Biological Synopsis of the colonial tunicates, *Botryllus schlosseri* and *Botrylloides violaceus*

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BIOLOGICAL SYNOPSIS OF THE COLONIAL TUNICATES,

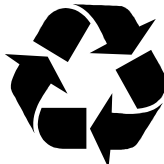***BOTRYLLUS SCHLOSSERI* AND *BOTRYLLOIDES VIOLACEUS***

by

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ABSTRACT

Two non-indigenous colonial ascidians *Botryllus schlosseri* (golden star tunicate) and *Botrylloides violaceus* (violet tunicate) are becoming increasingly abundant on both the east and west coasts of Canada. Their potential for rapid growth allows them to exploit new environments, potentially displacing native species and disrupting community dynamics. In particular, their tendency to colonize floating substrates and overgrow other organisms poses a threat to the viability of marine aquaculture operations. In both species colonies expand via the asexual budding of individual zooids or through fusion with closely-related colonies. This fusion process is controlled by a complex allorecognition system that allows the organism to detect the degree of genetic similarity between adjoining colonies. Both species are hermaphroditic and brood large larvae with a relatively short larval cycle and limited dispersal potential. Both species can tolerate a wide range of environmental conditions but growth rates increase substantially in warmer environments. Localized subpopulations may exhibit variable growth and reproductive patterns depending on the temperature and food regime. Colonial tunicates generally have few predators, particularly *B. violaceus* which may give it a competitive advantage over *B. schlosseri* in some environments. Major dispersal mechanisms are rafting on floating debris or hull fouling. No effective control mechanisms have been reported and any strategy that promotes fragmentation may only serve to enhance dispersal rates. Once these colonial tunicate species become established, they are extremely persistent and virtually impossible to eradicate; reducing the rate of dispersal is therefore imperative. More studies are required to document their life-history characteristics in Canadian waters, particularly such aspects as growth rates, spawning patterns, mortality events and overwintering survival strategies. Information on potential control measures and possible predators is also required to devise impact mitigation strategies for the aquaculture industry.

RÉSUMÉ

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Les deux ascidies coloniales non-indigènes *Botryllus schlosseri* (botrylle étoilé) et
Botrylloides violaceus (botrylloïde violet) deviennent de plus en plus abondantes sur les côtes
Est et Ouest du Canada. Leur potentiel de croissance rapide leur permet d'exploiter de
nouveaux environnements, possiblement déplaçant des espèces indigènes et perturbant la
dynamique des communautés. En particulier, leur tendance à coloniser les substrats flottants
et à envahir d'autres organismes constitue une menace à la viabilité des opérations aquacoles
marines. Les colonies des deux espèces croissent par bourgeonnement asexuel de différents
zooids ou par fusion avec les colonies reliées. Ce processus de fusion est commandé par un
système complexe d'allorecognition qui permet à l'organisme de détecter le degré de
similitude génétique avec une colonie contigüe. Les deux espèces sont hermaphrodites et
produisent de grosses larves avec un cycle relativement court et un potentiel limité de
dispersion. Les deux espèces peuvent tolérer un large éventail de conditions
environnementales mais les taux de croissance augmentent sensiblement dans des conditions
plus chaudes. Les sous-populations localisées peuvent présenter une croissance et un modèle
de reproduction variable selon le régime de température et de nourriture. Les tunicates
coloniaux ont généralement peu de prédateurs, en particulier *B. violaceus,* ce qui peut lui
donner un avantage concurrentiel par rapport à *B schlosseri* dans quelques environnements.
Les mécanismes principaux de dispersion sont le transport par débris flottant ou par
encrassement des coques des bateaux. Aucun mécanisme efficace de contrôle n'a été rapporté
et toute stratégie qui favorise la fragmentation ne peut seulement servir qu'à augmenter les
taux de dispersion. Une fois que ces espèces coloniales deviennent établies, elles sont
extrêmement persistantes et il est pratiquement impossible de les supprimer; il est donc
impératif de réduire leurs taux de dispersion. Plus d'études sont nécessaires pour documenter
les caractéristiques de leurs cycles de vie-histoire dans les eaux canadiennes, en particulier
des aspects tels que les taux de croissance, les modèles de reproduction, les taux de mortalité
et les stratégies de survie hivernale. Il est également nécessaire d'obtenir de l'information sur
des mesures de contrôle potentielles et des prédateurs possibles pour concevoir des stratégies
de réduction d'impact pour l'industrie aquacole.

1.0 INTRODUCTION

Over the past century there have been numerous biological introductions into the
Canadian marine environment, including several invasive species. Some of these, such as the
common periwinkle (*Littorina littorea*) have been present for over a century, whereas others
have only been detected recently, e.g. the green crab (*Carcinus maenas*) in 1951 (Audet et al.
2003) and the green fleece alga (*Codium fragile*) in 1989 (Bird 1993). Two species of non-
indigenous ascidians, specifically the solitary vase tunicate (*Ciona intestinalis*) and the
colonial golden star tunicate (*Botryllus schlosseri*) have been present since the early 1900's,
but are only now starting to exhibit the explosive population growth typical of invasive
species. Combined with the recent introduction of the solitary clubbed tunicate (*Styela
clava*) in 1998, and the colonial violet tunicate (*Botrylloides violaceus*) in 2001, invasive
tunicates have now become a serious biofouling concern for the aquaculture industry in
Atlantic Canada. Moreover, another colonial tunicate, *Didemnum* sp. is spreading rapidly
across Georges Bank, potentially jeopardizing the lucrative offshore scallop industry, and
posing an imminent threat to the marine ecosystem in eastern Canada. The purpose of the
following document is to review the life history characteristics of the two colonial ascidians,
B. schlosseri and *B. violaceus*, which are presently found in several regions of Atlantic
Canada as well as in British Columbia. Potential vectors and pathways for introduction and
dispersion will be discussed, as well as the effect of such factors as competition and
predation on population dynamics, and the urgent need for the development of impact
mitigation strategies.

1.1. NAME AND CLASSIFICATION

Taxonomic status according to the Integrated Taxonomic Information System (ITIS)
website (www.itis.usda.gov):

Kingdom:	Animalia
Phylum:	Chordata
Subphylum:	Tunicata
Class:	Ascidiacea
Order:	Stolidobranchia
Family:	Styelidae

Genus and Species: *Botryllus schlosseri*
Common Name: Golden Star Tunicate

Genus and Species: *Botrylloides violaceus*
Common Name: Violet Tunicate

B. schlosseri was first described by Pallas in 1766 under the name *Alcyonium schlosseri* (Millar 1966). *B. violaceus* was first officially distinguished as a separate species by Oka (1927), and the taxonomic details were later clarified by Saito et al. (1981). It has been frequently confused with similar temperate water species such as *Botrylloides leachi* and *Botrylloides diegensis* or even the boreal species *Botrylloides aureum.*

1.2. GENERAL DESCRIPTION

Colonial tunicates belonging to the genus *Botryllus* or *Botrylloides* are soft, smooth and fleshy in texture. They may take a variety of forms from thin flat encrusting mats to thick irregular lobes or projections depending on the shape of the substrate. In *B. schlosseri* the colonies are made up of small zooids (1-2 mm) or individuals arranged in a stellate or star-shaped pattern around a shared aperture or exhalent canal (Van Name 1945, Figures 1-2). By comparison, *B. violaceus* has larger zooids (2-4 mm) distributed in elongated irregular rows around a common aperture (Figures 1-2). A wide range of colour morphs are listed for *B. schlosseri* including purple, green and orange in New Zealand (Millar 1982) or blue, white and orange on the U.S. west coast (www.massbay.mit.edu). Records for the east coast of the U.S. indicate that *B. schlosseri* may have purple zooids with brown margins, red zooids with orange margins or appear purple-red throughout (Plough 1978). In Atlantic Canada, this species is typically bi-coloured with yellow or white zooids and brown, grey or black margins. Colonies of *B. violaceus* are typically monocoloured; the range of hues includes bright orange, burgundy, dull pink, lavender or purple (Lambert and Lambert 2003). Both species occur in sheltered areas on natural substrates such as algae or on artificial substrates such as floating docks or wharf pilings. *B. schlosseri* occurs subtidally down to depths of 200 m while *B. violaceus* is generally restricted to zones <50 m deep.

Figure 1. Photographs of *Botrylloides violaceus* and *Botryllus schlosseri* biofouling: (a) an aquaculture lantern net; (b) attached to a boat hull; and (c) a mass attached to seaweed.

Close-up photographs of *B. violaceus* (d) and *B. schlosseri* (e). Scale bars are 1 cm in (d) and 0.5 cm in (e).
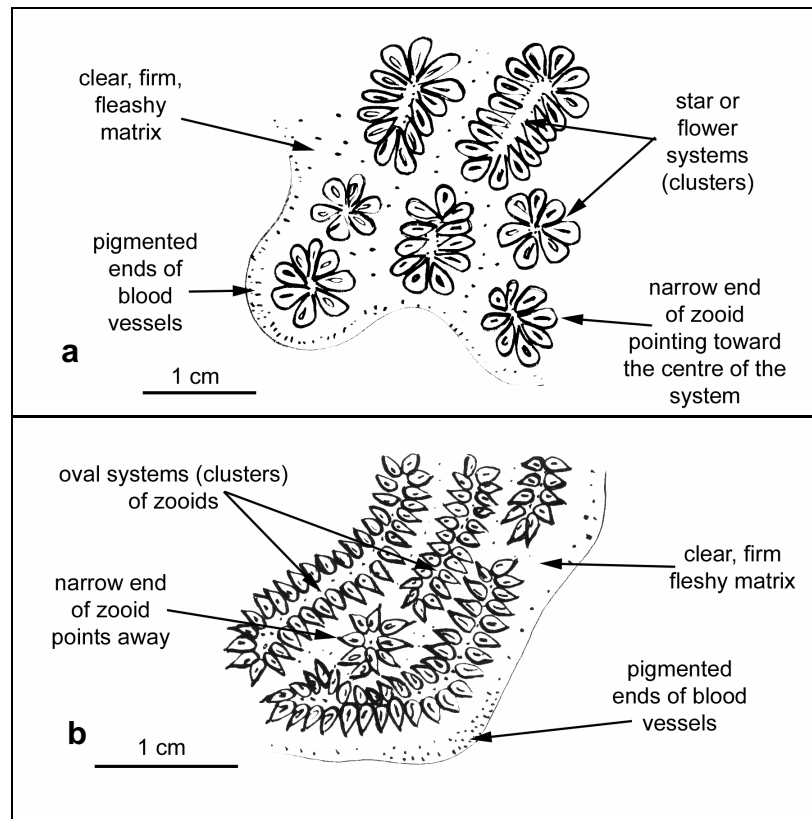
Figure 2. Top schematic views of (a) *B.schlosseri* and (b) *B. violaceus.* Note the difference in the orientation of the zooids within the matrix.

2.0 DISTRIBUTION

2.1. NATIVE DISTRIBUTION

B. schlosseri most likely originated from the Mediterranean Sea (Berrill 1950), but is now considered cosmopolitan as it can be found on all continents except Antarctica (Van Name 1945, Kott 1985). Current records are available for the Mediterranean Sea, the Black Sea, the Adriatic Sea (Italy and Greece), and the Suez Canal (www.deh.gov.au, www.iobis.org).

B. violaceus is believed to have originated from the Pacific Northwest, most likely from Japan (Oka 1927, Berrill 1950). It is considered a major biofouling species and a potential

problem for aquaculture operations in both Japanese (Tokioka 1953, Yamaguchi 1975, Arakawa 1990) and Chinese waters (Cao et al. 1998, 1999, Zhou et al. 2002).

2.2. NON-NATIVE DISTRIBUTION (EXCLUDING CANADA)

B. schlosseri

Outside of the Mediterranean Sea *B. schlosseri* is listed as occurring in the North Sea and the North Atlantic Ocean including the coasts of Norway (www.ascidiacea.com), Britain, France, Portugal, Spain, Denmark, Germany, and Sweden (www.iobis.org). In the case of North America, Bancroft (1903) reported that colonies in the northeast U.S. appeared to be identical to those observed in Naples (Italy). Plough (1978) reported that *B. schlosseri* occurs in the Gulf of Maine, off Georgia and south along the coast to Florida, although other surveys suggest that Chesapeake Bay is the southernmost limit (www.massbay.mit.edu).

The earliest observations from the west coast of North America are for San Francisco Bay in the early 1940's (Van Name 1945); apparently *B. schlosseri* was common in southern California harbours by the early 1960's (Lambert and Lambert 1998). Its subsequent appearance at an oyster farm in Washington State in the early 1970's was likely associated with the movement of oyster spat. Although it now occurs throughout the Puget Sound area (Cohen et al. 1998), it was not recorded in a recent survey of ascidians in Alaska (Hines and Ruiz 2000).

The earliest record for *B. schlosseri* in New Zealand is Michaelsen (1922) and the species is now widely distributed throughout the North and South Island (Millar 1982). Records for Australia suggest that it was observed as early as 1928 (Van Name 1945). Current reports indicate a widespread distribution from Queensland on the northeast coast extending southwards and around to the west coast, including Tasmania (www.deh.gov.au). However, it was noticeably absent from certain recent port surveys (e.g. Cohen et al. 2000a, 2000b) suggesting a patchy distribution.

There is little information as to the distribution of *B. schlosseri* in Asian waters. Uchida et al. (1952) described this colonial tunicate as a common fouling organism in the Inland Sea of Seto (Japan), and it is listed as occurring in Hong Kong (www.deh.gov.au), but there are few documented records.

B. violaceus

Although *B. violaceus* is listed as occurring on the northeast coast of Australia (Queensland) (www. iobis.org), Australian government surveys do not mention its presence (www.deh.gov.au). In general, the dispersal history of *B. violaceus* is difficult to trace as it has traditionally been confused with other *Botrylloides* species, particularly its congener *B. diegensis*. Ascidian taxonomic experts Lambert and Lambert (2003) noted that even in their 1998 survey of southern California, they failed to distinguish the non-indigenous *B. violaceus* from the native species *B. diegensis.*

The earliest report of *B. violaceus* in southern California was possibly Van Name (1945), although he failed to describe the large larvae diagnostic of this species. It was not definitively identified until the 1970's (Fay and Vallee 1979). Cohen and Carlton (1995) suggest that the earliest record was 1973 for San Francisco Bay. Currently *B. violaceus* occurs in large numbers along the west coast of North America from Alaska south to Ensenada (Mexico) (Cohen et al. 1998, Lambert and Sanayam 2001, Lambert and Lambert 2003).

The arrival of *B. violaceus* on the U.S. east coast is likewise not well documented due to confusion over species taxonomy. For example, Lesser et al. (1992) listed the boreal species "*Botrylloides aureum*" as a common member of the mussel culture fouling community in New England, and Berman et al. (1992) described the invasion and spread of "*Botrylloides diegensis*" in the Gulf of Maine; subsequent observations suggested that these species may have been mis-identified. At present *B. violaceus* occurs abundantly from Maine south to Rhode Island, and is often a dominant member of the biofouling community (Lambert and Lambert 2003, Pederson et al. 2005).

In European waters, *B. violaceus* was first documented in Venice Lagoon (Italy) in the 1990's (Zaniolo et al. 1998) and has recently been documented in the Netherlands (www.ascidians.com). Genetic tools such as microsatellites are being used to pinpoint the origin of various colonial species and identify the pathways that may be implicated in a given introduction (Stoner et al. 1997, Ben-Shlomo et al. 2001, Paz et al. 2003). For example, Stoner et al. (2002) used microsatellite techniques to demonstrate that the source of *B.*

schlosseri introduced to California was not from the U.S. east coast, as had been previously suggested (Boyd et al. 1990), but rather from European or Asian populations.

2.3. DISTRIBUTION IN CANADA

Both *B. schlosseri* and *B. violaceus* have been documented on the east and west coast of Canada, but their presence in Arctic waters has not been confirmed. Although *B. schlosseri* is listed as occurring in the Bay of Fundy (Logan et al. 1983) as well as northwards into the Gulf of St. Lawrence, it is generally indicated as rare (gmbis.marinebiodiversity.ca/BayOfFundy/background.html). In Nova Scotia, *B. schlosseri* has been observed for several decades at low levels along the Bay of Fundy coast, the Atlantic coast and in the Bras D'Or Lakes (Cape Breton).

Whiteaves (1900) reported the occurrence of *Botryllus* sp. at 90 m off Percé, Québec and at 150 m off Baie Comeau, Québec. However, Van Name (1945) argued that these colonial tunicates were *Botrylloides* sp. rather than *Botryllus* sp. In their comprehensive survey, Brunel et al. (1998) listed *B. schlosseri* as circalittoral (20-200 m) in the Estuary and Gulf of St. Lawrence, with specific records for the Upper North Shore of Québec, the southern Gaspé/ Baie des Chaleurs (Whiteaves 1900), and the west coast of Newfoundland (Hooper 1975). Although, there are no recent sightings of this species in shallow coastal regions of New Brunswick (N.B.) or north of Prince Edward Island (P.E.I.), it has been recently documented in various P.E.I. embayments including St Peter's Bay in 2001, Savage Harbour in 2004 and Cardigan and St. Mary rivers in 2005 (Figure 3).

Figure 3. Distribution of *B. schlosseri* in P.E.I. as of February 2006 (map courtesy of Neil MacNair, PEIDAF, Art Smith and Deryck Mills, DFO).

   B. violaceus was sighted for the first time on the Canadian Atlantic coast in 2001 in the Lunenburg and Mahone Bay area of N.S. (Carver, pers. obs.). A recent survey performed in November 2005 from the LaHave to Chester region (Figure 4) indicated the presence of both *B. violaceus* and *B. schlosseri* on most nautical buoys (Vercaemer, unpubl. obs.).
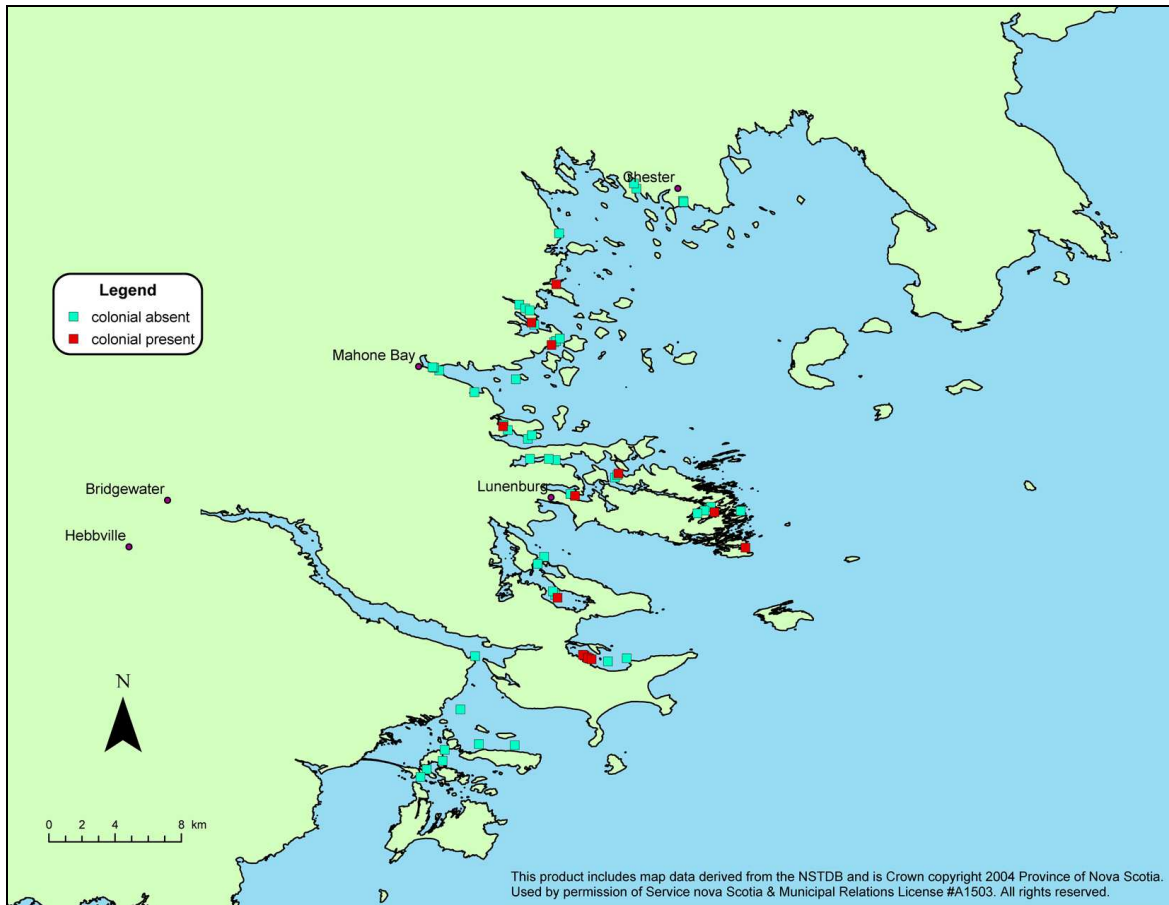
Figure 4. Results of the November 2005 small nautical buoy survey, between LaHave and Chester, N.S. Note the patchiness of the distribution of *B. schlosseri* and *B. violaceus*, referred to as "colonial" tunicates in the legend.

B. violaceus has been established in Savage Harbour, P.E.I. since the summer 2004 and has been reported in 2005 in several other P.E.I. bays including Cardigan River on the eastern end and the Summerside area on the south coast (Figure 5). To date, there have been no reported sightings of this species in N.B. or the Gulf of St. Lawrence north of P.E.I. Brunel et al. (1998) referred to the presence of the coldwater species *Botrylloides aureum* in the bathyal zone (200-500 m) of the Gulf of St. Lawrence based on records from Van Name (1910).
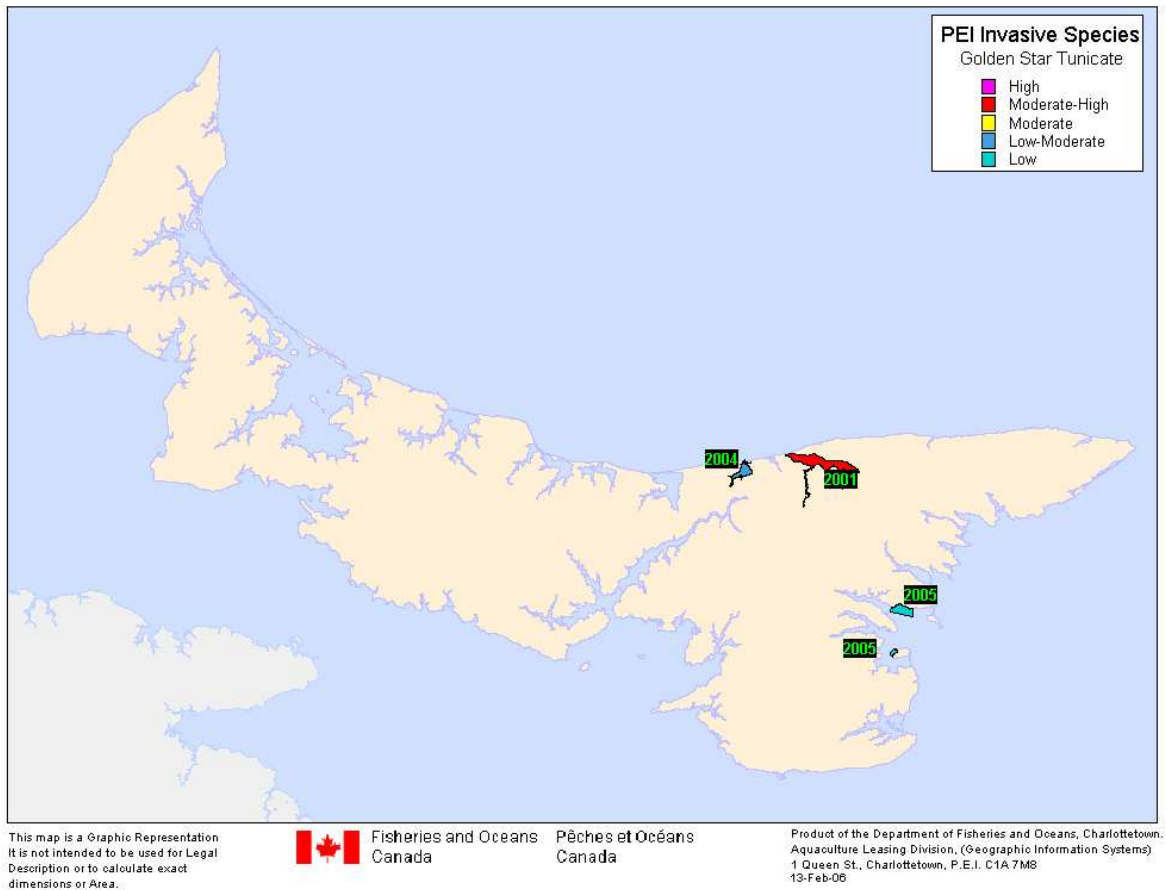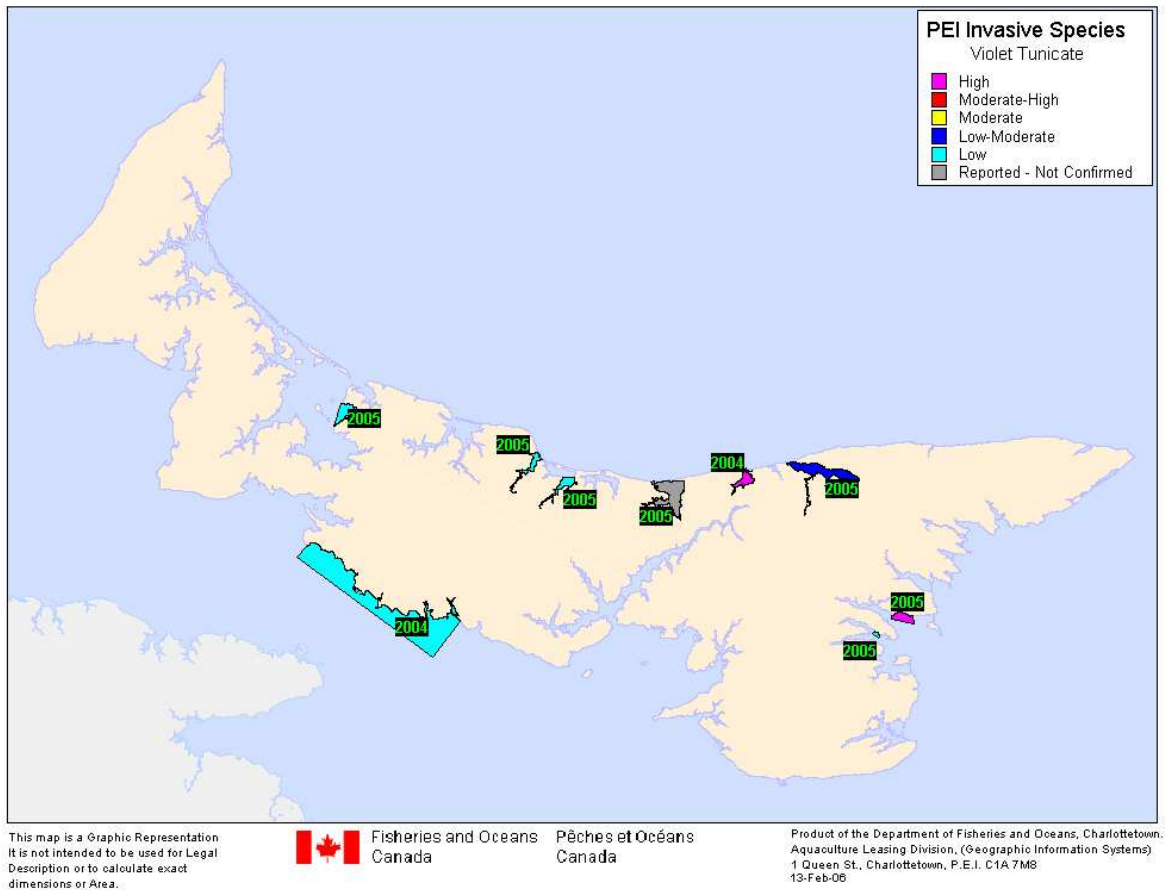
Figure 5. Distribution of *B. violaceus* in P.E.I. as of February 2006 (map courtesy of Neil
     MacNair, PEIDAF, Art Smith and Deryck Mills, DFO).

On the Canadian West coast *B. schlosseri* is common in harbours at the southern end of
Vancouver Island (Lambert and Lambert 1998), but is not cited as a major fouling problem
for aquaculture operations. In contrast, the more abundant colonial species *B. violaceus* is
considered a major fouling concern for both shellfish and finfish growers who rely on nets as
part of their culture technique (Figure 6).
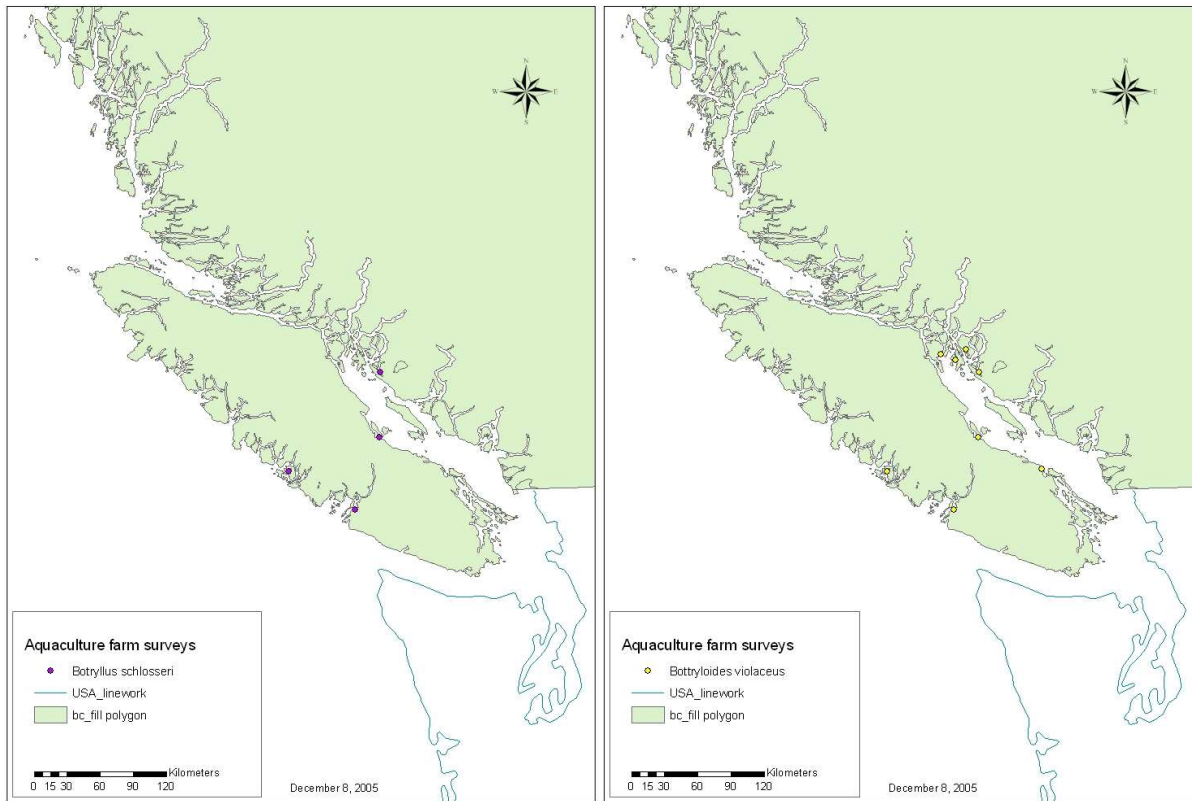
Figure 6. Results of the October 2005 aquaculture farm surveys in southern British Columbia (maps courtesy of Thomas Therriault, DFO-Pacific Biological station). Note that several recent sightings (spring 2006) of *B. violaceus* on the southwest coast of Vancouver Island are not indicated on this map.

3.0 BIOLOGY AND NATURAL HISTORY

3.1. BODY STRUCTURE

Both *B. schlosseri* and *B. violaceus* belong to the family Botryllidae and are characterized as colonial or compound ascidians with small zooids arranged within a common gelatinous matrix or tunic. The bulk of the tunic is composed of tunicin, a polysaccharide similar to cellulose (Hirose et al. 1991). Each individual zooid has an inhalent siphon but its atrial or exhalent siphon opens into a common atrial cavity which connects to the surface of the colony via a common exhalent canal (Figure 7). In *B. schlosseri* adjacent zooids share an exhalent siphon, whereas in *B. violaceus* each zooid has an independent siphon opening into the common cavity. By sharing a common vascular system and common exhalent canal, this morphological arrangement maximizes the number of zooids in a given area (Taneda and

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Watanabe 1992). Campbell et al. (1999) suggested that by diverting their exhalent siphons into a common chamber, the colony effectively augments the force of the exiting current thereby ensuring that waste products and larvae are propulsed or dispersed away from the main colony.
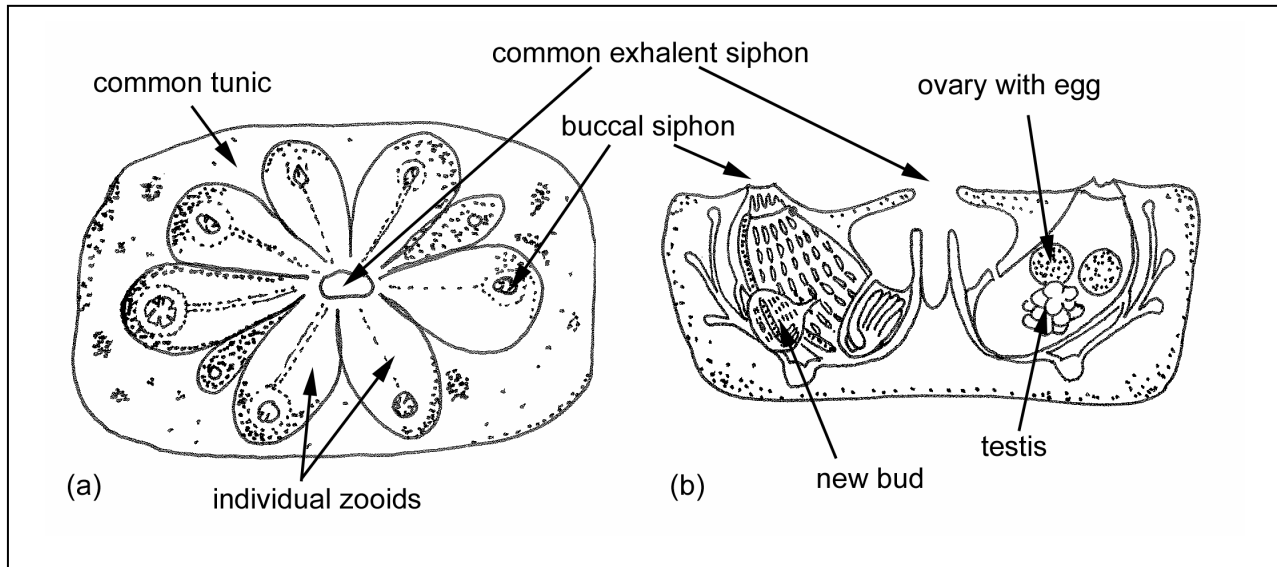
Figure 7. General top (a) and cross section (b) schematic views of a colonial tunicate (from Berrill 1941). Note the development of a new individual or bud from the atrial epithelium.

Attached to each mature zooid are 1-2 developing buds or blastozooids which will form the next generation (Figure 7b). The coordinated development of these buds with their adjoining atrial siphons leads to the formation of large colonial systems (Berrill 1941, Plough 1978). The zooids share a common vascular system that consists of blood vessels and enlarged sausage-shaped vascular ampullae along the periphery (Milkman 1967, Brunetti and Burighel 1969). This gelatinous matrix has the capacity to reconstruct the colony by vascular budding if all the zooids are lost (Sabbadin 1979). The internal structure of a zooid is dominated by the branchial sac, or pharynx, which is dedicated to the removal of food particles from the water column (Figure 8a). The water enters via the inhalant siphon, passes through the thin slits or stigmata in the wall of the branchial sac and then exits into the shared atrial cavity and out through the common exhalent canal. Faecal material is also released into the atrial cavity for subsequent discharge through the exhalent canal.
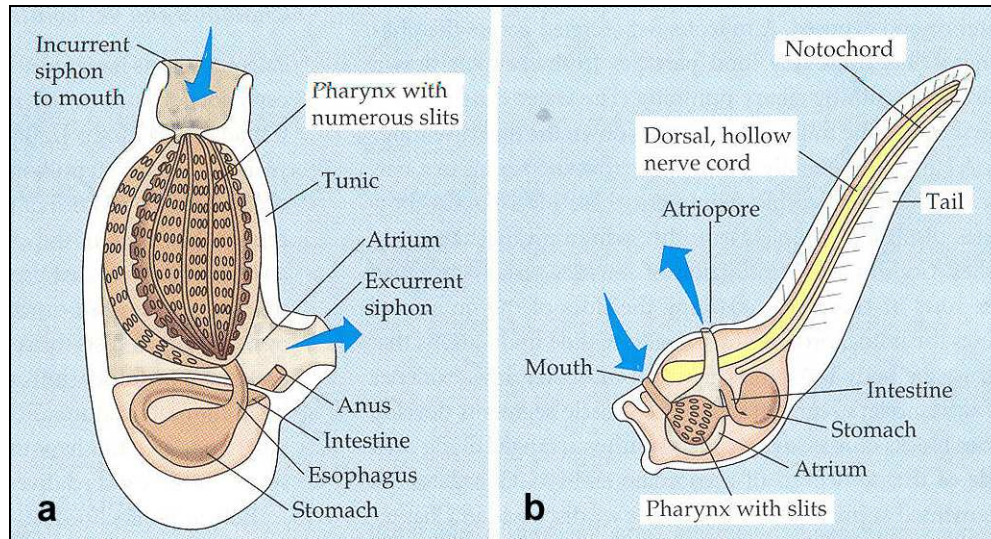
Figure 8: Morphological features of the adult (a) and larval stages (b) of a colonial tunicate (Campbell et al. 1999). Note the four features in the larvae that illustrate the common ancestry with vertebrates (the notochord, the dorsal nerve cord, the pharyngeal slits, and the post-anal tail).

Burighel et al. (2003) described a mechanoreceptor organ in the oral siphon of *B. schlosseri* and *B. violaceus*, believed to be related to the sensory cells in vertebrates which may provide a means of detecting movement and sound waves underwater. Detailed descriptions of the circulatory and nervous systems of *Botryllus* and *Botrylloides* are available in Mukai et al. (1978).

In both species male and female gonads are separate and located on either side of the body (Millar 1966). In the case of *B. schlosseri*, the adult female organs typically consist of one to three ovaries each containing one large egg, located on either side of the body anterior or dorsal to the testes. As many as four to six ovaries may be present on one side but then there are fewer on the other side (Van Name 1945). After the eggs are fertilized, the embryos are released into the atrial cavity where they develop into "tadpole" larvae (Figures 8b and 9). In contrast, *B. violaceus* has one ovary on either side of the body located posterior to the testes. Single eggs pass through the oviduct into a sac-like developmental or incubatory pouch that forms as an outgrowth of the body wall (Saito et al. 1981). Mature brooded larvae are evident through the translucent wall of the tunic. The two species can be easily distinguished on the basis of morphological structure of the tadpole larvae and early

Figure 9: Photographs of the ascidian *B. violaceus.* This specimen was identified by Gretchen Lambert, and photographed by Leslie H. Harris of the Natural History Museum Los Angeles County.

juvenile forms; for photos of larvae and young juveniles of *B. schlosseri* and *B. violaceus* see Bullard and Whitlatch (2004) or convoluta.ucdavis.edu/gallery/albums.php.

3.2. FEEDING

Although there are few data on the feeding rates of colonial tunicates, ascidians are known to be mucus filter feeders that can extract particles as small as 0.5 μm up to a limit set by the size of the oesophagus (Bone et al. 2003). The feeding currents are created by cilia in tracts located on either side of the stigmata in the walls of the branchial sac (Jorgensen 1984). Particles are collected on a mucus sheet that migrates by ciliary action across the internal surface of the branchial sac from where it is concentrated and directed into the stomach. This feeding net of fine mucus filaments allows the trapping of 2-3 μm particles at 100% efficiency. Few data are available on the feeding activity of colonial ascidians although Sherrard and LaBarbera (2005) recently compared flow rates in juveniles and adults.

3.3. SEXUAL REPRODUCTION AND DEVELOPMENT

B. schlosseri

Grosberg (1988) reported that food availability affects fecundity or egg production rates in *B. schlosseri.* Rinkevich et al. (1998) observed that 41% of *B. schlosseri* colonies on the eastern Mediterranean coast were not sexually reproductive, another 30% were male only and the remaining colonies were hermaphrodites. Variations in egg and sperm production

rates among the *B. schlosseri* colonies in the Damariscotta River, Maine, were also attributed to environmental factors (Stewart-Savage et al. 1999, 2001). When clones were transplanted into new environments, they altered their reproductive strategy; more productive and warmer environments tended to favor egg production whereas colder food-limited sites favoured male reproduction (Newlon et al. 2003).

Given the relationship between food availability and fecundity, it is predictable that reports on egg production per zooid in *B. schlosseri* vary considerably. In Israeli waters *B. schlosseri* colonies at the peak of reproduction develop 1.2 oocytes per zooid, and up to 58% produce >4 clutches or a maximum of 5 eggs per zooid (Rinkevich et al. 1998). According to Hiscock (2005) fecundity of *B. schlosseri* in British waters is 2-10 eggs per zooid. In a field study in California, Chadwick-Furman and Weissman (1995) documented that each *B. schlosseri* zooid produced up to 10 clutches, each with a maximum of 5 eggs resulting in a fecundity of up to 50 eggs per zooid or 8000 eggs per colony. Note that the eggs of *B. schlosseri* are relatively large (300 μm) and lecithotropic or yolky.

Botryllid colonial tunicates are cyclical hermaphrodites where the sperm mature 1-2 d after ovulation (Mukai 1977; Yund et al. 1997). Sperm is released throughout the sexual cycle but the amount varies through time for a given colony (Stewart-Savage and Yund 1997). *B. schlosseri* sperm have considerable dispersal potential; they are apparently quiescent until taken up by feeding zooids and only activated when exposed to ovulated eggs. They can be effective up to 28 h after release and can fertilise eggs at low concentrations (Johnson and Yund 2003, 2004). *B. schlosseri* can also concentrate sperm thereby increasing the probability of fertilization at low sperm densities (Phillippi et al. 2004). Using rare alleles as a tracer, Grosberg (1991) found, however, that the male effect declined rapidly 50 cm away from the colony, suggesting that actual sperm dispersal may be more limited than their longevity would imply. There is also evidence for the existence of sperm competition; the presence of other males has a negative impact on the fertilization success of local male colonies (Yund and McCartney 1994, Yund 1995, Atkinson and Yund 1996).

In contrast to other colonial tunicates, selfing can be induced and homozygous offspring can be obtained in *B. schlosseri* (Sabbadin 1989). However, Milkman (1967) argued that because the testes typically ripen 1-2 d after ovulation, self-fertilization is probably a rare

event. In this ovoviviparous species the fertilized eggs or embryos are retained in a cup-like organ within the atrial cavity of the parent colony during the development process; they do not have a feeding system but rather rely on the nutrients contained in the tunic surrounding them (Yund et al. 1997). The larval development period in the cup-like organ is approximately a week, although considerable variation in duration has been observed. Grave and Woodbridge (1924) investigated whether this variation was genetic, but found no evidence in the daughter colonies. Millar (1971) suggested that this variability may ensure that some larvae settle near the parental colony whereas others are more widely dispersed thereby enabling colonization of more distant habitats.

The tadpole larva of *B. schlosseri* has an ovoid trunk (0.5 mm long) with a single black sensory pigment spot or ocellus (Hiscock 2005). The larva has a rudimentary locomotor organ, or tail, which provides the means to orient themselves in the water column (McHenry 2005). After the tadpole is released it may swim freely for up to 36 h (Berrill 1950). Prior to metamorphosis the larvae attach to the substrate using adhesive papillae. Little is known regarding choice of substratum (i.e. gregarious settling behaviour); the presence of nerve fibres in the adhesive papillae (Grave and Woodbridge 1924) suggests that they may respond to solid substrates. Porter (2003) suggested that bacteria may provide some settlement cues to the larvae, but Schmidt and Warner (1986) found that larvae showed no significant preference for filmed surfaces. During this settlement period, a complex series of morphological changes occur including the absorption of the tail, the loss of the notochord, the expansion of the pharynx, and the development of the inhalent and exhalent siphons (Brunetti and Burighel 1969). The metamorphosing larva extends 8 ampullae and a functional zooid is formed within 3 d at 20-25°C (Figure 9). The larvae may settle near another colony and fuse or may grow to form a new colony (Rinkevich and Weissman 1987; Chadwick-Furman and Weissman 2003), but most larvae remain within a few meters of the parental colony (Grosberg 1987). Settlement on a dead colony usually translates into the death of the progeny (Rinkevich and Weissman 1987).

B. violaceus

B. violaceus normally produces one 80-μm alecithal or non-yolky egg (Manni et al. 1995), but occasionally two, which are ovulated into the atrial cavity and lodged within a

brood pouch in the tunic (Mukai 1977, 2004). The egg is fertilized within the brood pouch where it undergoes embryogenesis (Mukai et al. 1987). In this viviparous species the brood pouch eventually becomes detached from the atrial epithelium and becomes incorporated in the colonial tunic (Zaniolo et al. 1998). During the 1-mo gestation period the embryo receives nutrients from the blood flowing through the tunic; the embryos may reach 1-1.5 mm in diameter and are clearly evident through the wall of the tunic (Takeuchi 1980). Embryos in various stages of development may be observed within incubatory pouches in the same colony during the peak period of sexual reproduction (Saito et al. 1981). The mother zooids disintegrate approximately 5 d after ovulation, leaving only the brood pouches containing the developing larvae (Mukai et al. 1987).

In *B. violaceus* the free-swimming tadpole larvae break through the wall of the incubatory pouch to reach the exterior of the colony (Takeuchi 1980). The larvae are huge (2-3 mm) with 24-32 ampullae (Figure 10b); they swim for only a brief period (4-10 h) before using their elongated ampullae to attach to a suitable settlement substrate (Saito et al.1981). Yamaguchi (1975) observed that *B. violaceus* release their larvae at dawn, at which time they show marked positive phototaxis; larvae were observed to settle within a few hours of liberation. Takeuchi (1980) reported that larvae required only 1-2 d to attach and metamorphose into fully functional oozooids with branchial and atrial apertures. After 4-7 d blastozooids or buds develop at the base of the oozooids; at 7-10 d these blastozooids become functional and the primary oozooid degenerates.
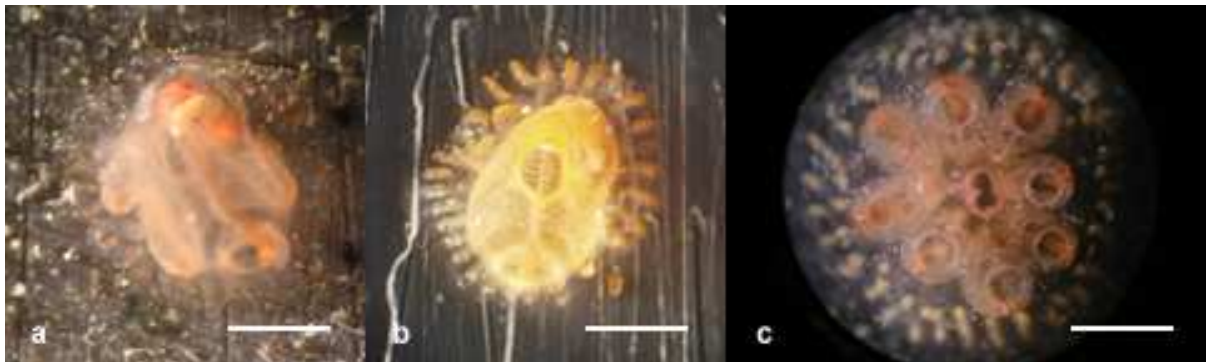

Figure 10. Photographs of recently settled (a) *B. schlosseri* (1-2 wk old, scale bar is 1mm), (b) *B. violaceus* (1 d old, scale bar is 0.7 mm) and (c) *B. violaceus* at 1-2 wk old, scale bar is 2 mm). Pictures taken by Marie Nydam and provided by the Stachowicz lab (reprinted with permission).

3.4. LIFE CYCLE: GROWTH, GENERATION TIME AND LONGEVITY

Colony growth occurs primarily by asexual budding or a process termed "blastogenesis" (Rabinowitz and Rinkevich 2004). As each new generation of blastozooids (or zooids) matures and begins to feed, the parent zooid is resorbed and new buds are formed (Berrill 1941). Thus, at any one time a colony is composed of three generations in three discrete stages of development: adult feeding zooids, primary buds connected to the adult, and secondary buds connected to the primary buds. The timing of the blastogenic process and the onset of the resorption phase is synchronous throughout the colony (Milkman 1967; Sabbadin 1979). In *B. schlossseri* each 5-7 d cycle (18°C) ends with a massive mortality of one generation over a 30-h period (Lauzon et al. 1992), or a "cell apoptosis" phase similar to the resorption of larval structures following metamorphosis (Zaniolo and Burighel 1976). Upon examining clonal replicates, Rinkevich et al. (1992) found a strong heritable basis underlying mortality, unlinked to reproductive effort or other life history traits. Furthermore, when a parent colony is experimentally separated into a number of clones, these subclones undergo senescence simultaneously (Lauzon et al. 2000).

Measurement of growth in botryllid colonies is complicated by the combined effects of one generation proliferating while another degenerates. Millar (1952) suggested making successive area measurements of intact or discrete colonies. Sabbadin (1957) tried to measure the growth of *B. schlosseri* by the number of zooids rather than by area but found this number to vary widely among colonies of similar age. Most growth estimates are based on zooid doubling time. For example, Grave (1933) observed that the number of zooids in *B. schlosseri* may double every 2-3 d; a fast-growing colony may attain 1000-2000 zooids within a month of establishment. Boyd et al. (1986) estimated a doubling time of 8 d for *B. schlosseri* at 12-17°C. Grosberg (1988) observed that during the early summer 80% of the colonies grew at rates exceeding 2.5 buds per zooid per asexual cycle, but this declined as the colonies entered their sexually reproductive phase. Rinkevich et al. (1998) noted that zooids at the colony's periphery developed twice the number of buds but fewer eggs than did zooids near the center.

Assessment of colony growth is also complicated by the possible fusion of colonies when they come into contact with clonal buds, kin buds or unrelated zooids. When this contact

occurs several types of interactions may ensue including full integration into a single colony, partial integration or complete exclusion (Mukai and Watanabe 1975, Rinkevich et al. 1993). In his review of Botryllid ascidians, Rinkevich (2005) described the sophisticated "allorecognition" system that allows the detection of genetic similarity and determines whether colonies either fuse to form a chimera (Rinkevich et al. 1993) or develop cytotoxic lesions at the contact zones. A fusion scenario may lead to the establishment of a common blood circulation and the complete integration of one colony into the other (Laird and Weissman 2004). Contact between unrelated colonies typically leads to a "haemolytic rejection reaction" (Hirose 2003) either upon contact or following fusion to form a chimera (Cima et al. 2004). Evidence suggests that the fusion and integration of clones or closely-related kin is beneficial to the colony whereas fusion between distantly-related colonies is associated with reduced fitness (Chadwick-Furman and Weissman 2003). Note that *B. violaceus* differs from *B. schlosseri* in that it only demonstrates allorecognition at the colony periphery; juxtaposition of cut surfaces resulted in fusion regardless of origin (Hirose et al. 1988).

In the case of *B. schlosseri*, the sexual reproductive cycle is initiated after 5-10 asexual growth cycles; this process is synchronized with the asexual cycle such that the maturation of a new generation of zooids coincides with ovulation (Milkman 1967). In *B. schlosseri* embryogenesis is completed and larvae are released just before the regression of the parent zooids, whereas in *B. violaceus*, the larval development period extends several weeks after the regression of the parent zooids. The duration of the breeding season in *B. schlosseri* varies with temperature, with more northerly cooler populations showing progressively shorter seasons; Millar (1971) summarized records from Naples, Italy (Jan-Dec), Venice, Italy (Apr-Nov), Brittany, France (Jun-Oct), Netherlands (Aug-Nov) and Millport, Scotland (Jun-Aug). Breeding patterns may also vary over short distances; Yund and Stires (2002) observed that sexual reproduction and larval settlement of *B. schlosseri* in the Damariscotta River estuary (Maine) began earlier in the summer in up-river populations which translated into a higher biomass than down-river populations. Two peaks in settlement density up-river (early July and early September) suggested that colonies may have completed two sexual generations, in contrast to a single generation at down-river sites. Rinkevich et al. (1998)

also observed that *B. schlosseri* populations in the eastern Mediterranean were divided into local subpopulations exhibiting microgeographic differences in life history patterns.

Chadwick-Furman and Weissman (1995) documented the growth, reproduction and senescence of 4 cohorts of *B. schlosseri* in Monterey Bay, California (USA). Exponential growth was confirmed from the juvenile to adult stage, reaching a colony size of 1400 zooids in 69 d and sexual maturity in 49 d. Following a period of continual reproduction, up to 70 d, the colony abruptly senesced and died while bearing eggs. Fall-born colonies exhibited an 8-mo lifespan compared to 3 mo for spring-born colonies, but the lifetime fecundity was similar between the two morphs. Other estimates of lifespan for *B. schlosseri* are 12-18 mo in the Mediterranean (Millar 1952, Sabbadin 1955), compared to <12 mo for British waters (Hiscock 2005). The factors that control colony longevity and possibly dormancy over the winter are less well understood. Millar (1971) observed that colonies may pass the winter in a relatively inactive condition, in some cases reduced to a mass of dormant buds. Grosberg (1988) noted that colonies which recruited after September 15 did not reproduce sexually until the following spring, but overwintering survival exceeded 90%.

Grosberg (1988, 1991) and Harvell and Grosberg (1988) described two different life-history morphs of *B. schlosseri* in Massachusetts. Semelparous colonies became reproductive early, showed a high reproductive output, and underwent rapid death. Iteroparous colonies grew at half the rate, had twice the lifespan, and invested 75% less in reproduction. Semelparous colonies dominated through mid-summer whereas the iteroparous colonies dominated in late summer. These different life history traits may reflect a possible fitness strategy to better cope with seasonal variation.

Relatively few studies have documented the life-history traits of *B. violaceus*, although Yamaguchi (1975) documented the life cycle of this species in Japanese waters. Following settlement the first zooid grew from 0.6 mm to a mature size of 2.5 mm within a week and then began producing buds asexually. The number of zooids increased exponentially thereafter until all the available space was occupied. The mean number of zooids exceeded 100 per colony within 2 wk in the summer (20-25°C) and 4 wk in the winter (14-20°C). Doubling time decreased by a factor of 3 with a 10°C increase in temperature. At 17°C individual colonies attained dimensions of 50-60 cm or 2000-3000 zooids only 8 wk after

larval metamorphosis. At this time, sexual reproduction and larval production was initiated and continued for 3 wk, followed by the regression and degeneration of the parent colonies. This cycle continued throughout the year with many generations per year. Yamaguchi (1975) suggested that substrate or space limitation may have triggered the switch from asexual colony growth to sexual reproduction.

The only information on the reproductive activity of *B. violaceus* in Canadian waters comes from a 2005 study in Savage Harbour, P.E.I. (MacNair, pers. comm.). A survey of colonies sampled from the surface of cultured mussels and aquaculture gear indicated the presence of mature eggs in all samples collected from mid-April to mid-September. Following mid-September the condition of the ovaries varied from no activity to the presence of mature eggs until the sampling was completed in mid-December. Developing larvae were observed in the colony tunic in late June and the first recruitment event occurred on July 14. Assuming a 1-mo maturation period for *B. violaceus* larvae, egg fertilization likely occurred in mid-June when water temperatures reached 15°C. Larval recruitment was ongoing until mid-October or approximately 1 mo after the cessation of egg production. Note that although some colonies continued to produce eggs through the fall, no larvae were observed.

3.5. HABITAT AND ENVIRONMENTAL TOLERANCE

The worldwide distribution of botryllid ascidians, from sub-arctic environments to temperate waters, is consistent with their tolerance to a wide range of environmental conditions. They occur on a variety of natural and artificial substrates such as rocks, algae, other organisms, floats, ship hulls and aquaculture equipment. Their phenotypic plasticity or the ability to adjust their growth and reproductive strategy in response to variable environmental conditions makes them remarkably resilient. According to Millar (1971) *B. schlosseri* can survive stressful environmental conditions by discontinuing sexual reproduction and reducing the rate of budding until there is only one bud per zooid. If all zooids are lost and only the vascular ampullae in the matrix survive, then vascular budding can generate a new colony when conditions improve (Oka and Watanabe 1957, 1959, Sabbadin 1979).

In general Botryllid ascidians thrive in warm productive environments (Chadwick-Furman and Weissman 1995). These conditions are associated with larger colonies and higher egg production compared to less productive conditions where colonies are smaller and have a higher proportion of male zooids (Stewart-Savage et al. 1999, Newlon et al. 2003). Brunetti et al. (1980) grew *B. schlosseri* at nine combinations of temperature (13°C to 25°C) and salinity (25°/oo to 40°/oo). No effect was detected up to the fifth blastogenic generation, but thereafter, high temperature and intermediate salinity stimulated gonad maturation whereas low temperature and high salinity stimulated colony growth. In general adult colonies tolerated a wider range of temperature and salinity conditions than did younger colonies. The distribution of *B. schlosseri* appears to be limited by salinities lower than 18°/oo, but the species can sustain high salinities (Hiscock 2005). Rasmussen (1997) documented an extensive population of *B. schlosseri* on the bottom of a Danish fjord system with high salinity and high summer temperatures. Few data are available on the temperature and salinity tolerance of *B. violaceus,* but Yamaguchi (1975) noted that the doubling time of colony growth decreased approximately 3-fold with a 10°C increase in temperature. Stachowicz et al. (2002b) observed that elevated seawater temperatures favoured the growth of *B. violaceus* over *B. schlosseri.*

According to Hiscock (2005) *B. schlosseri* may occur in areas with high sediment loads but is generally found on downward-facing or suspended surfaces that reduce the risk of smothering. Likewise, Yamaguchi (1975) noted that larvae of *B. violaceus* which settled on surfaces which were then placed in an upward-oriented position suffered from the accumulation of detritus whereas those placed in a downward-oriented position survived. Grosberg (1988) also noted that rates of settlement and subsequent survival were lower for *B. schlosseri* colonies developing on upward-oriented surfaces. Insufficient water flow and increased siltation particularly on benthic substrates would likely be detrimental to these species as silt may clog the branchial sac. Lambert and Lambert (2003) noted that *B. schlosseri* apparently died off following dredging in Newport Harbour (California). Despite its limited tolerance for high siltation, Naranjo et al. (1996) characterized *B. schlosseri* as one of the few species tolerant of extremely polluted conditions in Algeciras Harbour in southern Spain. Lambert and Lambert (2003) noted that although the diversity of the ascidian

community was relatively low at a highly polluted site in Mexico (Ensenada), the surviving assemblage included *B. schlosseri* and *B. violaceus*. Both these species can apparently outcompete other fouling organisms under suboptimal adverse conditions, including high sewage and heavy metal concentrations.

Colonies are very susceptible to desiccation, and are rarely observed in intertidal areas and only in damp shaded zones (Rinkevich et al. 1993). *B. schlosseri* can survive hypoxic conditions by changing the characteristics of their acid soluble carbohydrates (Beregovaya 2002).

3.6. ECOLOGY: INTERSPECIFIC INTERACTIONS

Artificial floating structures are often the first surfaces to be colonized by introduced species due to increased substrate availability, less competition, and lower predatory pressure than may be encountered in natural benthic communities (Connell and Glasby 1999, Connell 2000, 2001). Once established, colonial tunicate species have the potential to reach sexual maturity within a few weeks and rapidly establish broodstock populations. This propensity to expand at a rapid rate and overgrow existing biotic/abiotic substrates may lead to changes in community biodiversity. However, Berman et al. (1992) noted that while *B. violaceus* (mis-identified as *B. diegensis*) was initially extremely prevalent in the Gulf of Maine following its introduction, this dominant status did not persist. Apparently other factors such as competition and predation may play an important role in controlling the distribution and population dynamics of colonial tunicate species.

The cyclical nature in the colony size of colonial ascidians often leads to seasonal patterns of predominance and overgrowth of existing species (Harms and Anger 1983). In fouling communities, the composition and abundance of the established individuals may be important in determining larval settlement rates and subsequent juvenile growth and survival. Upon settlement, the <1-mm juvenile zooids must deal with a variety of factors including a suite of potential predators and competitors (Osman et al. 1992). Early studies showed that the presence of resident adult ascidians may influence larval settlement by affecting the amount of space available (Zajac et al. 1989; Osman and Whitlatch 1995a). For example,

Stachowicz et al. (2002a) observed decreased survival of *Botrylloides* recruits in communities with higher species richness due to reduced resource availability. However, recruitment is not specifically inhibited by the presence of other sessile species (Osman and Whitlatch 1995a). Bullard et al. (2004) confirmed that colonial tunicates do not have a specific mechanism to prevent or preclude settlement of other invertebrate larvae.

The patchy distribution of many ascidians is likely attributable to spatial variability in the abundance of predators and hence predation pressure on young recruits. In New England waters Osman and Whitlatch (1995b, 2004) observed that early post-settlement stages of the two species varied in their susceptibility to predation. *B. violaceus* was only vulnerable to predators for a few days to a week following settlement; evidence of partially eaten recruits of *B. violaceus* suggested that this species may be unpalatable or chemically defended as in some other species of ascidians (Pisut and Pawlik 2002, Tarjuelo et al. 2002). They also noted that *B. violaceus* exhibited similar survival on open or caged panels whereas *B. schlosseri* apparently suffered mortality due to browsing fish such as *Tautogolabrous adspersus* (cunner). *B. schlosseri* recruits deployed on panels on unscreened pilings were also vulnerable to small predatory gastropods including *Mitrella lunata* (lunar dove snail), *Anachis lafresnayi* and *Anachis avara* (greedy dove snail). Only when *B. schlosseri* colonies reached 3-4 wk of age did they exhibit similar levels of mortality to *B. violaceus*. In Japanese waters, Yamaguchi (1975) observed that young juveniles of *B. violaceus* were vulnerable to browsing fish, but adults were not eaten extensively.

Adult colonial tunicates generally have few predators, although the gastropod *Erato voluta* has been observed to feed on adult zooids of *B. schlosseri* by inserting its proboscis through the oral siphon (Fretter 1951). Other predators include turbellarian flatworms such as *Cycloporus papillosus* that attaches to the surface of the colony by its ventral sucker and extracts whole zooids (Jennings 1957). Yamaguchi (1975) noted that the polyclad flatworm *Cycloporus japonicus* was observed to feed on *B. violaceus* in the laboratory. Both species were preyed on by juvenile spider crab (*Libinia emarginata*) in laboratory trials (Osman and Whitlatch 2004). Gittenberg (pers. comm.) noted that predatory nudibranchs such as *Goniodoris castanea* were observed to feed on *B. schlosseri* in the Netherlands.

Teo and Ryland (1994) found that extracts of *B. schlosseri* tended to deter feeding behaviour in certain predators such as shore crabs and browsing fish. Pisut and Pawlik (2002) investigated whether metabolites in the body tissues or inorganic acids in the tunic played a role in protecting adult ascidians from predation but found no clear-cut effect. The only *Botrylloides* species tested (*B. nigrum*) possessed neither form of chemical defense. In contrast, Tarjuelo et al. (2002) did find a chemical basis for the unpalatability of certain colonial ascidians, but this may not apply to all colonial species.

3.7. DISEASES AND PARASITES

Few studies are available on the disease agents that may affect colonial tunicates. Moiseeva et al. (2004) described a progressive fatal disease called 'cup cell disease' where the time course from first appearance to death was 30 to 45 d. The disease-causing agent was believed to be a haplosporidian protist which can be transferred through seawater without direct contact between infected colonies. Levine (1981) examined 361 ascidian specimens of the 20 most common species in California and 14 species were parasitized at prevalence rates of 10% to 100%. Oosihi (1999) also described a copepod parasite *Botryllophilus ruber* on *B. schlosseri*, noting that an introduction of a species may actually come with an assemblage of parasites.

4.0 HUMAN USES

B. schlosseri has been extensively studied as a model system in the context of the evolution of allorecognition (De Tomaso et al. 1998, Cohen et al. 1998, Rinkevich 2002, De Tomaso and Weissman 2004). Bryan et al. (2003) found that compounds in the tunic of certain species such as *Botryllus planus* possessed chemical antifoulant properties. Granato et al. (2005) also reported several metabolites in colonial ascidians, but none of these were found to act against cancer cells. Matsuno and Sakaguchi (1984) isolated peridinin from *B. violaceus*.

5.0 POTENTIAL VECTORS FOR INTRODUCTION/DISPERSION

Dispersal of asexual buds (fragmentation) as well as larval swimming are two mechanisms by which colonial ascidians can expand their range into new environments. Rabinowitz and Rinkevich (2004) studied the fate of developing buds of *B. schlosseri* under

laboratory conditions and found that unattached buds could survive for up to 150 d whereas attached buds survived for only 35 d. By comparison, the dispersal potential of the larval tadpole stage that only swims freely for up to 36 h is relatively minor. Berrill (1950) argued that the larva of *B. schlosseri* is adapted for site selection and settlement rather than dispersal. Hiscock (2005) estimated a larval setting time of <1 d but a dispersal potential of 1-10 km depending on the local hydrodynamic conditions. Several population genetic studies suggest that larval dispersal is limited. Sabbadin and Graziani (1967) found that genetically-distinct sub-populations of *B. schlosseri* existed under similar ecological conditions within the Lagoon of Venice. Yund and O'Neil (2000) noted that genetic differentiation may occur over very short distances (8 to 21 m), and the patterns were consistent with inbreeding and genetic drift models. Grosholz (2001) observed significantly different survival and growth patterns in reciprocally-transplanted clones suggesting genetic differences between geographically-close populations. Studies focusing on the degree of genetic differentiation between populations using microsatellites (e.g. Ben-Shlomo et al. 2001) or allogenic responses (e.g. Rinkevich et al. 1992) may provide more insights into the dispersal potential of these two colonial tunicate species.

Natural pathways or mechanisms for introduction/dispersal include rafting on eelgrass, algae or other forms of floating debris (Van Name 1910). Worcester (1994) observed that colonies of *B. schlosseri* rafting on eelgrass contained brooded larvae that may have been released after the colony settled in a new habitat. Transport of free-swimming larvae in the ballast water of ships is unlikely because of their short larval cycle, but ballast may contain floating debris that could serve as a vector for adult forms. Another human-mediated pathway is the hull fouling of recreational watercraft, coastal fishing fleets and dredging barges. Lambert and Lambert (2003) noted that large numbers of boats with fouled hulls, especially small pleasure craft, likely provide new breeding stock to recolonize denuded areas and enhance gene flow among harbours. Slow-moving boats or towed barges may be more likely potential vectors than faster moving ships due to the reduced friction on the hull surface. Circumstantial evidence suggests that the appearance of both species in Savage Harbour on the north shore of P.E.I. in 2002 was linked to the presence of a dredging barge that had originated in the Bay of Fundy (MacNair, pers. comm.). Aquaculture activities

have also been responsible for the introduction of colonial tunicates. Polk (1962) reported that oysters imported from Holland were responsible for the introduction and rapid spread of *B. schlosseri* in Belgium in 1960. Likewise Lambert et al. (1987) suggested that the appearance of this species at an oyster farm in Washington State in the early 1970's was likely associated with the movement of oyster spat.

6.0 IMPACTS ASSOCIATED WITH INTRODUCTIONS

6.1. IMPACTS ON THE ENVIRONMENT

The introduction of a colonial tunicate species may have a negative impact on water quality depending on the size of the population and the hydrodynamics of the system. Under turbid conditions the increased rate of particle removal may improve water clarity thereby enhancing benthic macroalgal or eelgrass production. If food resources are limited, however, this increased demand may negatively impact resident filter feeders such as zooplankton and bivalve populations. A substantial increase in tunicate biomass may translate into greater biodeposition of fecal material that may in turn increase the risk of benthic habitat degradation. This problem may be exacerbated if colonial tunicates undergo a widespread mortality event and the decaying biomass accumulates on the bottom. Under extreme conditions, water quality could be impacted by the amount of ammonia excreted by the tunicate colonies.

6.2. IMPACTS ON OTHER SPECIES

Colonial tunicates compete for space by overgrowing and smothering existing species; in some cases the net impact may be a reduction in community species diversity. In a rapid assessment survey of native and non-native marine species of floating dock communities in New England, Pederson et al. (2005) found that *B. violaceus* and *B. schlosseri*, along with two other introduced compound ascidians, were very effective at overgrowing algae and other fouling organisms. These tunicate species represented 25% of the introduced species, whereas tunicates in general accounted for only 4% of all species.

Zajac et al. (1989) noted that *Botrylloides* sp. had a negative impact on the survival and growth of oyster spat probably as a result of localized food depletion rather than overgrowth. Arakawa (1990) noted that tunicates grew more rapidly than oyster spat and

effectively interfered with their survival. Anecdotal reports from the Bras D'Or Lakes (N.S.) also suggested that *B. schlosseri* can overgrow and smother young oyster spat on shell collectors (Stuart, pers. comm.). In the case of juveniles and adults, the potential impacts of tunicate smothering behaviour vary among studies. For example, Dalby and Young (1993) observed no consistent pattern in terms of the impact of colonial ascidians on oysters; overgrowth did not always lead to mortality and in some cases growth and survival were enhanced. In comparative trials in P.E.I. where *B. violaceus* was actively cleaned from the surface of cultured mussels, MacNair (pers. comm.) reported no significant positive impact on growth, meat yield or survival relative to heavily fouled mussels. Likewise Bullard (pers. comm.) found that overgrowth of young cultured oysters by the colonial tunicate *Didemnum sp*. had no negative impact on performance. Conversely, in New Zealand, Coutts and Sinner (2004) reported that an infestation of the colonial tunicate *Didemnum vexillum* on the green mussel (*Perna canaliculus*) lead to a loss of condition and in some cases mortality.

Sebens (1997) noted in extensive diving surveys that the combined presence of sea urchins and *Botrylloides* severely impacted the indigenous assemblage, and the botrylloids seemed immune to urchin grazing. It was argued that, even in the absence of the urchins, the capacity of *Botrylloides* to overgrow and outcompete indigenous species may have compromised the former natural habitat.

Less well-documented chemical interactions include the observation that *B. schlosseri* metabolites may have toxic effects on barnacle nauplii, copepods and mussel gills (Braiko et al. 1988). Also, Teo and Ryland (1995) suggested that extracts of *B. schlosseri* displayed moderate levels of toxicity against invertebrate larvae. In contrast, Keough (1998) found that the presence of *B. schlosseri* had a positive impact on the settlement of other fouling organisms, specifically arborescent bryozoans (*Bugula* spp.).

6.3. IMPACTS ON INDUSTRY

It is generally acknowledged that the colonial tunicates, *B. violaceus* and *B. schlosseri*, have the potential to impact marine industrial activities, but there are few documented studies. Various surveys have listed their occurrence as a nuisance fouling organism on the hulls of ships, floating docks and nautical buoys (Lambert and Lambert 2003, Pederson et al.

2005). In shellfish aquaculture operations, they may overgrow seed collectors thereby smothering young juveniles or excluding the settlement of the desired species. Finfish nets or shellfish cages may become infested with a mat of colonial tunicates that effectively eliminates the flow of oxygen and particles through the mesh. In one case they were observed to cover 90-100% of the cage surface and weigh up to 6 $kg.m^2$ (Cao et al. 1998). The actual impact on the growth and survival of the caged inventory is likely important but no estimates are available. Other costs directly related to tunicate biofouling include the increased labour and maintenance activities required to keep the equipment clean.

6.4. CONTROL METHODS

In general, the strategy for controlling the spread of an invasive species is based on three principles: eradicate, contain and mitigate. Eradication is typically an option in the early stage of an introduction, but becomes very difficult with the geographic dispersal of the species. Containment implies the restriction of the space occupied by the invasive species. This is particularly difficult in the case of colonial tunicates that have the ability to spread asexually by means of budding, fragmentation or larval dispersal. Finally, mitigation involves the development of management strategies to reduce the impact of the invasive species; unless an introduction can be quickly eradicated, this is often the only remaining option.

Ideally, natural predators such as small gastropods are the preferred control method for eliminating biofouling organisms from aquaculture equipment, but colonial tunicates are apparently unpalatable to many predatory species. In terms of active control measures, Lambert (pers. comm.) suggested that *B. violaceus* should be treated with freshwater when it overgrows oyster spat. In a survey of possible methods for controlling the proliferation of *B. violaceus* on cultured mussels in P.E.I. (MacNair, pers. comm.), spraying or dipping in vinegar (5% acetic acid) for 15 sec was found to be substantially more effective than freshwater, brine or lime treatments. Note, however, that this approach was not recommended for juvenile mussels and possibly other shellfish which may be vulnerable to the effects of vinegar; preliminary trials should be undertaken to assess the impact on the cultured species. For a more detailed discussion of possible strategies for eliminating tunicates, see Carver et al. (2006).

Although not very practical as a control measure, Rinkevich and Weissman (1990) studied the effect of X-ray irradiation on *B. schlosseri* colony survival; colonies exposed to more than 5,000 rads died within 19 d whereas exposure to less than 2000 rads did not cause mortality. Cima et al. (1995) reported that *B. schlosseri* is sensitive to organotics, especially butyltins. Terlizzi et al. (1997) recommended the use of easy-release silicone coatings on ship hulls to reduce the risk of fouling and dispersal of species such as *B. schlosseri*. Preparation or cleaning protocols should be devised for mussel seed as well as other commercial species to reduce the risk of transferring these colonial tunicate species to new areas within Atlantic Canada.

Once colonial tunicates are introduced and become established, they are likely to persist despite treatments such as de-fouling, since the surrounding structures may act as a source of subsequent infestations. Coutts and Sinner (2004) actually caution that de-fouling operations could exacerbate the problem futher since colonial tunicates tend to disperse by fragmentation. Fortunately, in Atlantic Canada both species apparently become dormant or die-back during the winter months, although specific estimates of mortality are not available.

6.5. IMPACT SUMMARY

Introduction of any species into a new environment may lead to a disruption in community structure/dynamics but such interactions are varied, complex, and difficult to predict. Competition for space or food resources may lead to the displacement of native species, while a population explosion may lead to excessive organic enrichment of the system. These impacts may translate into biological, economic and/or human health risks.

In general, the recent spread of *B. schlosseri* and *B. violaceus* into new regions of Atlantic Canada is arousing concern because of their reputation for overgrowing other species and possibly jeopardizing their survival. It would appear that the ongoing expansion of the aquaculture industry has effectively increased the availability of suitable settlement substrate thereby encouraging the development of these colonial tunicate populations. Yet the longterm implications for the aquaculture industry and other marine industries remain to be determined. More information on various aspects of the life history and ecology of these species, particularly their impacts on the growth and survival of cultured shellfish, would

improve our ability to assess their potential impact on the marine ecosystem in Atlantic Canada. It would also provide a better platform for devising management strategies to reduce the risk of continued spread and mitigate the impact of these species in affected areas.

7.0 CONSERVATION STATUS

B. schlosseri and *B. violaceus* are both considered "globally secure" or without conservation status.

8.0 SUMMARY

Anecdotal reports from the Atlantic coast of N.S. and more recently P.E.I. suggest that the colonial tunicates *B. schlosseri* and *B. violaceus* are now exhibiting the rapid population growth often associated with invasive species. Studies from other regions suggest that adjacent subpopulations of these species may exhibit different life history traits depending on food resources and temperature conditions. This variability highlights the urgent need for studies documenting the growth, generation time and longevity of local populations in Atlantic Canada. Likewise, more information is required to accurately assess the impact of these species on the growth and survival of cultured shellfish. This information is critical to devising control measures to reduce the risk of further dispersion as well as developing effective mitigation and eradication strategies to minimize their impacts on aquaculture industry and the structure of marine communities in Atlantic Canada.

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