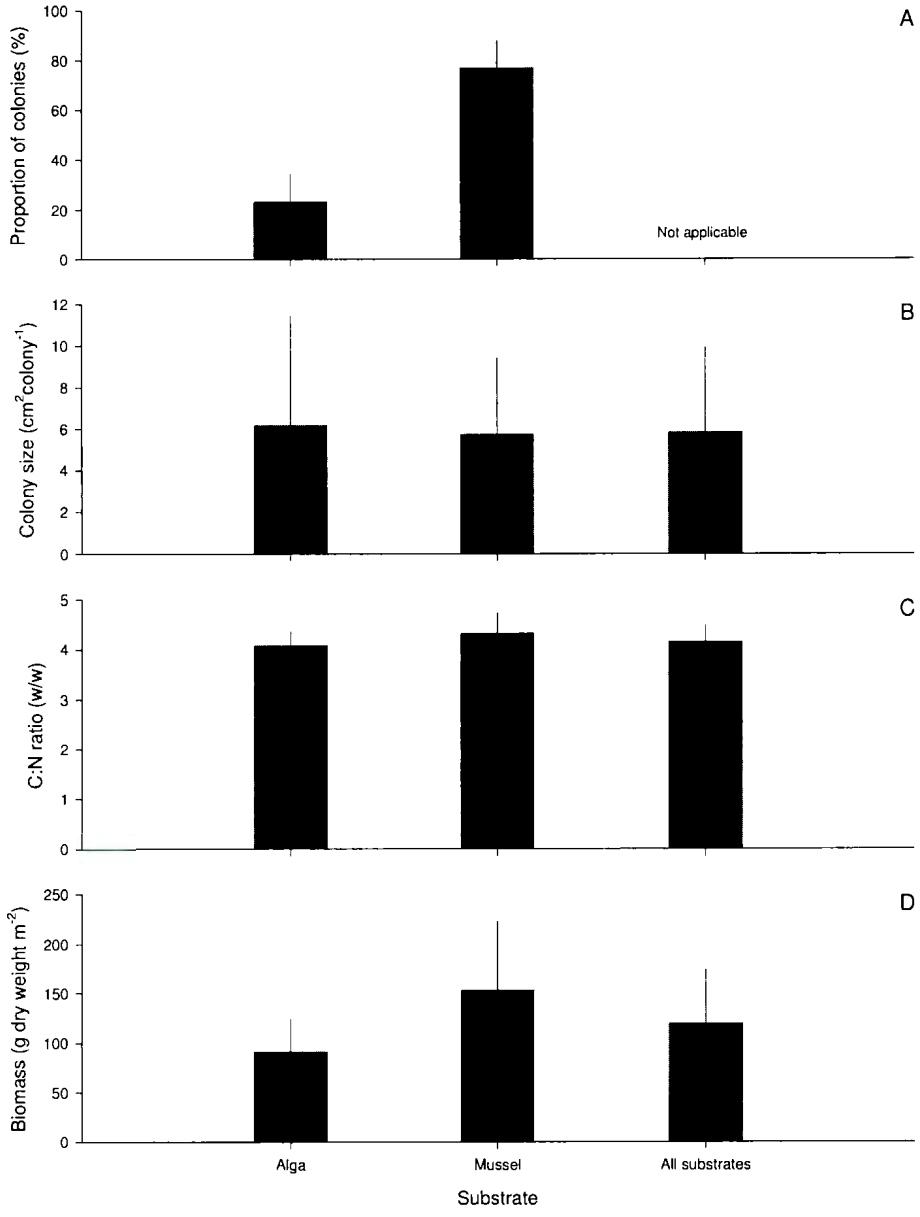

Figure A-5 *Botryllus schlosseri* on alga, mussel, and all substrates pooled together. (A) Mean proportion of colonies; (B) mean colony size; (C) C:N ratio; (D) mean biomass. Bars represent one SD.

APPENDIX B – METHODS FOR MODELING ZERO-INFLATED CONTINUOUS ECOLOGICAL DATA

Abstract

Statistical methods for modelling zero-inflated count data may or may not be applicable for zero-inflated continuous data. However, conditional models for count data can be adapted to model zero-inflated continuous data, by splitting the analysis into two components: (1) a binomial logit generalised linear model (GLM) on binary data for the first component and (2) a GLM on zero-truncated data for the second component. Tweedie GLM is a potential alternative to conditional models because it can admit exact zeros. Here, Tweedie GLM and conditional models were examined with 4 ecological datasets that consisted of zero-inflated continuous data. Firstly, the goodness-of-fit values of Tweedie GLM were compared to those of log-normal GLM and gamma GLM on natural logarithm transformed zero-inflated continuous data. Gamma GLM on $ln(x+1)$ transformed zero-inflated continuous data had an overall good fit among the models examined. Secondly, the goodness-of-fit values were compared among Tweedie GLM, log-normal GLM, and gamma GLM on natural logarithm transformed zero-truncated data to evaluate the second component of the conditional model. Log-normal GLM on natural logarithm transformed zero-truncated data had an overall good fit among the models examined. In summary, application of (a) Tweedie GLM on transformed zero-inflated continuous data and

(b) conditional models are inherently different, but both methods can be adapted with preference to conditional models.

B-1 Introduction

The task of modelling zero-inflated (i.e., excess zeros) and continuous (i.e., non-count) ecological data can be challenging, especially if conventional transformations of the data fail to normalise the distribution. One underlying assumption of an analysis of variance (ANOVA) is that the values are distributed normally, although ANOVA is often reasonably robust. The generalised linear model (GLM) is an alternative to non-parametric approaches. GLMs have gained popularity to model data that are not normally distributed by specifying distribution families and link function parameters.

A number of methods using GLM are available to model zero-inflated count data, but they may or may not be applicable for zero-inflated continuous data. Conditional models, which split the analysis into two components, have been a longstanding approach. Zuur et al. (2010) encourage ecologists to model zero-inflated count data with GLM. Depending on the statistical package, distribution families (also referred to as probability distributions) that can be applied with GLM include: Poisson, negative binomial (NB), general Poisson (GP), zero-inflated Poisson (ZIP), zero-inflated negative binomial (ZINB), and zero-inflated general Poisson (ZIGP; Özmen and Famoye 2007). Additionally, compound distribution families, particularly the Tweedie distribution, are suitable for modelling continuous data containing exact zeros. Here, I adapted (1) GLM using the Tweedie distribution

family and (2) the two-component conditional models to model zero-inflated continuous ecological data.

The canonical link function for the Tweedie distribution family is the log link function. Unlike the log-normal and gamma distribution families, Tweedie is a compound Poisson-gamma distribution that allows for exact zeros. GLM using the Tweedie distribution (referred to as Tweedie GLM) has been successfully used for count data in disciplines such as fisheries science (catch data) and meteorology (rainfall data).

Two-component conditional models are also known as hurdle models, compatible models, or simply conditional models. The zero mass is referred to as the hurdle. Zeros can be either structural zeros, sampling zeros, or false zeros (Martin et al. 2005). Structural zeros are true zeros and sampling zeros are zeros that occur by chance. False zeros are a matter of detection, e.g., when values are too small and evade detection. For the first component of the conditional model, the presence and absence data (binary data) is modelled with GLM using the binomial distribution (referred to as binomial logit GLM) and the canonical logit (logistic) link function. The second component considers only non-zero values (zero-truncated data). For count data, the zero-truncated data can be modelled with GLM using Poisson (truncated Poisson), NB (truncated NB), extended Poisson, log-normal, or gamma distribution families (Mayer et al. 2005). Gamma GLM tends to fit better than log-normal GLM on zero-truncated data (Myers and Pepin 1990). Log-normal GLM (with the hyphen) and lognormal GLM (without the hyphen) should be distinguished. Log-

normal GLM consists of the normal distribution family and the log link function. Lognormal GLM consists of the normal distribution family and the canonical identity link function on logarithm transformed data (Hardin and Hilbe 2007).

B-2 Methods and results

B-2-1 Data

Four datasets were considered for the comparison of statistical methods to model zero-inflated continuous ecological data for a colonial ascidian, *Botryllus schlosseri*, in Arnold's Cove, Placentia Bay, NL, Canada: recruitment on different substrate types (RDST) during the 2010 field season, recruitment on PVC (RPVC) during the 2010 and 2011 field seasons, density of colonies on wharf pilings (DCWP), and colony cover on wharf pilings (CCWP). Data collected during the non-recruitment season were removed from the RDST and RPVC datasets. For the RDST dataset, categorical independent variables are sampling date (five classes), sampling location (three classes), depth (three classes), and substrate type (three classes). For the RPVC dataset, categorical independent variables are year (two classes), month (four classes), sampling location (three classes), and depth (three classes). For the DCWP and CCWP datasets, categorical independent variables are sampling date (10 classes) and sampling zone (three classes). All models include main effects and all two-way interaction effects. Full factorial models were not considered because the sample size for each unique combination of factors was small and because three-way and higher interaction terms are difficult to interpret ecologically. Descriptive

statistics for these datasets are summarised in Table B-1. Their respective histogram distributions are skewed to the right (positive skew; Figure B-1).

Table B-1 Descriptive statistics of zero-inflated continuous datasets: recruitment on different substrate types (RDST) during the 2010 field season, recruitment on PVC (RPVC) during the 2010 and 2011 field seasons, density of colonies on wharf pilings (DCWP), and colony cover on wharf pilings (CCWP). Zero values during the non-recruitment period were excluded in the RDST and RPVC datasets.

	RDST (recruits $m^{-2}d^{-1}$)	RPVC (recruits $m^{-2}d^{-1}$)	DCWP (colonies m^{-2})	CCWP (%)
Population of zeros	59.3 %	33.3 %	56.4 %	56.4 %
Mean	4.5 ± 8.6	10.4 ± 14.0	26.3 ± 40.3	1.5 ± 2.6
Mean (> 0)	11.1 ± 10.5	15.5 ± 14.6	60.2 ± 41.0	3.5 ± 3.0
Median (> 0)	8.5	11.8	55.5	2.7
Maximum	74.4	74.4	249.7	20.3
Skewness	3.583	2.179	1.975	2.680
Skewness (> 0)	2.787	1.959	1.603	2.147

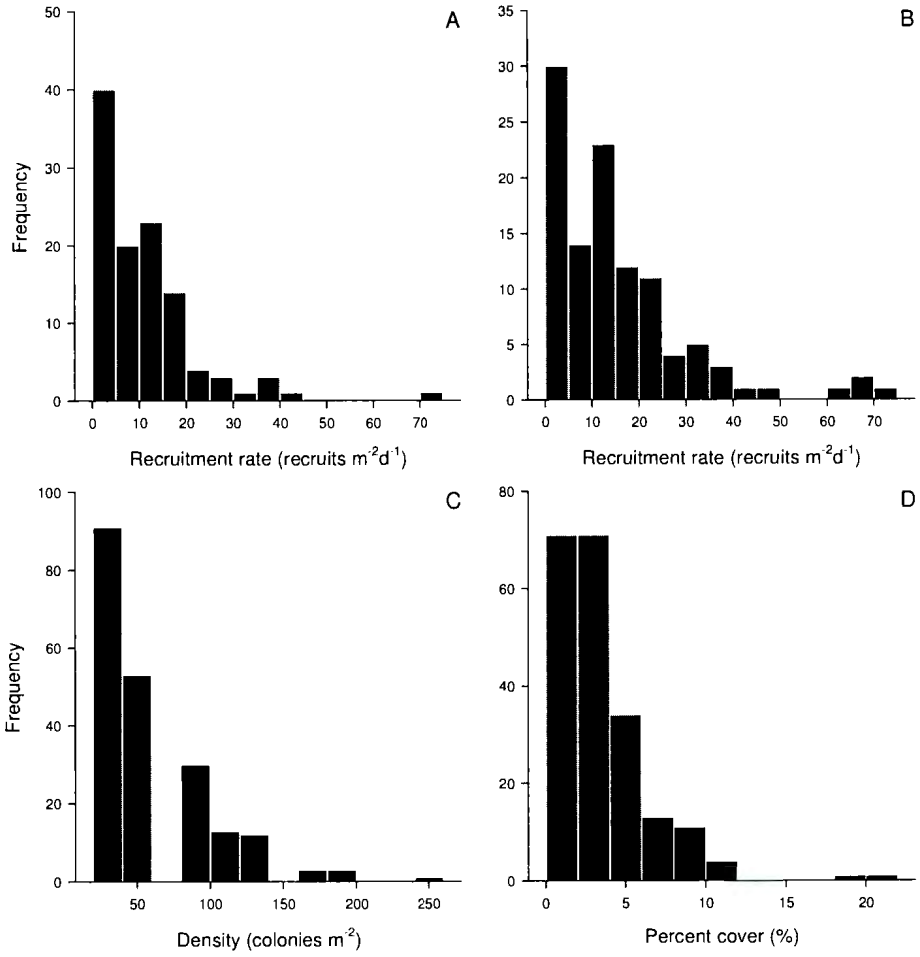

Figure B-1 Histogram distribution of zero-truncated datasets: (A) recruitment on different substrate types (RDST) during the 2010 field season; (B) recruitment on PVC (RPVC) during the 2010 and 2011 field seasons; (C) density of colonies on wharf pilings (DCWP); (D) colony cover on wharf pilings (CCWP).

B-2-2 Shapiro-Wilk test for normality

A majority of the transformations that were applied to the datasets failed the Shapiro-Wilk test for normality (Table B-2). Transformations that were applied were: (1) natural logarithm, (2) power, (3) square root, and (4) fourth root. Transformation of datasets that contained exact zero values incorporated a constant. Constants that were used included C = 0.1, C = 1, and C = 10. With the exception of natural logarithm and fourth root transformed zero-truncated CCWP dataset, all other transformed ecological datasets were not normally distributed.

Table B-2 Shapiro-Wilk test for normality on untransformed (x) and natural logarithm and fourth root transformed data. Transformed data that passed the test are indicated with an asterisk (*). All power and square root transformations failed the test (results not shown).

	W-Statistics									
	Zero-inflated continuous data							Zero-truncated data		
Dataset	x	$ln(x+0.1)$	$ln(x+1)$	$ln(x+10)$	$(x+0.1)^{0.25}$	$(x+1)^{0.25}$	$(x+10)^{0.25}$	x	$ln(x)$	$(x)^{0.25}$
RDST	0.583	0.719	0.745	0.711	0.747	0.744	0.692	0.729	0.913	0.906
RPVC	0.724	0.815	0.882	0.878	0.888	0.899	0.859	0.795	0.963	0.960
DCWP	0.698	0.695	0.721	0.759	0.741	0.754	0.766	0.777	0.836	0.835
CCWP	0.649	0.749	0.763	0.711	0.768	0.756	0.699	0.818	0.989*	0.992*

B-2-3 GLM on zero-inflated continuous data

Untransformed and natural logarithm (*ln*) transformed zero-inflated datasets were modelled in SPSS 19. The models consisted of Tweedie GLM, log-normal GLM and gamma GLM. The log link function was used in the gamma GLM because the canonical inverse link function was not available in SPSS 19. The log likelihood goodness-of-fit values were recorded and compared to find the model with the best overall fit. Gamma GLM on *ln(x+1)* transformed data performed well with an overall good fit among the models examined (Table B-3). GLM on untransformed and *ln(x+10)* transformed data did not have consistent goodness-of-fit values (most values greatly deviated from zero).

B-2-4 Two-component conditional models

Conditional models were modelled in SPSS 19 by splitting the analysis into two components: (1) a binomial logit GLM on binary data for the first component and (2) a GLM on zero-truncated data for the second component. The binary data consisted of presence and absence values and was modelled with a binomial logit GLM. Tweedie GLM, log-normal GLM, and gamma GLM on untransformed and natural logarithm transformed zero-truncated data were modelled for the second component of the conditional model. The log likelihood goodness-of-fit values were recorded and compared to find the model with the best overall fit. Log-normal GLM on *ln(x)* transformed data performed well with an overall good fit among the models examined (Table B-3).

Table B-3 Log likelihood goodness-of-fit values among Tweedie GLM, log-normal GLM, and gamma GLM on zero-inflated continuous data, and zero-truncated data for the second component of the two-component conditional model. RDST = recruitment on different substrate types; RPVC = recruitment on PVC; DCWP = density of colonies on wharf pilings; CCWP = colony cover on wharf pilings.

		Log likelihood goodness-of-fit					
		Zero-inflated continuous data				Zero-truncated data	
GLM distribution	Dataset	Untransformed	*ln*(x+0.1)	*ln*(x+1)	*ln*(x+10)	Untransformed	*ln*(x)
Tweedie	RDST	-4.3×10^2	-5.6×10^1	-2.8×10^2	3.2×10^1	-2.9×10^2	-5.8×10^1
	RPVC	-4.2×10^2	-7.5×10^1	-2.5×10^2	-2.5×10^1	-3.3×10^2	-7.7×10^1
	DCWP	-1.3×10^3	-1.6×10^2	-7.8×10^2	-5.6×10^2	-9.9×10^2	-1.6×10^2
	CCWP	-7.4×10^2	-1.6×10^2	-5.5×10^2	1.9×10^2	-4.4×10^2	-1.4×10^2
Log-normal	RDST	-7.4×10^2	-5.6×10^2	-2.6×10^2	1.3×10^1	-3.2×10^2	-5.7×10^1
	RPVC	-5.6×10^2	-3.1×10^2	-1.8×10^2	-3.3×10^1	-3.7×10^2	-7.4×10^1
	DCWP	-2.4×10^3	-1.2×10^3	-9.3×10^2	-5.9×10^2	-1.0×10^3	-1.7×10^2
	CCWP	-1.1×10^3	-2.9×10^3	-4.9×10^2	1.7×10^2	-5.0×10^2	-2.4×10^2
Gamma	RDST	-2.9×10^2	-5.7×10^1	-4.3×10^1	3.8×10^1	-2.9×10^2	-5.9×10^1
	RPVC	-3.3×10^2	-7.8×10^1	-6.4×10^1	-2.3×10^1	-3.3×10^2	-8.0×10^1
	DCWP	-9.8×10^2	-1.6×10^2	-1.6×10^2	-5.5×10^2	-9.8×10^2	-1.6×10^2
	CCWP	-4.3×10^2	-1.8×10^2	-1.6×10^2	1.9×10^2	-4.3×10^2	-1.6×10^2

B-3 Discussion

Gamma GLM on *ln(x+1)* transformed zero-inflated continuous data performed better than Tweedie GLM and log-normal GLM and than on untransformed or other natural logarithm transformed data with C = 0.1 and C = 10. For the second component of the conditional model, log-normal GLM on *ln(x)* transformed zero-truncated performed better than Tweedie GLM and gamma GLM and than on untransformed data.

The two methods (GLM on zero-inflated continuous data and two-component conditional models) led to two potentially different interpretations. However, Tweedie GLM cannot compute data with high levels of excess zeros despite the advantage of admitting exact zeros and the ability to fit data with over-dispersion. Cunningham and Lindenmayer (2005) suggest that the simplicity in analysing and interpreting two-component conditional models is an advantage. Min and Agresti (2002) termed non-negative continuous data with a zero mass as semi-continuous data if exact zeros are structural zeros. However, the exact zeros from this study are chiefly considered as sampling zeros and or false zeros. Nonetheless, a comparative review of methods to model semi-continuous data identified the advantage of the two-component conditional model to be: (1) the data is modelled in its original form, (2) simple to fit, and (3) relatively simple to interpret (Min and Agresti 2002). In conclusion, the application of conditional models is preferred over GLM (e.g., Tweedie GLM) for zero-inflated data.

APPENDIX C – THE OOZOOID AND THE FIRST BLASTOZOOID OF *BOTRYLLUS SCHLOSSERI*

Abstract

The colonial ascidian, *Botryllus schlosseri*, is a temperate species that has been expanding its range into subarctic waters. The relatively short productive and long winter seasons should exert strong selective pressure for genotypic and phenotypic adaptations. Retardation in the development rate of the oozooid is a possible adaptation to the subarctic marine environment, which could substantially change the interpretation of the recruitment patterns presented in Chapter 3. Therefore, in this study, I investigated the oozooid and the first blastozooids of *B. schlosseri* originating from a subarctic population. In the laboratory, I observed and documented the early life history from larval settlement through metamorphosis to the end of the first blastogenic cycle. The morphology of the larva and the oozooid reared from colonies of subarctic origin was identical to those reared from colonies of temperate origin as described in the literature. Furthermore, the oozooid of subarctic origin lived for 9-10 d at 20°C before undergoing the first blastogenic cycle, which is similar to the oozooid of temperate origin at the same temperature. Colonies with two or more blastozooids were first observed on days 16-17 after initial settlement. These findings suggest that there are no marked differences in the development of the oozooid of subarctic and that of temperate origin.

C-1 Introduction

Botryllus schlosseri (Pallas, 1766) is a temperate ascidian species native to the Mediterranean Sea (Carver et al. 2006a, Lejeusne et al. 2011). For over 50 years, *B. schlosseri* has maintained a cosmopolitan distribution that is mainly in temperate waters, yet northerly populations persist in the boreal waters of Alaska, northern Japan, and Norwegian fjords. The extensive populations in the coastal waters of insular Newfoundland represent an unprecedented expansion of its global range into subarctic waters. The success of *B. schlosseri* in a novel system may be largely attributable to its broad physiological tolerance to environmental conditions. In particular, it is relatively eurythermal and euryhaline (Brunetti et al. 1980). Strong evolutionary pressures are likely to be exerted on *B. schlosseri* under subarctic conditions that are characterised by short productive seasons. Such pressures may result in genotypic and phenotypic adaptations. For example, lower temperatures may delay sexual maturity or result in reduced development (e.g., growth trajectory, lifespan) and somatic growth rates. Variability in development rates of oozooids from Arnold's Cove, Placentia Bay, NL, Canada, could affect the interpretation of recruitment patterns discussed in Chapter 3. For instance, a substantial retardation in the development of the oozooid from larval settlement to the end of the first blastogenic cycle represents a potential time lag in detecting recruitment within the 1-1.5 month deployment period. Thus, in this study, I tested the hypotheses that (1) complete metamorphosis from larva to a functional oozooid is greater than 1 d, (2) the first blastozooids are less than 1 mm in diameter, and (3) the development from

larval settlement to the first blastozooid takes longer than one week at 20°C in *B. schlosseri* from insular Newfoundland.

C-2 Materials and methods

C-2-1 Source of larvae

Colonies of *Botryllus schlosseri* were collected on July 15 (n = 3) and August 1 (n = 1), 2011, in Arnold's Cove, Placentia Bay, NL, Canada, when seawater temperatures were 11 and 14°C respectively. These colonies were fully immersed in ambient seawater in a 40 L insulated container during the 1.5 h transport from the field to the laboratory. At the laboratory, these colonies were placed in a common holding tank (500 L) filled with static seawater (source = Logy Bay, NL, Canada) and held at 20°C. A mixed diet of Instant Algae® (Shellfish Diet 1800™) that consisted of *Isochrysis* spp., *Pavlova* spp., *Thalassiosira weissflogii*, and *Tetraselmis* spp. was supplied (10-15 mL) after a manual change of water every weekday. Discard water was chlorinated at a minimum of 5 ppm for at least 12 h before discharge. These colonies were the source of larvae for the experiments. The release of larvae was induced by shining two sources of microscope fibre optic, all-spectrum light (Micro-Lite FL2000) in air for 10-15 minutes at a distance of ca. 5 cm from the parent colony, which was being held in static ambient seawater in a glass beaker. The application of this method yielded 30-60 larvae per colony. Selected larvae and oozooids (immersed in seawater) were photographed through a stereo microscope with a 7.1 megapixel Canon PowerShot SD750 digital camera.

C-2-2 Analysis of larval settlement

Following their release on July 19 (n = 8) and on August 8, 2011 (n = 10), larvae were individually transferred with a pipette into a small Petri dish filled with ambient seawater. The maximum chord length of the larval head, tail, and total body length was measured under a stereo microscope to the nearest 0.1 mm. Measurements were taken on July 19 of larvae 1 h after release. Measurements were taken on August 8 of sessile individuals during the metamorphosis of the larva into the oozooid 1 h after settlement.

C-2-3 Analysis of development rate

Following their release on July 19 (cohort A on 15 dishes), July 25 (cohort B on five dishes), and August 8 (cohort C on seven dishes), 2011, larvae were haphazardly organised into groups of 5-10 per Petri dish filled with ambient seawater. Spots were drawn with a permanent ink marker on the underside of the dish to identify the location of each newly-settled individual. The dishes were placed in a common holding tank as described above on the same day the larvae were released. Individuals that settled on the dishes after being placed in the holding tank were not analysed. The maximum chord length of the oozooid (the body inside the tunic) and its respective tunic was measured daily, except on weekends, under a stereo microscope to the nearest 0.1 mm. A total of 354 measurements (cohort A = 259, cohort B = 58, and cohort C = 37) were recorded during the course of this study. The body length to tunic length ratio was determined for each oozooid and first

blastozooid. The study was terminated when colonies consisted of two or more blastozooids.

C-3 Results

C-3-1 Larval settlement

All 18 larvae reared at 20°C settled on Petri dishes ca. 1 h (maximum of ca. 3 h) after release. The maximum chord length of the larval head tended to increase (ca. 125%) and that of the tail tended to decrease (ca. 75%) during the metamorphosis of the larva into the oozooid (Figures C-1 and C-2).

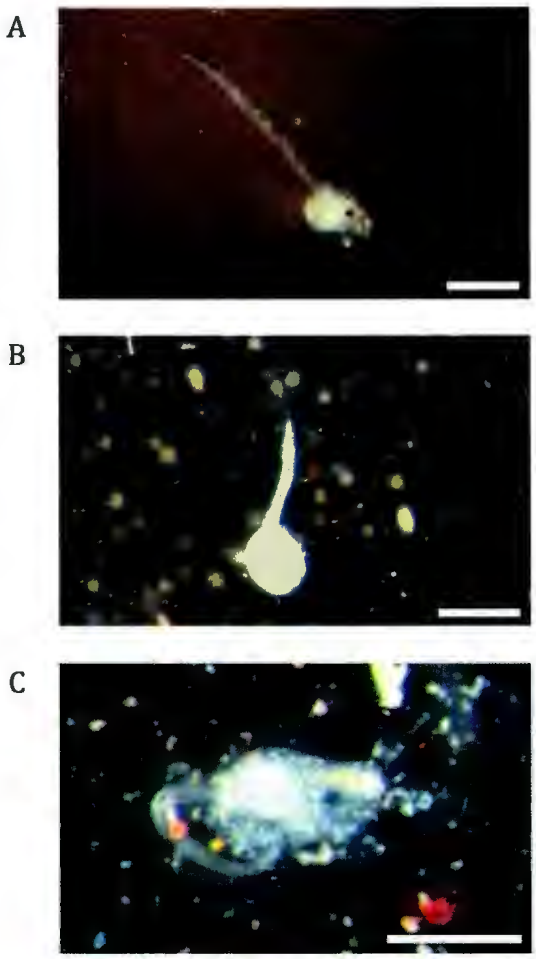

Figure C-1 Larval settlement and metamorphosis in *Botryllus schlosseri*. (A) Larva; (B) sessile individual undergoing metamorphosis 1 h after settlement; (C) sessile individual undergoing metamorphosis more than 1 h after settlement. Scale bars = 0.5 mm.

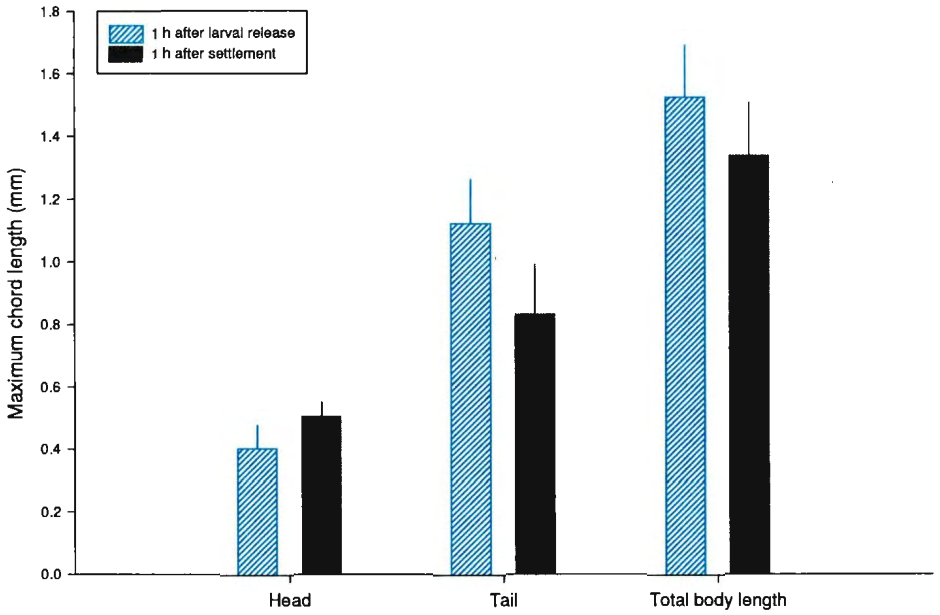

Figure C-2 Anatomical changes in *Botryllus schlosseri* during metamorphosis from the larva into the oozooid. Head, tail, and total body lengths of the larva 1 h after release (n = 8) and the sessile individual undergoing metamorphosis 1 h after settlement (n = 10). Bars represent one SD.

C-3-2 Oozooid and first blastozooid development

The development of the oozooid and the first blastozooids until the end of the first blastogenic cycle is summarised in Table C-1 and Figure C-3. The growth trajectory is shown in Figure C-4. The non-feeding oozooid exhibited a photolith and eight ampullae from 7 h to 1 d after settlement. A beating heart and body contractions were first observed on day 2. Well-formed siphons were evident by day 3, i.e., the oozooid became functional. High mortality and signs of de-growth were noted from day 6. Primary buds (one bud per oozooid) were visible as early as day 7. The next day, fecal pellets accumulated around the oozooid. The oozooid lived for 9-10 d before undergoing the first blastogenic cycle. First blastozooids can be identified by the stigmata oriented parallel to the body axis (Manni and Burighel 2006). Gradual addition of ampullae around the blastozooid occurred from day 10 onwards. The end of the first blastogenic cycle occurred as early as day 16-17, which resulted in colonies with ≥ 2 blastozooids. Lastly, the body length to tunic length ratio appeared to be more stable several days before (days 6-10) and after (day 13 onwards) the start of the first blastogenic cycle (Figure C-4).

Table C-1 Observations in the development of the oozooid and first blastozooid of *Botryllus schlosseri* of subarctic origin until the end of the first blastogenic cycle.

Time (d)	Observation
0.3-1	Photolith present, 8 ampullae
2	Beating of heart and body contractions
3	Well-formed siphons
6	High mortality rate and signs of de-growth
7	1 primary bud on oozooid
8	Accumulation of fecal pellets around oozooid
9-10	Start of first blastogenic cycle
10	> 8 ampullae per blastozooid
13	≥ 1 primary bud(s) on blastozooid
16-17	End of first blastogenic cycle, ≥ 2 blastozooids per colony

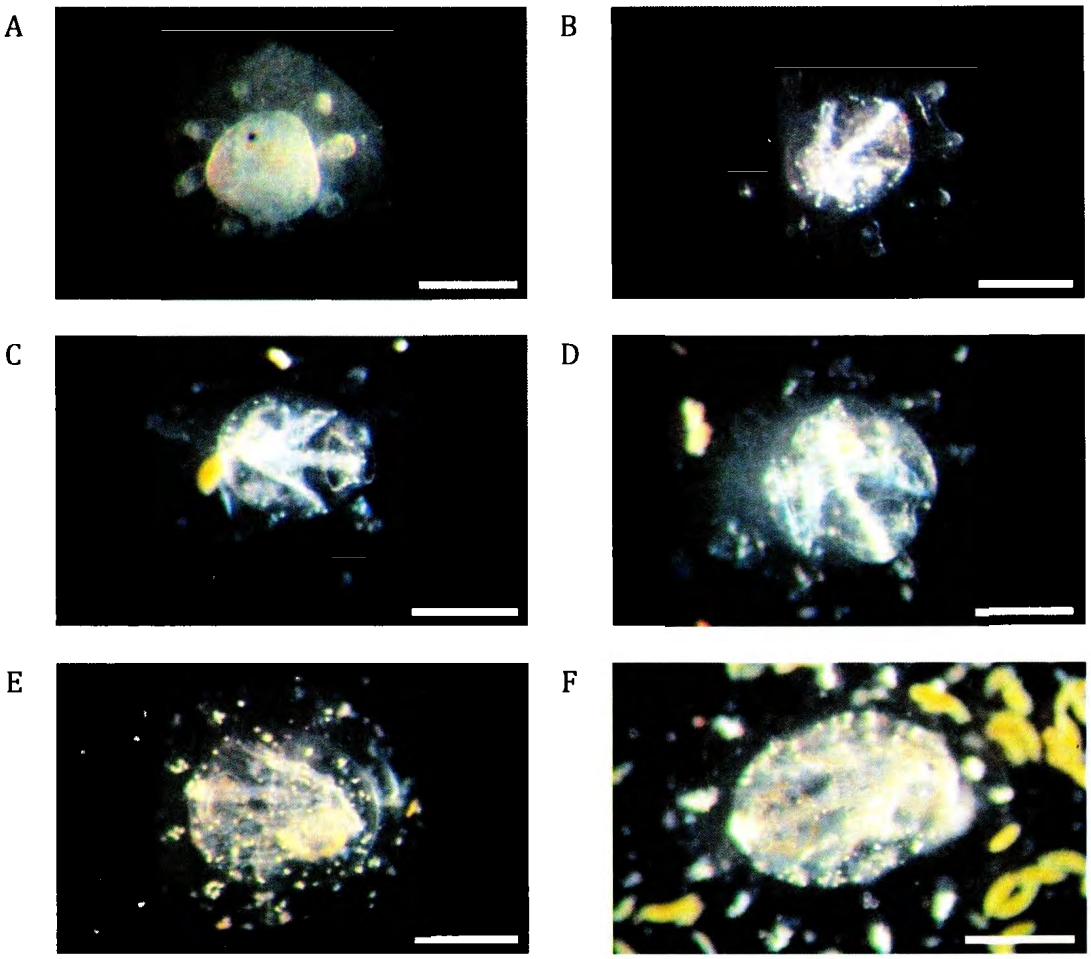

Figure C-3 Oozooid and first blastozooid of *Botryllus schlosseri*. (A) Non-feeding oozooid on day 1; (B) functional oozooid on day 2; (C) oozooid on day 3; (D) oozooid on day 7; (E) first blastozooid on day 10; (F) first blastozooid on day 13. Scale bars = 0.5 mm.

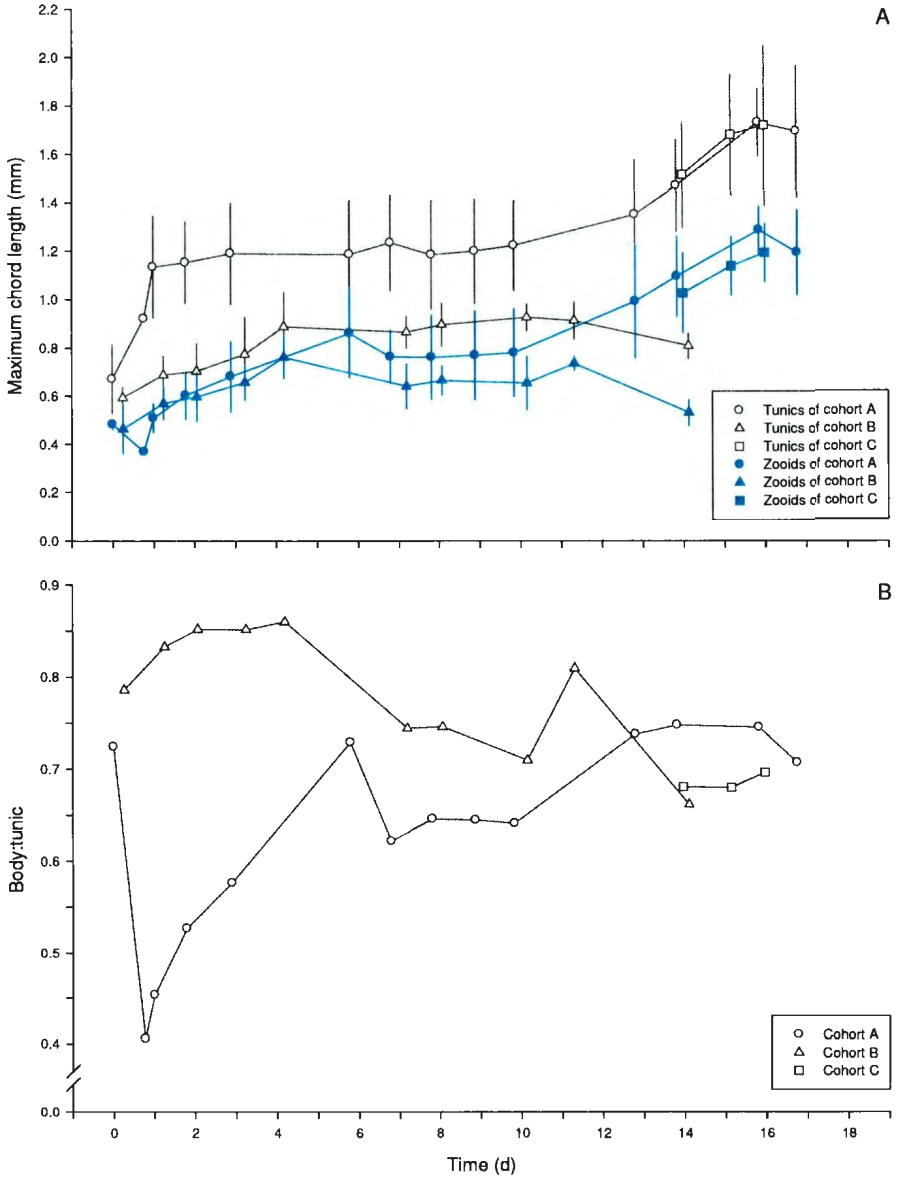

Figure C-4 Development of the oozooid and first blastozooids of *Botryllus schlosseri.* (A) Growth trajectory of oozooids (until days 9-10) and first blastozooids (days 9-10 onwards) and their respective tunics; (B) relationship between the length of the body (the zooid inside the tunic) and the tunic expressed as a ratio (body:tunic). Bars represent one SD. Cohort A: mean n = 19 (range 2-34) observations per data point; cohort B: mean n = 6 (range 2-10); an cohort C: mean n = 12 (range 10-16).

C-4 Discussion

The time taken for complete metamorphosis of the settling larva into an oozooid and the lifespan of the oozooid of *Botryllus schlosseri* of subarctic origin were the same as oozooids from temperate waters at similar temperatures. According to the literature, the larva of *B. schlosseri* settles at greatest frequency ca. 1-3 h (ranging from 13 minutes to 27.7 h) after release under laboratory conditions (Grave and Woodbridge 1924). The duration of the larval stage is about 1 h (Ben-Shlomo et al. 2010); however, older literature often cites 24 or 36 h (Millar 1971, Boyd et al. 1986, Grosberg 1988).

Metamorphosis is complete within 36-48 h of settlement, resulting in a fully functional oozooid about 0.5 mm long (Boyd et al. 1990, Manni et al. 2007). This is consistent with my study in which functional oozooids were observed by day three. The duration of the oozooid stage is ca. one week at 19-20°C (Watterson 1945, Boyd et al. 1990, Rabinowitz and Rinkevich 2004, Manni et al. 2007). This indicates that there was no apparent difference in development rate between the oozooids of *Botryllus schlosseri* of subarctic and those of temperate origin at the same temperature.

Although the cohort of larvae that I observed originated from a single colony, there was a possibility of genetic variability because the eggs may have been fertilised by sperm from other colonies in the field or in the holding tank. Therefore, it is assumed that each larva was a genetically unique individual. This study did not determine the rate of larval and oozooid mortality.

In conclusion, this study does not support my first hypothesis that metamorphosis from larva to functional oozooid is greater than 1 d as predicted in the literature. Larvae settle and metamorphose into functional oozooids within 24 h of larval release in subarctic waters of insular Newfoundland. Furthermore, this study does not support my second hypothesis that the first blastozooid is less than 1 mm long, suggesting that its development is not delayed. First blastozooids can reach a length of ca. 1 mm, which is within the detection limits of the imaging methodology used in Chapter 3. Lastly, this study does not support my third hypothesis that the appearance of the first blastozooid at 20°C occurs later than ca. 1 week as predicted in the literature. This indicates that development of the oozooids and first blastozooids at 20°C is not longer than is typical of temperate populations. First blastozooids were observed from days 9-10 post-settlement until days 16-17, which suggests that recruits (including small colonies) on artificial plates used in Chapter 3 should be observable within the four week deployment.

APPENDIX D – DICHOTOMOUS KEY TO MORPHOTYPES OF *BOTRYLLUS SCHLOSSERI*

Abstract

The colonial ascidian, *Botryllus schlosseri*, exhibits a wide range of colour patterns, which can be a source of taxonomic confusion. The aim of this investigation was to identify morphotypes of *B. schlosseri* and to develop a dichotomous key to assist in future field identifications. Colonies attached to algae, mussels, and wharf structures were collected and photographed with a digital camera. A total of six discrete, two-state character traits that were easily discerned from examining photographs of colonies, were selected. In insular Newfoundland, I examined photographs of colonies that were collected in Arnold's Cove, Placentia Bay, NL, Canada, (on November 24, 2009, and October 12, 2010) and in Foxtrap, Conception Bay, NL, Canada, (on December 5, 2011). All 130 colonies were scored for the presence or absence of each trait. A total of 10 morphotypes was determined, which offers an insight into the diversity of colour patterns that can be observed in colonies of *B. schlosseri* in insular Newfoundland.

D-1 Introduction

Colour polymorphism, coupled with high genetic variability, has been widely reported in colonies of *Botryllus schlosseri* (Pallas, 1766). This polymorphism can contribute to taxonomic confusion. For instance, *B. schlosseri* from the south coast of insular Newfoundland exhibits high genetic variability in the cytochrome *c* oxidase

subunit I (COI), a mitochondrial gene sequence (Callahan et al. 2010). In fact, the genetic diversity of *B. schlosseri* is greater than in *Botrylloides violaceus*, a closely related ascidian species (Callahan et al. 2010, Lejeusne et al. 2011). Boyd et al. (1990) reported that, with the exception of colour, the morphology and development of *B. schlosseri* from Monterey, California, and from Woods Hole, Massachusetts, were identical despite these two populations being in separate oceans. Furthermore, genetic evidence suggested that *B. schlosseri*, as a non-indigenous species, on the west and east coasts of North America originated from different populations (Stoner et al. 2002).

Variability in colour patterns in colonies of *Botryllus schlosseri* is attributable to granules, which are sometimes referred to as either pigmented cells or nephrocytes (Watterson 1945). Differently pigmented granules, which can be blue (granular), orange (carotenoid in solution), or white (granular, purine derivative), are embedded in the epithelium of the zooid (Milkman 1967). Hence, the zooid and the ampullae can be covered with a combination of different types of granules, which can result in discontinuous colour patterns such as intersiphonal bands, inhalent rings, or peristomatic rings.

An intersiphonal band is defined as the dorsal double band that occupies the space between the cloaca and the inhalent siphon in each zooid (Figure D-1). The natural frequency of colonies that exhibit intersiphonal bands may differ among populations (Boyd et al. 1990). Watterson (1945) noted that early drawings of *Botryllus schlosseri* clearly show the presence of these bands, and he described their

development. An inhalent ring is defined as the aggregation of pigmented granules around the inhalent siphon region (Figure D-2), and a peristomatic ring the aggregation of pigmented granules around the cloaca (Figure D-3).

The fundamental objectives of this study were to determine the number of morphotypes of *Botryllus schlosseri* from insular Newfoundland, and to develop a dichotomous key to the morphotypes, which may assist in future field identification.

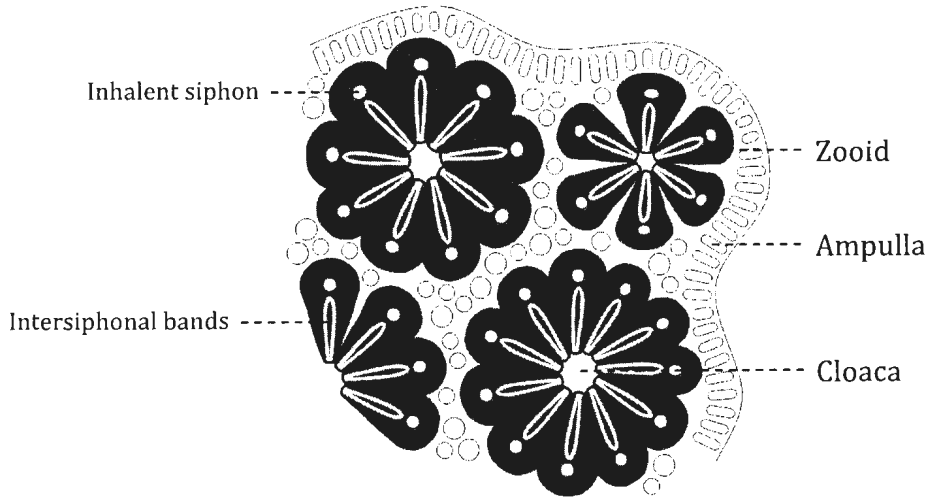

Figure D-1 Drawing of colony with intersiphonal bands.

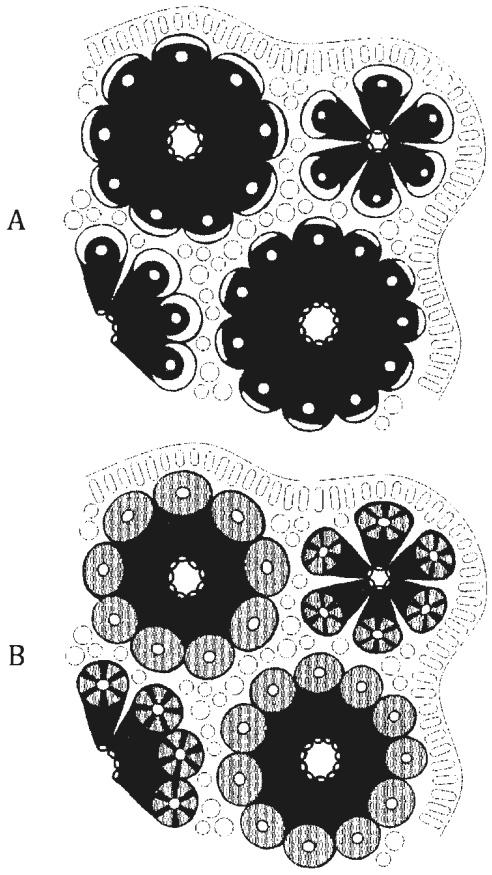

Figure D-2 Drawings of colonies with (A) pigmentation along the edge of the inhalent-siphon region and (B) pigmentation occupying the entire inhalent-siphon region.

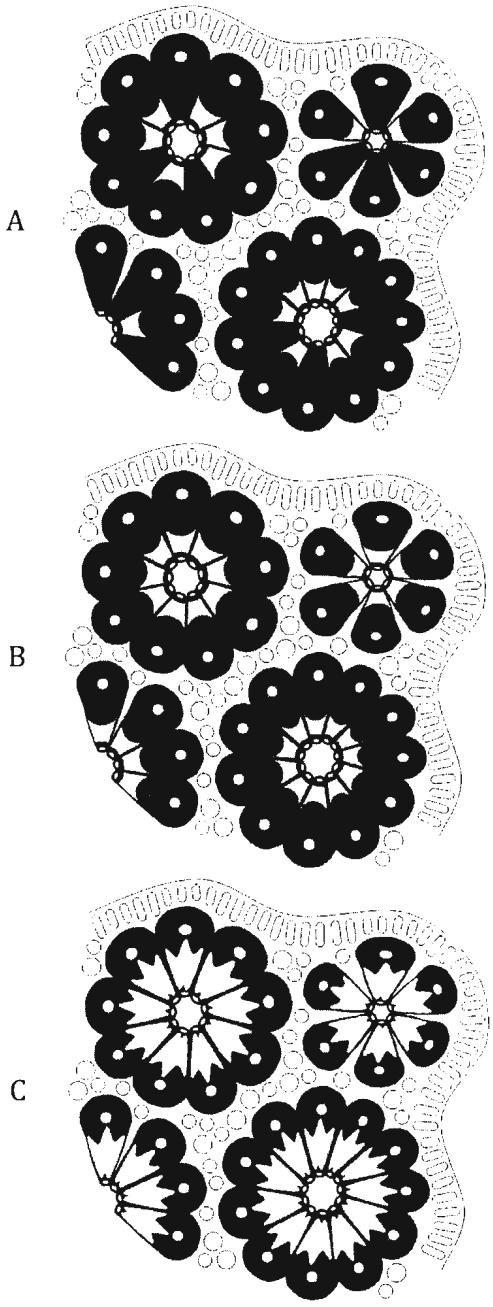

Figure D-3 Drawings of colonies with (A) incomplete pigmentation, (B) simple pigmentation, and (C) elaborate pigmentation around the cloaca.

D-2 Methods and results

D-2-1 Image analysis

Colonies of *Botryllus schlosseri* were collected on November 24, 2009, and October 12, 2010, in Arnold's Cove, Placentia Bay, NL, Canada, (47°45' N, 54°00' W) and on December 5, 2011, in Foxtrap, Conception Bay, NL, Canada, (47°31' N, 53°00' W). Images of whole colonies were taken using a 10 megapixel Canon PowerShot G11 digital camera (with 5x wide-angle zoom) and viewed with ImageJ (version 1.44) software. Colour and pattern details on colonies were normally viewed between 100-170% of full size on a 61 cm widescreen LCD monitor.

From photographs, all 130 colonies (n = 24 in 2009, n = 77 in 2010, and n = 29 in 2011) were scored for the presence or absence of six discrete two-state character traits: (1) intersiphonal bands, (2) inhalent rings, (3) peristomatic rings, (4) orange bands or rings, (5) translucent zooids, and (6) orange zooids. These traits were selected because they were easily discerned from the photographs. Twelve unique combinations of traits were found, but all morphs with intersiphonal bands were considered as a single morphotype, and all morphs with translucent zooids also combined into one morphotype, making a total of 10 morphotypes (Table D-1).

Table D-1 Morphotypes of *Botryllus schlosseri* and their respective two-state character traits. Morphotypes are listed in the order as they appear in the dichotomous key.

Morphotype	Character traits						*n*		
	Intersiphonal bands	Inhalent rings	Peristomatic rings	Colour of bands and or rings	Colour of zooids	Opacity of zooids	2009	2010	2011
Uniform orange morph	Absent	Absent	Absent	Not orange	Orange	Opaque	1	1	3
Orange peristomatic-ringed morph	Absent	Present	Present	Orange	Not orange	Opaque	0	0	2
Inhalent- and peristomatic-ringed orange morph	Absent	Present	Present	Not orange	Orange	Opaque	0	1	0
Inhalent-ringed orange morph	Absent	Present	Absent	Not orange	Orange	Opaque	1	2	1
White translucent morph	Absent	Present	Present	Not orange	Not orange	Translucent	0	2	1
Uniform morph	Absent	Absent	Absent	Not orange	Not orange	Opaque	2	4	0
Intersiphonal-banded morph	Present	Absent	Absent	Not orange	Not orange	Opaque	0	2	2
Inhalent-ringed morph	Absent	Present	Absent	Not orange	Not orange	Opaque	5	13	0
Inhalent- and peristomatic-ringed morph	Absent	Present	Present	Not orange	Not orange	Opaque	14	49	20
Peristomatic-ringed morph	Absent	Absent	Present	Not orange	Not orange	Opaque	1	3	0

D-2-2 Dichotomous key

A colony consists of several anatomical structures with which users of this dichotomous key should be familiar (see Section 1.4 in Chapter 1). Granules may be distributed at densities varying from sparse to dense. Consequently, inhalent rings and peristomatic rings may range from faint to solid and from simple to elaborate (Figures D-2 and D-3). Colonies with intersiphonal bands are relatively rare in populations from insular Newfoundland. Ampullae may or may not be visible in a given specimen. Moreover, users should be able to distinguish between pigmented ampullae and pigmented zooid systems visually because the key is concerned with colour patterns of zooid systems. The key consists of a series of paired decisions (in the form of written statements) by which the identity of a morphotype is resolved. The key is as follows:

1-A. Orange pigmentation present 2-A

1-B Orange pigmentation absent 5-A

2-A Zooid systems in the colony are uniformly orange in colour .....

..... uniform orange morph (Plate I)

2-B Zooid systems in the colony are not uniformly orange in colour 3-A

3-A Zooids are orange 4-A

3-B Zooids are not orange ..... orange peristomatic-ringed morph (Plate II)

4-A Peristomatic ring present .....

..... inhalent- and peristomatic-ringed orange morph (Plate III)

4-B Peristomatic ring absent ..... inhalent-ringed orange morph (Plate IV)

5-A Zooids are translucent with white pigmentation present .....

..... white translucent morph (Plate V)

5-B Zooids are not translucent with white pigmentation present 6-A

6-A Zooid systems in the colony are uniform in colour ... uniform morph (Plate VI)

6-B Zooid systems in the colony are not uniform in colour 7-A

7-A Intersiphonal bands present ..... intersiphonal-banded morph (Plate VII)

7-B Intersiphonal bands absent 8-A

8-A Peristomatic rings present 9-A

8-B Peristomatic rings absent ..... inhalent-ringed morph (Plate VIII)

9-A Inhalent rings present ..... inhalent- and peristomatic-ringed morph (Plate IX)

9-B Inhalent rings absent ..... peristomatic-ringed morph (Plate X)

D-2-3 Plates

The following plates can be used to verify the final decisions that arise from using the dichotomous key (Plates I to X). Magnified images of the colony are shown to draw attention to colour patterns such that whole colonies are not shown.

Plate I Uniform orange morph: photograph (left) and drawing (right). Colony was photographed in Arnold's Cove, Placentia Bay, NL, Canada, on November 24, 2009. Scale bar = 1 cm.

Plate II Orange peristomatic-ringed morph: photograph (left) and drawing (right). Colony was photographed in Foxtrap, Conception Bay, NL, Canada, on December 5, 2011. Scale bar = 1 cm.

Plate III Inhalent- and peristomatic-ringed orange morph: photograph (left) and drawing (right). Colony was photographed in Arnold's Cove, Placentia Bay, NL, Canada, on October 12, 2010. Scale bar = 1 cm.

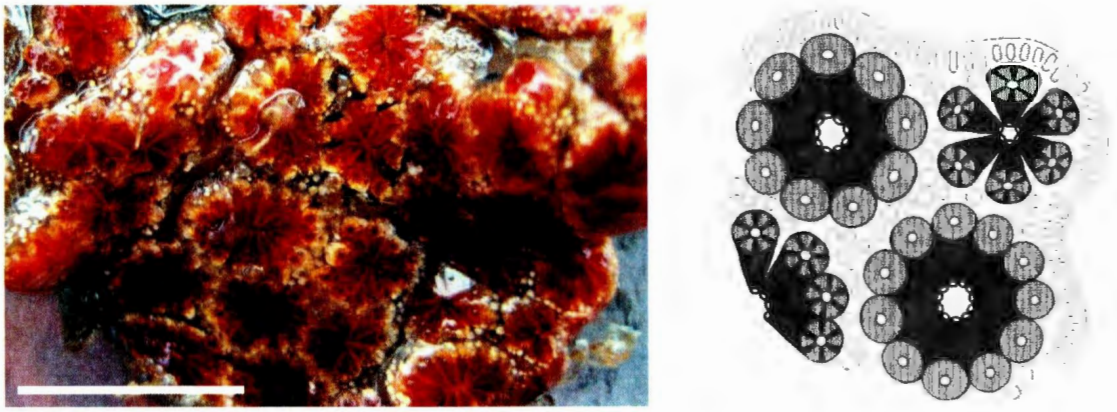

Plate IV Inhalent-ringed orange morph: photograph (left) and drawing (right). Colony was photographed in Arnold's Cove, Placentia Bay, NL, Canada, on November 24, 2009. Scale bar = 1 cm.

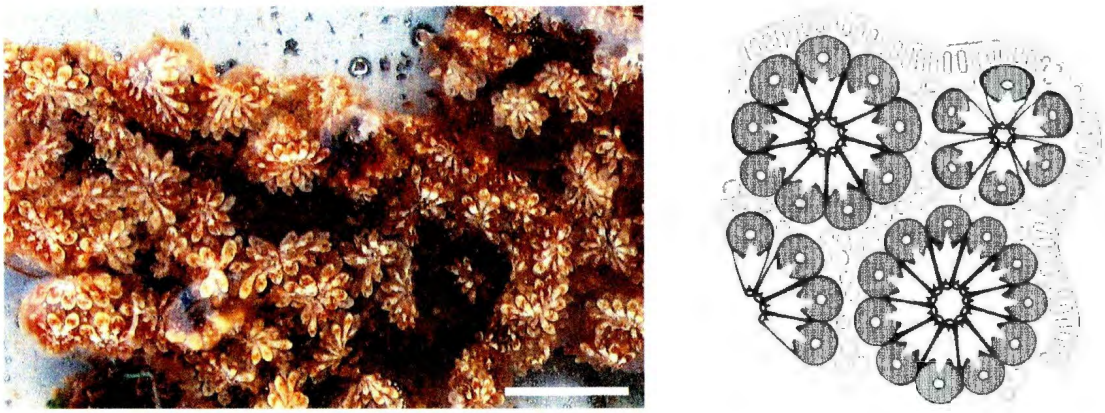

Plate V White translucent morph: photograph (left) and drawing (right). Colony was photographed in Arnold's Cove, Placentia Bay, NL, Canada, on October 12, 2010. Scale bar = 1 cm.

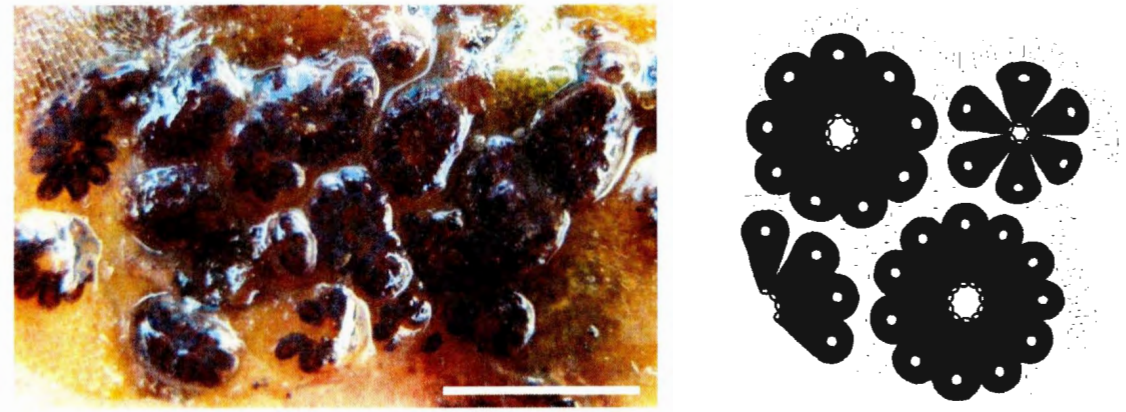

Plate VI Uniform morph: photograph (left) and drawing (right). Colony was photographed in Arnold's Cove, Placentia Bay, NL, Canada, on November 24, 2009. Scale bar = 1 cm.

Plate VII Intersiphonal-banded morph: photograph (left) and drawing (right). Colony was photographed in Arnold's Cove, Placentia Bay, NL, Canada, on October 12, 2010. Scale bar = 1 cm.

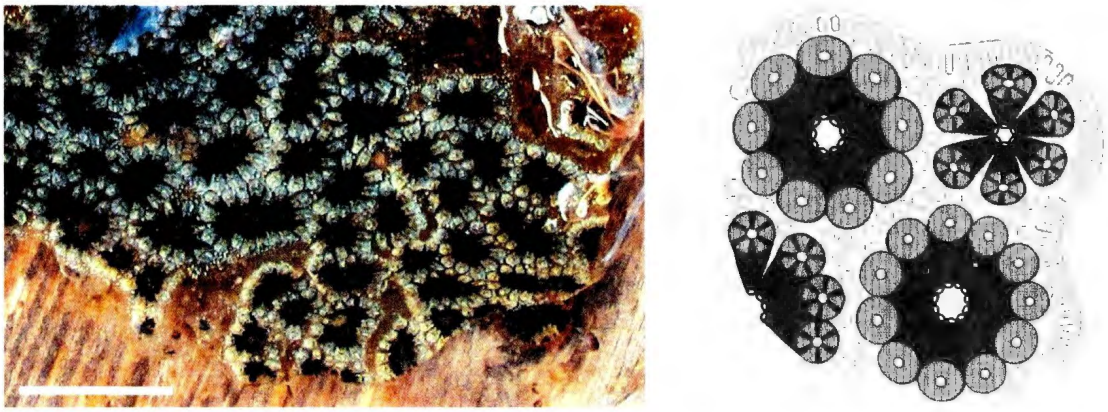

Plate VIII Inhalent-ringed morph: photograph (left) and drawing (right). Colony was photographed in Arnold's Cove, Placentia Bay, NL, Canada, on October 12, 2010. Scale bar = 1 cm.

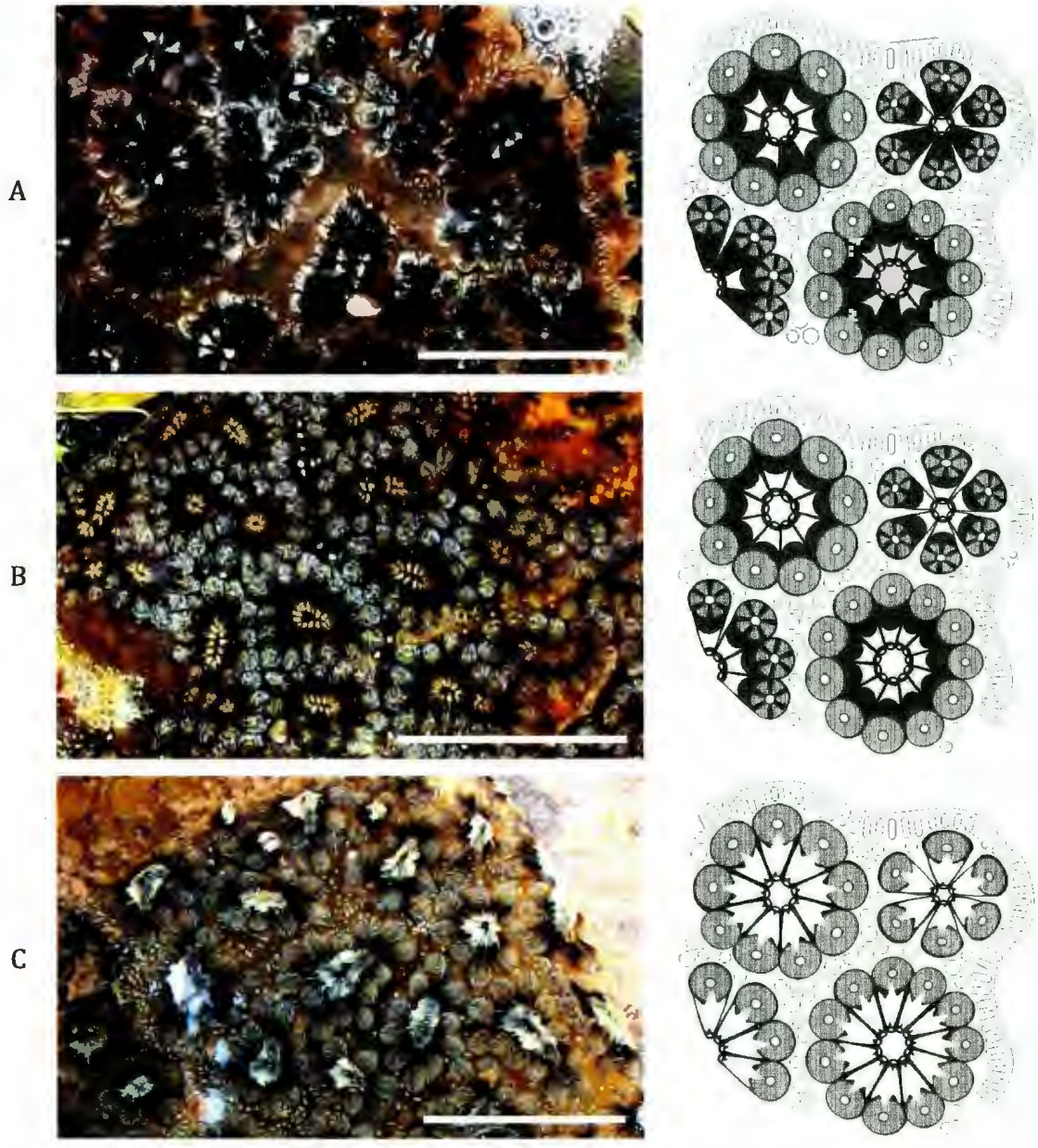

Plate IX Inhalent- and peristomatic-ringed morph: photographs (left) and drawings (right). Variations of peristomatic rings: (A) incomplete pigmentation, (B) simple pigmentation, and (C) elaborate pigmentation around the cloaca. Colonies were photographed in Arnold's Cove, Placentia Bay, NL, Canada, on October 12, 2010. Scale bars = 1 cm.

Plate X Peristomatic-ringed morph: photograph (left) and drawing (right). Colony was photographed in Arnold's Cove, Placentia Bay, NL, Canada, on October 12, 2010. Scale bar = 1 cm.

D-3 Discussion

The collection process targeted the less common morphotypes of *Botryllus schlosseri*, so the natural frequencies of all morphotypes were not determined. However, the proportion of each morphotype provides some insight into those that were more frequently encountered and those that were relatively rare (Table D-1).

There were potentially 64 unique combinations of the six discrete two-state character traits (2^6). New morphotypes may exist in other populations. For example, the 2011 collection of colonies from Foxtrap included a morphotype not previously encountered in the 2009 and 2010 collections from Arnold's Cove. Therefore, the key is limited to colonies collected during the autumn and winter seasons from two populations of *Botryllus schlosseri* in the waters of Newfoundland.

The diversity of colour polymorphism was remarkably high. The hypothesis that different morphotypes represent divergent species of *Botryllus* can be resolved with sufficiently sensitive genetic analyses (Applin et al., unpubished data), and with detailed life-history investigations (Lowen et al. 2010, 2011, 2012). In this study, the colour of zooids can be generally categorised as (1) orange or red, (2) translucent or very light brown, and (3) neither 1 nor 2. The last category includes zooids that were green, brown, purple, and black. Hence, a colour standard placed in the image frame of photographs of colonies can be used to compare and contrast subtle differences in colour (Stevens et al. 2007).

In conclusion, this study objectively identified morphotypes of *Botryllus schlosseri*. Future work should test the efficacy of the dichotomous key with naive and expert human subjects. The ability to recognise anatomical structures is essential for an effective use of the key. Such a test should consist of 2 components: (1) subjects should be tested on the ability to identify structures and colour patterns correctly, and (2) subjects should be tested to identify correctly the morphotype from a series of unidentified photographs of colonies. It may be advisable to test each subject with a different subset of photographs or with a different random order of the same photographs.

BIBLIOGRAPHY

Aitken, A. E., and Fournier, J. 1993. Macrobenthos communities of Cambridge, McBeth and Itirbilung Fjords, Baffin Island, Northwest Territories, Canada. Arctic 46 (1): 60-71.

Anger, K. 1978. Development of a subtidal epifaunal community at the island of Helgoland. Helgoland Marine Research 31 (4): 457-470.

Appeltans, W., Bouchet, P., Boxshall, G. A., De Broyer, C., de Voogd, N. J., Gordon, D. P., Hoeksema, B. W., Horton, T., Kennedy, M., Mees, J., Poore, G. C. B., Read, G., Stöhr, S., Walter, T. C., and Costello, M. J. (eds.). 2012. World Register of Marine Species. Accessed on January 15, 2012, at http://www.marinespecies.org/.

Applin, G. 2011. Newfoundland's alien tunicates: An overview of a collaborative research program to understand and control the spread of the golden star and violet tunicate: Development of TaqMan probes for early detection of invasive eggs and larvae. The Cold Harvester, Spring 2011: 24.

Arens, C. J., Paetzold, S. C., Ramsay, A., and Davidson, J. 2011. Pressurized seawater as an antifouling treatment against the colonial tunicates *Botrylloides violaceus* and *Botryllus schlosseri* in mussel aquaculture. Aquatic Invasions 6 (4): 465-476.

Atkinson, E. G., and Wacasey, J. W. 1989. Benthic invertebrates collected from Hudson Strait, Foxe Channel and Foxe Basin, Canada, 1949 to 1970. Canadian Data Report of Fisheries and Aquatic Sciences 746, 98 pp.

Atlantic Reference Centre. 2006. Atlantic Reference Centre Museum of Canadian Atlantic Organisms: Invertebrates and Fishes Data. Accessed on January 15, 2012, at http://www.gbif.org/.

Baines, T. 2007. An assessment of the risk of aquatic invasive species introduction to Newfoundland waters. Newfoundland Aquaculture Industry Association, Technical Report, St. John's, 24 pp.

Ballarin, L., Burighel, P., and Cima, F. 2008. A tale of death and life: Natural apoptosis in the colonial ascidian *Botryllus schlosseri* (Urochordata, Ascidiacea). Current Pharmaceutical Design 14 (2): 138-147.

Ben-Shlomo, R., Douek, J., and Rinkevich, B. 2001. Heterozygote deficiency and chimerism in remote populations of a colonial ascidian from New Zealand. Marine Ecology Progress Series 209: 109-117.

Ben-Shlomo, R., Reem, E., Douek, J., and Rinkevich, B. 2010. Population genetics of the invasive ascidian *Botryllus schlosseri* from South American coasts. Marine Ecology Progress Series 412: 85-92.

Berrill, N. J. 1928. The identification and validity of certain species of ascidians. Journal of the Marine Biological Association of the United Kingdom 15 (1): 159-175.

Berrill, N. J. 1950. The Tunicata: With an account of the British species. The Ray Society, London, 354 pp.

Bernier, R. Y., Locke, A., and Hanson, J. M. 2009. Lobsters and crabs as potential vectors for tunicate dispersal in the southern Gulf of St. Lawrence, Canada. Aquatic Invasions 4 (1): 105-110.

Blum, J. C., Chang, A. L., Liljesthröm, M., Schenkm, M. E., Steinberg, M. K., and Ruiz, G. M. 2007. The non-native solitary ascidian *Ciona intestinalis* (L.) depresses species richness. Journal of Experimental Marine Biology and Ecology 342 (1): 5-14.

Bock, D. G. 2010. Invasion genetics of botryllid colonial ascidians in North America. Master of Science thesis, University of Windsor, Windsor, 121 pp.

Boyd, H. C., Brown, S. K., Harp, J. A., and Weissman, I. L. 1986. Growth and sexual maturation of laboratory-cultured Monterey *Botryllus schlosseri*. The Biological Bulletin 170 (1): 91-109.

Boyd, H. C., Weissman, I. L., and Saito, Y. 1990. Morphologic and genetic verification that Monterey *Botryllus* and Woods Hole *Botryllus* are the same species. The Biological Bulletin 178 (3): 239-250.

Brunel, P., Bossé, L., and Lamarche, G. 1998. Catalogue of the marine invertebrates of the Estuary and Gulf of Saint Lawrence. Canadian Special Publication of Fisheries and Aquatic Sciences 126, 405 pp.

Brunetti, R. 1974. Observations on the life cycle of *Botryllus schlosseri* (Pallas) (Ascidiacea) in the Venetian Lagoon. Bolletino di zoologia 41 (3): 225-251.

Brunetti, R., Beghi, L., and Bressan, M., and Marin, M. G. 1980. Combined effects of temperature and salinity on colonies of *Botryllus schlosseri* and *Botrylloides leachi* (Ascidiacea) from the Venetian Lagoon. Marine Ecology – Progress Series 2 (4): 303-314.

Brunetti, R., Marin, M. G., and Bressan, M. 1984. Combined effects of temperature and salinity on sexual reproduction and colonial growth of *Botryllus schlosseri* (Tunicata). Bolletino di zoologia 51 (3-4): 405-411.

Bullard, S. G., Lambert, G., Carman, M. R., Byrnes, J., Whitlatch, R. B., Ruiz, G., Miller, R. J., Harris, L., Valentine, P. C., Collie, J. S., Pederson, J., McNaught, D. C., Cohen, A. N., Asch, R. G., Dijkstra, J., and Heinonen, K. 2007. The colonial ascidian *Didemnum* sp. A: Current distribution, basic biology and potential threat to marine communities of the northeast and west coasts of North America. Journal of Experimental Marine Biology and Ecology 342 (1): 99-108.

Caddy, J. F. 1970. Records of associated fauna in scallop dredge hauls from the Bay of Fundy. Fisheries Research Board of Canada, Technical Report No. 225, 11 pp.

Callahan, A. G. 2009. Harbour survey and genetic analysis of non-indigenous ascidian tunicates in Newfoundland. Master of Science thesis, Memorial University of Newfoundland, St. John's, 86 pp.

Callahan, A. G., Deibel, D., McKenzie, C. H., Hall, J. R., and Rise, M. L. 2010. Survey of harbours in Newfoundland for indigenous and non-indigenous ascidians and an analysis of their cytochrome *c* oxidase I gene sequences. Aquatic Invasions 5 (1): 31-39.

Carlton, J. T. 1979. History, biogeography, and ecology of the introduced marine and estuarine invertebrates of the Pacific coast of North America. Doctor of Philosophy thesis, University of California, Davis, 904 pp.

Carlton, J. T. 2009. Deep invasion ecology and the assembly of communities in historical time. In: Rilov, G., and Crooks, J. A. (eds.), Biological invasions in marine ecosystems. Ecological Studies 204 (1): 13-56.

Carlton, J. T., and Hodder, J. 1995. Biogeography and dispersal of coastal marine organisms: Experimental studies on a replica of a 16th-century sailing vessel. Marine Biology 121 (4): 721-730.

Carman, M. R., Hoagland, K. E., Green-Beach, E., and Grunden, D. W. 2009. Tunicate faunas of two North Atlantic-New England islands: Martha's Vineyard, Massachusetts and Block Island, Rhode Island. Aquatic Invasions 4 (1): 65-70.

Carman, M. R., and Grunden, D. W. 2010. First occurrence of the invasive tunicate *Didemnum vexillum* in eelgrass habitat. Aquatic Invasions 5 (1): 23-29.

Carman, M. R., Morris, J. A., Karney, R. C., and Grunden, D. W. 2010. An initial assessment of native and invasive tunicates in shellfish aquaculture of the North American east coast. Journal of Applied Ichthyology 26 (Supplement s2): 8-11.

Carver, C. E., Mallet, A. L., and Vercaemer, B. 2006a. Biological synopsis of the colonial tunicates, *Botryllus schlosseri* and *Botrylloides violaceus*. Canadian Manuscript Report of Fisheries and Aquatic Sciences 2747, 42 pp.

Carver, C. E., Mallet, A. L., and Vercaemer, B. 2006b. Biological synopsis of the solitary tunicate *Ciona intestinalis*. Canadian Manuscript Report of Fisheries and Aquatic Sciences 2746, 55 pp.

Catto, N. R., Hooper, R. G., Anderson, M. R., Scruton, D. A., Meade, J. D., Ollerhead, L. M. N., and Williams, U. P. 1999. Biological and geomorphological classification of Placentia Bay: A preliminary assessment. Canadian Technical Report of Fisheries and Aquatic Sciences 2289, 35 pp.

Canadian Council of Fisheries and Aquaculture Ministers-Aquatic Invasive Species Task Group. 2004. A Canadian action plan to address the threat of aquatic invasive species. Canadian Council of Fisheries and Aquaculture Ministers-Aquatic Invasive Species Task Group, Ottawa, 26 pp.

Chadwick-Furman, N. E., and Weissman, I. L. 1995. Life histories and senescence of *Botryllus schlosseri* (Chordata, Ascidiacea) in Monterey Bay. The Biological Bulletin 189 (1): 36-41.

Claar, D. C., Edwards, K. F., and Stachowicz, J. J. 2011. Positive and negative effects of a dominant competitor on the settlement, growth, and survival of competing species in an epibenthic community. Journal of Experimental Marine Biology and Ecology 399 (2): 130-134.

Clancey, L., and Hinton, R. 2003. Distribution of the tunicate, *Ciona intestinalis*, in Nova Scotia. Nova Scotia Department of Fisheries and Agriculture, Halifax, 6 pp.

Clark, D., and Branton, B. 2007. DFO Maritime Research Vessel Trawl Surveys Fish Observations. Accessed on January 15, 2012, at http://www.iobis.org/.

Clarke, C. L., and Therriault, T. W. 2007. Biological synopsis of the invasive tunicate *Styela clava* (Herdman 1881). Canadian Manuscript Report of Fisheries and Aquatic Sciences 2807, 23 pp.

Clarke Murray, C., Pakhomov, Evgeny, A., and Therriault, T. W. 2011. Recreational boating: A large unregulated vector transporting marine invasive species. Diversity and Distributions 17 (6): 1161-1172.

Cohen, A. N., and Carlton, J. T. 1995. Biological study: Nonindigenous aquatic species in a United States estuary: A case study of the biological invasions of the San Francisco Bay and Delta. United States Fish and Wildlife Service, Washington, D. C., and The National Sea Grant College Program, Connecticut Sea Grant, Groton, 246 pp.

Cohen, J. E. 1969. Natural primate troops and a stochastic population model. The American Naturalist 103 (933): 455-477.

Colautti, R. I., Bailey, S. A., van Overdijk, C. D. A., Amundsen, K., and MacIsaac, H. J. 2006. Characterised and projected costs of nonindigenous species in Canada. Biological Invasions 8 (1): 45 -59.

Cole, L. L. 1989. Catalog of tunicate type specimens in the United States National Museum collections. Smithsonian Contributions to Zoology 487, 12 pp.

Committee on the Status of Endangered Wildlife in Canada. 2011. COSEWIC assessment and status report on the Olympia oyster *Ostrea lurida* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, 56 pp.

Couthouy, J. P. 1838. Descriptions of new species of mollusca and shells, and remarks on several polypi found in Massachusetts Bay. Boston Journal of Natural History 2 (1): 53-111.

Crisp, D. J., and Ghobashy, A. F. A. A. 1971. Responses of the larvae of *Diplosoma listerianum* to light and gravity. In: Crisp, D. K. (ed.), Fourth European Marine Symposium, pp. 443-465.

Cunningham, R. B., and Lindenmayer, D. B. 2005. Modelling count data of rare species: Some statistical issues. Ecology 86 (5): 1135-1142.

Dalby Jr., J. E., and Young, C. M. 1992. Role of early post-settlement mortality in setting the upper depth limit of ascidians in Florida epifaunal communities. Marine Ecology Progress Series 80 (2-3): 221-228.

Daniel, K. S., and Therriault, T. W. 2007. Biological synopsis of the invasive tunicate *Didemnum* sp. Canadian Manuscript Report of Fisheries and Aquatic Sciences 2788, 53 pp.

Davis, M. H., and Davis, M. E. 2009. *Styela clava* (Tunicata, Ascidiacea) – a new threat to the Mediterranean shellfish industry? Aquatic Invasions 4 (1): 283-289.

Davis, M. H., and Davis, M. E. 2010. The impact of the ascidian *Styela clava* Herdman on shellfish farming in the Bassin de Thau, France. Journal of Applied Ichthyology 26 (Supplement s2): 12-18.

Department of Invertebrate Zoology. 2007. NMNH Invertebrate Zoology Collections. Accessed on January 15, 2012, at http://www.iobis.org/ and http://www.gbif.org/.

Dijkstra, J., Harris, L. G., and Westerman, E. 2007. Distribution and long-term temporal patterns of four invasive colonial ascidians in the Gulf of Maine. Journal of Experimental Marine Biology and Ecology 342 (1): 61-68.

Dobretsov, S. V. 1999. Effects of macroalgae and biofilm on settlement of blue mussel (*Mytilus edulis* L.) larvae. Biofouling: The Journal of Bioadhesion and Biofilm Research 14 (2): 153-165.

Dunbar, M. J. 1953. Arctic and subarctic marine ecology: Immediate problems. Arctic 6 (2): 75-90.

Dworjanyn, S. A., de Nys, R., and Steinberg, P. D. 2006. Chemically mediated antifouling in the red alga *Delisea pulchra*. Marine Ecology Progress Series 318: 153-163.

Epelbaum, A., Herborg, L. M., Therriault, T. W., and Pearce, C. M. 2009c. Temperature and salinity effects on growth, survival, reproduction, and potential distribution of two non-indigenous botryllid ascidians in British Columbia. Journal of Experimental Marine Biology and Ecology 369 (1): 43-52.

Epelbaum, A., Pearce, C. M., Barker, D. J., Paulson, A., and Therriault, T. W. 2009a. Susceptibility of non-indigenous ascidian species in British Columbia (Canada) to invertebrate predation. Marine Biology 156 (6): 1311-1320.

Epelbaum, A., Therriault, T. W., Paulson, A., and Pearce, C. M. 2009b. Botryllid tunicates: Culture techniques and experimental procedures. Aquatic Invasions 4 (1): 111-120.

Fisheries and Oceans Canada. 2011a. Effects of aquatic invasive species. Accessed on February 15, 2012, at http://www.nfl.dfo-mpo.gc.ca/e0017567/.

Fisheries and Oceans Canada. 2011b. Aquatic invasive species: Golden star tunicate in Newfoundland and Labrador waters. Fisheries and Oceans Canada, Ottawa, 2 pp.

Fisheries and Oceans Canada. 2011c. Aquatic invasive species: Violet tunicate in Newfoundland and Labrador waters. Fisheries and Oceans Canada, Ottawa, 2 pp.

Fisheries and Oceans Canada. 2012. Proceedings of a workshop on the Canadian Aquatic Invasive Species Rapid Response Framework. Canadian Science Advisory Secretariat, Proceeding Series 2012/016, 70 pp.

Gaertner, J. 1774. Zoophyta quaedam minuta. In: Pallas, P. S., Spicilegia zoologica quibus novae imprimis et obscurae animalium species iconibus, descriptionibus atque commentariis illustrantur, fasciculus decimus. G. A. Lange, Berolini, pp. 34-41.

Ganong, W. F. 1885. On the zoology of the invertebrate animals of Passamaquoddy Bay. Bulletin of the Natural History Society of New Brunswick 4: 87-97.

GBIF. 2012. Global Biodiversity Information Facility. Accessed on January 15, 2012, at http://www.gbif.org/.

Gittenberger, A., and Moons, J. J. S. 2011. Settlement and possible competition for space between the invasive violet tunicate *Botrylloides violaceus* and the native star tunicate *Botryllus schlosseri* in The Netherlands. Aquatic Invasions 6 (4): 435-440.

Gittenberger, A., and van der Stelt, R. C. 2011. Artificial structures in harbors and their associated fauna. Aquatic Invasions 6 (4): 413-420.

Goldstien, S. J., Dupont, L., Viard, F., Hallas, P. J., Nishikawa, T., Schiel, D. R., Gemmell, N. J., and Bishop, J. D. D. 2011. Global phylogeography of the widely introduced north west Pacific ascidian *Styela clava*. PLoS ONE 6 (2): e16755. doi:10.1371/journal.pone.0016755.

Gosner, K. L. 1971. Guide to identification of marine and estuarine invertebrates: Cape Hatteras to the Bay of Fundy. John Wiley and Sons, New York, 693 pp.

Gould, A. A. 1870. Report on the invertebrate of Massachusetts, second edition, comprising the mollusca. Wright and Potter, Boston, 524 pp.

Grave, C. 1937. Notes on the culture of eight species of ascidians. In: Galtsoff, P. S., Lutz, F. E., Welch, P. S., and Needham, J. G. (eds.), Culture Methods for Invertebrate Animals. Comstock Publishing Company, Ithaca, pp. 560-564.

Grave, C., and Woodbridge, H. 1924. *Botryllus schlosseri* (Pallas): The behavior and morphology of the free-swimming larva. Journal of Morphology and Physiology 39 (1): 207-247.

Green, P. T., O'Dowd, D. J., Abbott, K. L., Jeffery, M., Retallick, K., and Mac Nally, R. 2011. Invasional meltdown: Invader-invader mutualism facilitates a secondary invasion. Ecology 92 (9): 1758-1768.

Grosberg, R. K. 1981. Competitive ability influences habitat choice in marine invertebrates. Nature 290 (5808): 700-702.

Grosberg, R. K. 1982. Ecological, genetical and developmental factors regulating life history variation within a population of the colonial ascidian *Botryllus schlosseri* (Pallas) Savigny. Doctor of Philosophy thesis, Yale University, New Haven, 252 pp.

Grosberg, R. K. 1988. Life-history variation within a population of the colonial ascidian *Botryllus schlosseri*. I. The genetic and environmental control of seasonal variation. Evolution 42 (5): 900-920.

Grosberg, R. K. 1991. Sperm-mediated gene flow and the genetic structure of a population of the colonial ascidian *Botryllus schlosseri*. Evolution 45 (1): 130-142.

Haahr, M. 2012. Random.org – true random number service. Accessed on February 20, 2010, at http://www.random.org/.

Hardin, J. W., and Hilbe, J. M. 2007. Generalized linear models and extensions, second edition. Stata Press, College Station, 387 pp.

Harms, J., and Anger, K. 1983. Seasonal, annual, and spatial variation in the development of hard bottom communities. Helgoland Marine Research 36 (2): 137-150.

Hatfield, C., Logan, A., and Thomas, M. L. H. 1992. Ascidian depth zonation on sublittoral hard substrates off Deer Island, New Brunswick, Canada. Estuarine, Coastal and Shelf Science 34 (2): 197-202.

Haydar, D. 2010. What is natural? The scale and consequences of marine bioinvasions in the North Atlantic Ocean. Doctor of Philosophy thesis, Rijksuniversiteit Groningen, Groningen, 183 pp.

Hooper, R. 1975. Bonne Bay marine resources: An ecological and biological assessment. Parks Canada, Atlantic Region Office, 295 pp.

Howes, S., Herbinger, C. M., Darnell, P., and Vercaemer, B. 2007. Spatial and temporal patterns of recruitment of the tunicate *Ciona intestinalis* on a mussel farm in

Nova Scotia, Canada. Journal of Experimental Marine Biology and Ecology 342 (1): 85-92.

Huntsman, A. G. 1912. Ascidians from the coasts of Canada. Transactions of the Royal Canadian Institute 9 (12): 111-148.

Huntsman, A. G. 1922a. Arctic ascidians. Report of the Canadian Arctic Expedition 1913-18, Volume VI: Fishes and tunicates, Part B: Ascidiacea. F. A. Acland, Ottawa, 14 pp.

Huntsman, A. G. 1922b. Results of the Hudson Bay Expedition, 1920: IV. The Ascidiacea. Contributions to Canadian Biology and Fisheries 1 (1): 27-38.

Integrated Taxonomic Information System. 2012. Integrated Taxonomic Information System. Accessed on January 15, 2012, at http://www.itis.gov/.

Izquierdo-Muñoz, A., Diaz-Valdés M., and Ramos-Esplá, A. A. 2009. Recent non-indigenous ascidians in the Mediterranean Sea. Aquatic Invasions 4 (1): 59-64.

Kawamura, K., and Sunanaga, T. 2010. Hemoblasts in colonial tunicates: Are they stem cells or tissue-restricted progenitor cells? Development, Growth & Differentiation 52 (1): 69-76.

Keough, M. J. 1998. Responses of settling invertebrate larvae to the presence of established recruits. Journal of Experimental Marine Biology and Ecology. 231 (1): 1-19.

Keough, M. J., and Raimondi, P. T. 1996. Responses of settling invertebrate larvae to bioorganic films: Effects of large-scale variation in films. Journal of Experimental Marine Biology and Ecology 207 (1-2): 59-78.

Kindle, E. M. 1917. Notes on the bottom environment of the marine invertebrates of western Nova Scotia. The Ottawa Naturalist 30 (12): 149-154.

Korczynski, R. E. 1989. A new species of solitary ascidian (Ascidacea: Pyuridae) from Mason Bay, Northwest Territories, Canada. Journal of Natural History 23 (1): 219-224.

Krebs, C. J. 2001. Ecology: The experimental analysis of distribution and abundance, fifth edition. Benjamin Cummings, San Francisco, 695 pp.

Lam, K., Shin, P. K. S., Bradbeer, R., Randall, D., Ku, K. K. K., Hodgson, P., and Cheung, S. G. 2006. A comparison of video and point intercept transect methods for monitoring subtropical coral communities. Journal of Experimental Marine Biology and Ecology 333 (1): 115-128.

Lambert, C. C., and Lambert, G. 1998. Non-indigenous ascidians in southern California harbors and marinas. Marine Biology 130 (4): 675-688.

Lambert, C. C., and Lambert, G. 2003. Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Marine Ecology Progress Series 259: 145-161.

Lambert, G. 2001. A global review of ascidian introduction and their possible impact on the endemic fauna. In: Sawada, H., Yokosawa, H., and Lambert C. C. (eds.), The Biology of Ascidians. Springer Verlag, Tokyo, pp. 249-257.

Lambert, G. 2002. Nonindigenous ascidians in tropical waters. Pacific Science 56 (3): 291-298.

Lambert, G. 2005. Ecology and natural history of the protochordates. Canadian Journal of Zoology 83 (1): 34-50.

Lambert, G. 2007. Invasive sea squirts: A growing global problem. Journal of Experimental Marine Biology and Ecology 342 (1): 3-4.

Lambert, G. 2009. Adventures of a sea squirt sleuth: Unraveling the identity of *Didemnum vexillum*, a global ascidian invader. Aquatic Invasions 4 (1): 5-28.

Lambert, G., Shenkar, N., and Swalla, B. J. 2010. First Pacific record of the north Atlantic ascidian *Molgula citrina* – bioinvasion or circumpolar distribution? Aquatic Invasions 5 (4): 369-378.

Lauzon, R. J., Patton, C. W., and Weissman, I. L. 1993. A morphological and immunohistochemical study of programmed cell death in *Botryllus schlosseri* (Tunicata, Ascidiacea). Cell and Tissue Research 272 (1): 115-127.

LeGresley, M. M., Martin, J. L., McCurdy, P., Thorpe, B., and Chang, B. D. 2008. Non-indigenous tunicate species in the Bay of Fundy, eastern Canada. ICES Journal of Marine Science 65 (5): 770-774.

Lejeusne, C., Bock, D. G., Therriault, T. W., MacIsaac, H. J., and Cristescu, M. E. 2011. Comparative phylogeography of two colonial ascidians reveals contrasting invasion histories in North America. Biological Invasions 13 (3): 635-650.

Linkletter, L. E., Lord, E. I., and Dadswell, M. J. 1977. A checklist and catalogue of the marine fauna and flora of the Lower Bay of Fundy shore of New Brunswick. The Huntsman Marine Laboratory, St. Andrews, 68 pp.

Linnæus, C. 1758. Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata. L. Salvius, Holmiæ, 824 pp.

Locke, A. 2009. A screening procedure for potential tunicate invaders of Atlantic Canada. Aquatic Invasions 4 (1): 71-79.

Locke, A., Hanson, J. M., Ellis, K. M., Thompson, J., and Rochette, R. 2007. Invasion of the southern Gulf of St. Lawrence by the clubbed tunicate (*Styela clava* Herdman): Potential mechanisms for invasions of Prince Edward Island estuaries. Journal of Experimental Marine Biology and Ecology 342 (1): 69-77.

Locke, A., and Carman, M. 2009. An overview of the 2nd International Invasive Sea Squirt Conference: What we learned. Aquatic Invasions 4 (1): 1-4.

Logan, A. 1988. A sublittoral hard substrate epibenthic community below 30 m in Head Harbour Passage, New Brunswick, Canada. Estuarine, Coastal and Shelf Science 27 (4): 445-459.

Logan, A., Page, F. H., and Thomas, M. L. H. 1984. Depth zonation of epibenthos on sublittoral hard substrates off Deer Island, Bay of Fundy, Canada. Estuarine, Coastal and Shelf Science 18 (5): 571-592.

López-Legentil, S., Turon, X., and Planes, S. 2006. Genetic structure of the star sea squirt, *Botryllus schlosseri*, introduced in southern European harbours. Molecular Ecology 15 (13): 3957-3967.

Lowen, J. B., Deibel, D., Rise, M. L., Thompson, R., Ma, K. C. K., and McKenzie, C. H. 2010. Life-history, population dynamics, diversity, and abundance of *Botryllus schlosseri* in a sub-arctic environment (Arnold's Cove, Placentia Bay, NL). Aquaculture Canada 2010 and Cold Harvest 2010, St. John's, Newfoundland and Labrador, Canada.

Lowen, J. B. 2011. Newfoundland's alien tunicates: An overview of a collaborative research program to understand and control the spread of the golden star and violet tunicate: Life-history constraints affecting invasion success in the golden star tunicate. The Cold Harvester, Spring 2011: 23.

Lowen, J. B., Deibel, D., Ma, K. C. K., McKenzie, C. H., and Thompson, R. 2011. Reproductive constraints of the golden star tunicate in Arnold's Cove, Newfoundland. The 6th International Tunicate Meeting, Montreal, Quebec, Canada.

Lowen, J. B., Deibel, D., Ma, K. C. K., McKenzie, C. H., and Thompson, R. 2012. Life-history constraints affecting invasion success in the ascidian *Botryllus schlosseri*. The 41st Annual Benthic Ecology Meeting, Norfolk, Virginia, USA.

Lutz-Collins, V., Ramsay, A., Quijón, P. A., and Davidson, J. 2009. Invasive tunicates fouling mussel lines: Evidence of their impact on native tunicates and other epifaunal invertebrates. Aquatic Invasions 4 (1): 213-220.

Ma, K. C. K. 2011. Newfoundland's alien tunicates: An overview of a collaborative research program to understand and control the spread of the golden star and violet tunicate: Abundance and recruitment. The Cold Harvester, Spring 2011: 22-23.

Mackenzie, A. B. 2011. Biological synopsis of the compound sea squirt (*Diplosoma listerianum*). Canadian Manuscript Report of Fisheries and Aquatic Sciences 2966, 18 pp.

Manni, L., Zaniolo, G., and Burighel, P. 1993. Egg envelope cytodifferentiation in the colonial ascidian *Botryllus schlosseri* (Tunicata). Acta Zoologica 74 (2): 103-113.

Manni, L., Zaniolo, G., and Burighel, P. 1994. Ultrastructural study of oogenesis in the compound ascidian *Botryllus schlosseri* (Tunicata). Acta Zoologica 75 (2): 101-113.

Manni, L., and Burighel, P. 2006. Common and divergent pathways in alternative developmental processes of ascidians. BioEssays 28 (9): 902-912.

Manni, L., Zaniolo, G., Cima, F., Burighel, P., and Ballarin, L. 2007. *Botryllus schlosseri*: A model ascidian for the study of asexual reproduction. Developmental Dynamics 236 (2): 335-352.

Martin, J. L., LeGresley, M. M., Cooper, J. A., Thorpe, B., Locke, A., Simard, N., Sephton, D., Bernier, R., Bérubé, I., Hill, B., Keays, J., Knox, D., Landry, T., Lander, T., Nadeau, A., and Watson, E. J. 2010. Rapid assessment for *Didemnum vexillum* in southwest New Brunswick. Canadian Technical Report of Fisheries and Aquatic Sciences 2882, 16 pp.

Martin, J. L., LeGresley, M. M., Thorpe, B., and McCurdy, P. 2011. Non-indigenous tunicates in the Bay of Fundy, eastern Canada (2006-2009). Aquatic Invasions 6 (4): 405-412.

Martin, T. G., Wintle, B. A., Rhodes, J. R., Kuhnert, P. M., Field, S. A., Low-Choy, S. J., Tyre, A. J., and Possingham, H. P. 2005. Zero tolerance ecology: Improving ecological inference by modelling the source of zero observations. Ecology Letters 8 (11): 1235-1246.

Mayer, D., Roy, D., Robins, J., Halliday, I., and Sellin, M. 2005. Modelling zero-inflated fish counts in estuaries - a comparison of alternate statistical distributions. In: Zerger, A., and Argent, R. M. (eds.), MODSIM 2005 International Congress on Modelling and Simulation. Modelling and Simulation Society of Australia and New Zealand, pp. 2581-2587.

McNaught, D. C., and Norden, W. S. 2011. Generalised regional spatial patterns of larval recruitment of invasive ascidians, mussels, and other organisms along the coast of Maine. Aquatic Invasions 6 (4): 519-523.

Milkman, R. 1967. Genetic and developmental studies on *Botryllus schlosseri*. The Biological Bulletin 132 (2): 229-243.

Millar, R. H. 1952. The annual growth and reproductive cycle in four ascidians. Journal of the Marine Biological Association of the United Kingdom 31 (1): 41-61.

Millar, R. H. 1966. Tunicata Ascidiacea. Marine invertebrates of Scandinavia 1. Universitetsforlaget, Oslo, 123 pp.

Millar, R. H. 1970. British ascidians. Synopses of the British Fauna (New Series) 1. Academic Press, London, 88 pp.

Millar, R. H. 1971. The biology of ascidians. Advances in Marine Biology 9: 1-100.

Min, Y., and Agresti, A. 2002. Modeling nonnegative data with clumping at zero: A survey. Journal of the Iranian Statistical Society 1 (1-2): 7-33.

Morris Jr., J. A., Carman, M. R., Hoagland, K. E., Green-Beach, E. R. M., and Karney, R. C. 2009. Impact of the invasive colonial tunicate *Didemnum vexillum* on the recruitment of the bay scallop (*Argopecten irradians irradians*) and

implications for recruitment of the sea scallop (*Placopecten magellanicus*) on Georges Bank. Aquatic Invasions 4 (1): 207-211.

Mukai, H. 1977. Comparative studies on the structure of reproductive organs of four botryllid ascidians. Journal of Morphology 152 (3): 363-379.

Myers, R. A., and Pepin, P. 1990. The robustness of lognormal-based estimators of abundance. Biometrics 46 (4): 1185-1192.

Nadeau, M. 2008. Évaluation de la présence et de la progression des principales espèces de tuniciers envahissants aux Îles-de-la-Madeleine avec des efforts particuliers sur l'ascidie jaune (*Ciona intestinalis*). Societé de développement de l'industrie maricole inc., Cap-aux-Meules, 7 pp.

Nandakumar, K. 1996. Importance of timing of panel exposure on the competitive outcome and succession of sessile organisms. Marine Ecology Progress Series 131 (1-3): 191-203.

Osman, R. W., Munguia, P., Whitlatch, R. B., Zajac, R. N., and Hamilton, J. 2010. Thresholds and multiple community states in marine fouling communities: Integrating natural history with management strategies. Marine Ecology Progress Series 413: 277-289.

Osman, R. W., and Whitlatch, R. B. 1998. Local control of recruitment in an epifaunal community and the consequences to colonization processes. Hydrobiologia 375/376: 113-123.

Osman, R. W., and Whitlatch, R. B. 2004. The control of the development of a marine benthic community by predation on recruits. Journal of Experimental Marine Biology and Ecology 311 (1): 117-145.

Osman, R. W., and Whitlatch, R. B. 2007. Variation in the ability of *Didemnum* sp. to invade established communities. Journal of Experimental Marine Biology and Ecology 342 (1): 40-53.

Otsuka, C. M., and Dauer, D. M. 1982. Fouling community dynamics in Lynnhaven Bay, Virginia. Estuaries 5 (1): 10-22.

Özmen, İ., and Famoye, F. 2007. Count regression models with an application to zoological data containing structural zeros. Journal of Data Science 5 (4): 491-502.

Packard Jr., A. S. 1863. A list of animals dredged near Caribou Island, southern Labrador, during July and August, 1860. The Canadian Naturalist and Geologist 8 (6): 401-429.

Packard Jr., A. S. 1867. Observations on glacial phenomena of Labrador and Maine, with a view of the recent invertebrate fauna of Labrador. Memoirs Read Before the Boston Society of Natural History 1 (2): 210-303.

Paetzold, S. C., and Davidson, J. 2010. Viability of golden star tunicate fragments after high-pressure water treatment. Aquaculture 303 (1-4): 105-107.

Pallas, P. S. 1766. Elenchus Zoophytorum sistens generum adumbrations generaliores et specierum cognitarum succinctas descriptions cum selectis auctorum synonymis. P. van Cleef, The Hague, 451 pp.

Pancer, Z., Gershon, H., and Rinkevich, B. 1995. Coexistence and possible parasitism of somatic and germ cell lines in chimeras of the colonial urochordate *Botryllus schlosseri*. The Biological Bulletin 189 (2): 106-112.

Pellegrin, N., Boutillier, J., Lauzier, R., Verrin, S., and Johannessen, D. 2007. Appendix F: Invertebrates. In: Lucas, B. G., Verrin, S., and Brown, R. (eds.), Ecosystem overview: Pacific North Coast Integrated Management Area (PNCIMA). Canadian Technical Report of Fisheries and Aquatic Sciences 2667, 37 pp.

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

Pohle, G., Van Guelpen, L., Martin, A., Welshman, D., and McGuire, A. 2004. Bay of Fundy Species List. Accessed on January 15, 2012, at http://www.iobis.org/.

Pulliam, H. R. 1988. Sources, sinks, and population regulation. The American Naturalist 132 (5): 652-661.

Rabinowitz, C., and Rinkevich, B. 2004. *In vitro* delayed senescence of extirpated buds from zooids of the colonial tunicate *Botryllus schlosseri*. The Journal of Experimental Biology 207 (9): 1523-1532.

Ramsay, A., Davidson, J., Landry, T., and Arsenault, G. 2008. Process of invasiveness among exotic tunicates in Prince Edward Island, Canada. Biological Invasions 10 (8): 1311-1316.

Rinkevich, B. 2005. Natural chimerism in colonial urochordates. Journal of Experimental Marine Biology and Ecology 322 (2): 93-109.

Rinkevich, B., Lauzon, R. J., Brown, B. W. M., and Weissman, I. L. 1992. Evidence for a programmed life span in a colonial protochordate. Proceedings of the National Academy of Sciences of the United States of America 89 (8): 3546-3550.

Rinkevich, B., Porat, R., and Goren, M. 1998. Ecological and life history characteristics of *Botryllus schlosseri* (Tunicata) populations inhabiting undersurface shallow-water stones. Marine Ecology 19 (2): 129-145.

Rinkevich, B., and Saito, Y. 1992. Self-nonself recognition in the colonial protochordate *Botryllus schlosseri* from Mutsu Bay, Japan. Zoological Science 9 (5): 983-988.

Rinkevich, B., and Yankelevich, I. 2004. Environmental split between germ cell parasitism and somatic cell synergism in chimeras of a colonial urochordate. The Journal of Experimental Biology 207 (20): 3531-3536.

Ruis, M., Branch, G. M., Griffiths, C. L., and Turon, X. 2010. Larval settlement behaviour in six gregarious ascidians in relation to adult distribution. Marine Ecology Progress Series 418: 151-163.

Ruiz, G. M., Fofonoff, P. W., Steves, B., Huber, T., Larson, K., McCann, L., Hitchcock, N. G., Hines, A. H., and Carlton, J. T. 2005. North American Sessile Marine Invertebrate Survey. Accessed on January 15, 2012, at http://www.iobis.org/ and http://www.gbif.org/.

Sabbadin, A. 1971. Self- and cross-fertilization in the compound ascidian *Botryllus schlosseri*. Developmental Biology 24 (3): 379-391.

Sabbadin, A., and Astorri, C. 1988. Chimeras and histocompatibility in the colonial ascidian *Botryllus schlosseri*. Developmental and Comparative Immunology 12 (4): 737-747.

Satoh, N. 1994. Developmental biology of ascidians. Cambridge University Press, New York, 234 pp.

Saito, Y., and Okuyama, M. 2003. Studies on Japanese botryllid ascidians. IV. A new species of the genus *Botryllus* with a unique colony shape, from the vicinity of Shimoda. Zoological Science 20 (9): 1153-1161.

Schander, C., and Willassen, E. 2005. What can biological barcoding do for marine biology? Marine Biology Research 1 (1): 79-83.

Schlosser, J. A., and Ellis, J. 1756. An account of a curious, fleshy, coral-like substance: In a letter to Mr. Peter Collinson, F. R. S. from Dr. John Albert Schlosser, M. D. F. R. S. with some observations on it communicated to Mr. Collinson by Mr. John Ellis, F. R. S. Philosophical Transactions 49: 449-452.

Schmidt, G. H. 1982. Random and aggregative settlement in some sessile marine invertebrates. Marine Ecology – Progress Series 9 (1): 97-100.

Schmidt, G. H., and Warner, G. F. 1986. Spatial competition between colonial ascidians: The importance of stand-off. Marine Ecology – Progress Series 31 (1): 101-104.

Sephton, D., Vercaemer, B., Nicolas, J. M., and Keays, J. 2011. Monitoring for invasive tunicates in Nova Scotia, Canada (2006-2009). Aquatic Invasions 6 (4): 391-403.

Shenkar, N., and Swalla, B. J. 2011. Global diversity of Ascidiacea. PLoS ONE 6 (6): e20657. doi:10.1371/journal.pone.0020657.

Sorrentino, M., Manni, L., Lane, N. J., and Burighel, P. 2000. Evolution of cerebral vesicles and their sensory organs in an ascidian larva. Acta Zoologica 81 (3): 243-258.

Stachowicz, J. J., Whitlatch, R. B., and Osman, R. W. 1999. Species diversity and invasion resistance in a marine ecosystem. Science 286 (5444): 1577-1579.

Stachowicz, J. J., Fried, H., Osman, R. W., and Whitlatch, R. B. 2002a. Biodiversity, invasion resistance, and marine ecosystem function: Reconciling pattern and process. Ecology 83 (9): 2575-2590.

Stachowicz, J. J., Terwin, J. R., Whitlatch, R. B., and Osman, R. W. 2002b. Linking climate change and biological invasions: Ocean warming facilitates nonindigenous species invasions. Proceedings of the National Academy of Sciences of the United States of America 99 (24): 15497-15500.

Stafford, J. 1906. On the fauna of the Atlantic coast of Canada: Third report – Gaspe, 1905-1906. Contributions to Canadian Biology and Fisheries B (1): 45-67.

Stevens, M., Párraga, C. A., Cuthill, I. C., Partridge, J. C., and Troscianko, T. S. 2007. Using digital photography to study animal coloration. Biological Journal of the Linnean Society 90 (2): 211-237.

Stewart-Savage, J., Phillippi, A., and Yund, P. O. 2001. Delayed insemination results in embryo mortality in a brooding ascidian. The Biological Bulletin 201 (1): 52-58.

Stimpson, W. 1852. Several new ascidians from the coast of the United States. Proceedings of the Boston Society of Natural History 4: 228-232.

Stimpson, W. 1854. Synopsis of the marine Invertebrata of Grand Manan: Or the region about the mouth of the Bay of Fundy, New Brunswick. Smithsonian Contributions to Knowledge 6, 66 pp.

Stoner, D. S., Ben-Shlomo, R., Rinkevich, B., and Weissman, I. L. 2002. Genetic variability of *Botryllus schlosseri* invasions to the east and west coasts of the USA. Marine Ecology Progress Series 243: 93-100.

Sumner, F. B., Osburn, R. C., and Cole, L. J. 1913. A biological survey of the waters of Woods Hole and vicinity. Section I. Physical and zoological. Bulletin of the Bureau of Fisheries 31 (1): 11-441.

Terwin, J. R., Whitlatch, R. B., and Stachowicz, J. J. 2000. Long-term patterns of recruitment in a fouling community of eastern Long Island Sound. In: Van Patten, M. (ed.), Proceedings of the Fifth Biennial Long Island Sound Research Conference, pp. 5-9.

Therriault, T. W., and Herborg L.-M. 2008a. Risk assessment of two solitary and three colonial tunicates in both Atlantic and Pacific Canadian waters. Fisheries and Oceans Canada, Canadian Science Advisory Secretariat, Research Document 2007/063, 64 pp.

Therriault, T. W., and Herborg, L.-M. 2008b. Predicting the potential distribution of the vase tunicate *Ciona intestinalis* in Canadian waters: Informing a risk assessment. ICES Journal of Marine Science 65 (5): 788-794.

Todd, C. D., and Turner, S. J. 1988. Ecology of intertidal and sublittoral cryptic epifaunal assemblages. II. Nonlethal overgrowth of encrusting bryozoans by colonial ascidians. Journal of Experimental Marine Biology and Ecology 115 (2): 113-126.

Trason, W. B. 1964. Ascidians of the Canadian Arctic waters. Journal of the Fisheries Board of Canada 21 (6): 1505-1517.

Trimble, A. C., Ruesink, J. L., and Dumbauld, B. R. 2009. Factors preventing the recovery of a historically overexploited shellfish species, *Ostrea lurida* Carpenter 1864. Journal of Shellfish Research 28 (1): 97-106.

Tyrrell, M. C., and Byers, J. E. 2007. Do artificial substrates favor nonindigenous fouling species over native species? Journal of Experimental Marine Biology and Ecology 342 (1): 54-60.

United States Navy. 1951. Report on marine borers and fouling organisms in 56 important harbors and tabular summaries of marine borer data from 160 widespread locations. Bureau of Yards and Docks, Department of the Navy, Washington, D.C., 326 pp.

Vanden Berghe, E. (ed.). 2007. The Ocean Biogeographic Information System. Accessed on January 15, 2012, at http://www.iobis.org/.

Van Name, W. G. 1910. Compound ascidians of the coasts of New England and neighboring British Provinces. Proceedings of the Boston Society of Natural History 34 (11): 339-424.

Van Name, W. G. 1912. Simple ascidians of the coasts of New England and neighboring British Provinces. Proceedings of the Boston Society of Natural History 34 (13): 439-619.

Van Name, W. G. 1921. Ascidians of the West Indian region and southeastern United States. Bulletin of the American Museum of Natural History 44: 283-494.

Van Name, W. G. 1945. The North and South American ascidians. Bulletin of the American Museum of Natural History 84: 1-476.

Verrill, A. E. 1871. Descriptions of some imperfectly known ascidians from New England. The American Journal of Science and Arts 1 (2): 54-58, 93-100, 211-212, 288-294, 443-446.

Verrill, A. E., and Smith, S. I. 1874. Report upon the invertebrate animals of Vineyard Sound and adjacent waters, with an account of the physical features of the region. Government Printing Office, Washington, 478 pp.

Visscher, J. P. 1928. Nature and extent of fouling of ships' bottoms. Bulletin of the Bureau of Fisheries 43 (2): 193-252.

Voskoboynik, A., Simon-Blecher, N., Soen, Y., Rinkevich, B., De Tomaso, A. W., Ishizuka, K. J., and Weissman, I. L. 2007. Striving for normality: Whole body regeneration through a series of abnormal generations. The FASEB Journal 21 (7): 1335-1344.

Voultsiadou, E., and Vafidis, D. 2007. Marine invertebrate diversity in Aristotle's zoology. Contributions to Zoology 76 (2): 103-120.

Wahl, M. 1989. Marine epibiosis. I. Fouling and antifouling: Some basic aspects. Marine Ecology Progress Series 58 (1-2): 175-189.

Watterson, R. L. 1945. Asexual reproduction in the colonial tunicate, *Botryllus schlosseri* (Pallas) Savigny, with special reference to the developmental history of intersiphonal bands of pigment cells. The Biological Bulletin 88 (1): 71-103.

Westerman, E. 2007. Impacts of changing water temperatures on the life histories of two invasive ascidians in the Gulf of Maine: *Botryllus schlosseri* and *Botrylloides violaceus*. Master of Science thesis, University of New Hampshire, Durham, 136 pp.

Westerman, E. L., Whitlatch, R., Dijkstra, J. A., and Harris, L. G. 2009. Variation in brooding period masks similarities in response to changing temperature. Marine Ecology Progress Series 391: 13-19.

Whiteaves, J. F. 1901. Catalogue of the marine Invertebrata of eastern Canada. Geological Survey of Canada 772: 1-271.

Whitlatch, R. B., and Osman, R. W. 2009. Post-settlement predation on ascidian recruits: Predator responses to changing prey density. Aquatic Invasions 4 (1): 121-131.

Wieczorek, S. K., and Todd, C. D. 1997. Inhibition and facilitation of bryozoan and ascidian settlement by natural multi-species biofilms: Effects of film age and the roles of active and passive larval attachment. Marine Biology 128 (3): 463-473.

Willis, J. E., Stewart-Clark, S., Greenwood, S. J., Davidson, J., and Quijon, P. 2011. A PCR-based assay to facilitate early detection of *Diplosoma listerianum* in Atlantic Canada. Aquatic Invasions 6 (1): 7-16.

Wong, M. C., and Vercaemer, B. 2012. Effects of invasive colonial tunicates and a native sponge on the growth, survival, and light attenuation of eelgrass (*Zostera marina*). Aquatic Invasions 7 (3):315-326.

Woods Hole Oceanographic Institute. 1952. Marine fouling and its prevention. United States Naval Institute, Annapolis, 388 pp.

Worcester, S. E. 1994. Adult rafting versus larval swimming: Dispersal and recruitment of a botryllid ascidian on eelgrass. Marine Biology 121 (2): 309-317.

Yale Peabody Museum. 2009. Peabody Invertebrate Zoology DiGIR Service. Accessed on January 15, 2012, at http://www.gbif.org/.

Yan, S. K., and Huang, Z. G. 1993. Biofouling of ships in Daya Bay, China. In: Morton, B. (ed.), The Marine Biology of the South China Sea. Proceedings of the First International Conference on the Marine Biology of Hong Kong and the South China Sea, Hong Kong University Press, Hong Kong, pp. 131-135.

Yund, P. O., Marcum, Y., and Stewart-Savage, J. 1997. Life-history variation in a colonial ascidian: Broad-sense heritabilities and tradeoffs in allocation to

asexual growth and male and female reproduction. The Biological Bulletin 192 (2): 290-299.

Yund, P. O., and Stires, A. 2002. Spatial variation in population dynamics in a colonial ascidian (*Botryllus schlosseri*). Marine Biology 141 (5): 955-963.

Zhan, A., MacIsaac, H. J., and Cristescu, M. E. 2010. Invasion genetics of the *Ciona intestinalis* species complex: From regional endemism to global homogeneity. Molecular Ecology 19 (21): 4678-4694.

Zuur, A. L., Ieno, E. N., and Elphick, C. S. 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1 (1): 3-14.